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ABSTRACT

The present study compares the impact of thermal and high pressure high temperature (HPHT) processing on volatile profile (via a non-targeted headspace fingerprinting) and structural and nutritional quality parameter (via targeted approaches) of orange and yellow carrot purees. The effect of oil enrichment was also considered. Since oil enrichment affects compounds volatility, the effect of oil was not studied when comparing the volatile fraction. For the targeted part, as yellow carrot purees were shown to contain a very low amount of carotenoids, focus was given to orange carrot purees. The results of the non-targeted approach demonstrated HPHT processing exerts a distinct effect on the volatile fractions compared to thermal processing. In addition, different colored carrot varieties are characterized by distinct headspace fingerprints. From a structural point of view, limited or no difference could be observed between orange carrot purees treated with HPHT or HT processes, both for samples without and with oil. From nutritional point of view, only in samples with oil, significant isomerisation of all-*trans*-β-carotene occurred due to both processing. Overall, for this type of product and for the selected conditions, HPHT processing seems to have a different impact on the volatile profile but rather similar impact on the structural and nutritional attributes compared to thermal processing.

1. Introduction

1.1 High pressure high temperature (HPHT) treatment as a potential alternative for thermal sterilization

Sterilization treatments aim to inactivate both vegetative cells and microbial spores, resulting in food products which are shelf-stable. In order to obtain this stability in low-acid products with high water content, thermal processing is commonly applied. The high thermal load which especially needs to be applied to products with slow heat transfer results in considerable quality changes compared to the untreated product [1]. Improving the heat transfer is one of the strategies which have been followed in optimizing thermal processes. Since the year 2000, high pressure (e.g. 600 MPa) combined with high temperature (e.g. 121 °C) has been discussed in literature as an alternative for thermal processing [2-5]. Some authors addressed this technique as a 'high pressure-assisted thermal process' describing the high pressure high temperature (HPHT) treatment as a more optimal thermal treatment in which pressure is used to quickly increase the temperature of the product due to compression heating [2, 6]. Others have been studying the effect of pressure at high temperature and reported a clear pressure effect on quality changes at that particular high temperature [3, 7-9]. Both increasing and decreasing effects on the reaction rate constants of high pressure at high temperature have been described [10].

In the end, the impact of a HPHT process on the food product will be the integrated impact of pressure, temperature and time. In order to compare the process impact of this novel technique to its traditional thermal counterpart, process conditions should be selected on a fair basis, for example equivalent microbial inactivation resulting in products with a similar microbial shelf-life [11-13]. However, to obtain insight in the

individual effect of the process variables, the importance of kinetic experiments in which all process variables are accurately monitored should not be forgotten [10].

1.2 Carrots (Daucus carota) are one of the most popular root vegetables

Carrots can be considered as primary vegetables in many countries. In recent decades, carrots gained popularity due to the awareness of their nutritional value and health-related benefits [14]. Carrots exist in different varieties, such as orange, yellow, red, white and purple. This genetic variation combined with cultivation conditions and exposure to ethylene affect the sensorial (e.g. volatiles and polyacetylenes) and nutritional (e.g. carotenoids, vitamins and minerals) quality parameters [15-17]. It has been described in literature that food processing techniques affect (negatively/positively) these quality parameters. On the one hand, as a result of conversion of carotenoids (*trans* to (poly-)*cis*) and degradation reactions, the beneficial biological activity of the carotenoids is altered (e.g. reduced antioxidant and provitamin A capacity) [18-21]. On the other hand, processing has been shown to positively affect carotenoid bioaccessibility and bioavailability in most cases [22].

Comparing the process impact on carrot quality parameters between conventional thermal and HPHT processes has been the topic of interest of other researchers before. However, research studies in which the comparison was performed based on the principle of equivalence are scarcely found in literature. Recently, fair comparisons have been reported in studies of Knockaert et al. [8] (targeted approach, carrot pieces), Vervoort et al. [24] (targeted approach, orange juice), Timmermans et al. [11] (targeted approach, orange juice), Vervoort et al. [13] (targeted approach, carrot pieces), Vervoort et al. [25] (untargeted approach, carrot pieces) and Kebede et al. [26-28] (untargeted

approach, wide range of vegetables). It is thus clear that additional investigations in this context are needed in order to evaluate using science-based evidence the potential of HPHT processing. Besides, since the quality parameters differ from variety to variety, more than one carrot cultivar should be taken into account.

In this perspective, the main objective of the present work was to compare the effect of thermal and HPHT processing on a range of important quality attributes. Aiming for a fair comparison, processing conditions resulting in equivalent processes in terms of microbial inactivation were selected. Taking into account the possible difference in chemical composition among varieties, the focus is given to carrots with different colour varieties, i.e. orange and yellow carrots. Given the fact that oil might have an effect on the stability of lipophilic nutrients, the enrichment of oil to the carrot purees was considered as well. The novelty of the present work is that, the comparison in the impact of conventional and novel sterilization techniques was performed from both non-targeted and targeted approaches. In the former case, the impact on volatile fractions of differently processed carrot puree was compared using an untargeted fingerprinting approach (integration of headspace (HS) solid-phase micro-extraction (SPME) GC-MS method and multivariate statistical data analysis). In the latter case, a targeted approach to analyze specific quality related parameters (color, carotenoid profile, particle size distribution, microscopy) was performed. A schematic overview of the research plan of this work can be found in **Fig. 1**.

2 Materials and methods

2.1 Sample preparation

Single batches of fresh orange and yellow carrots (*Daucus carota* cv. Nerac and cv. Yellow mellow, respectively) were bought at a local market and stored at 4 °C. Carrots

were peeled, cut into slices as homogeneously as possible, packed in plastic bags and blanched at 95 °C for 8 minutes. At all times orange and yellow carrots were kept separately. Blanched carrot bags were frozen in liquid nitrogen and stored at -40 °C until use. Singular carrot purees were prepared by blending blanched carrots with water (1:1) for 1 min in a kitchen blender. In the case of the carrot purees enriched with oil, they were stirred with 5% (w/w) extra virgin olive oil for 15 min at room temperature.

The carrot puree was homogenised at 10 MPa for 1 cycle using a high-pressure homogeniser (Panda 2K; Gea Niro Soavi, Mechelen, Belgium). Since the product temperature usually increases after homogenisation, the sample inlet and outlet were thermostated at 4 °C and the pressure was controlled on a digital display.

2.2 Processing

Sterilization treatments were performed using both traditional technologies (thermal treatments) and novel technologies (HPHT treatment). Aiming fair comparison of the process impact, an equivalent industrially relevant process value F_0 = 3 min was put forward for both processing targeting inactivation of spores of *Clostridium botulinum*. For both treatment types a particular reactor holding temperature (T_h) of 117 °C was selected. Each treatment was repeated six times for valid statistical data analysis afterwards. Due to the lack of reliable kinetic data as a result of incomplete understanding of the combined effect of pressure and temperature on *Clostridium botulinum* spore inactivation [10], in the present work, the HPHT was considered as pressure assisted thermal processing. Due to their inert nature, glass jars for the thermal and Teflon sample holders for the HPHT processing were selected. A comparison of the monitored profiles of the process variables during both treatments, can be found in **Fig. 2**.

2.2.1 Thermal processing

For the thermal treatments, glass jars (100 mL volume, 95 mm height and 45 mm diameter) were filled with 85 ± 0.5 g carrot puree and treated in a static steriflow pilot retort (Barriquand, Paris, France). Temperature profiles of the retort and at the coldest point in the glass jars (1 cm above the bottom) were controlled by type-T thermocouples (Ellab, Hillerod, Denmark). The total process time (t_p) was on average 60 minutes (**Fig. 2**).

2.2.2 High pressure high temperature processing

High pressure sterilization was carried out using laboratory-scale 6-vessel high pressure equipment (custom-made, Resato, Roden, the Netherlands) with propylene glycol fluid (PG fluid, Resato, The Netherlands) as the pressure medium. The HPHT equipment allows computer controlled pressure build-up to 800 MPa, temperature control up to 120 °C and data logging of both sample pressure and temperature (**Fig. 2**). For pressure increase, the equipment consists of a pressure prefill pump which builds up the pressure to 150 MPa with a single piston displacement after which a high pressure intensifier can further built up the pressure at a particular selected pressure build-up rate. The high pressure sterilization processes were performed at 600 MPa combined with a process temperature of 117 °C. Teflon cylindrical tubes (12 mm internal diameter, 4 mm thickness, 85 mm length) were filled with carrot puree, closed with a movable cap, vacuum sealed with double plastic bags, equilibrated at 10 °C and placed in the pressure vessels equilibrated at the process temperature. The pressure build-up was started when the temperature registered in the vessels by type-J thermocouples (Ellab) reached 75 °C (i.e. initial temperature). Pressure was immediately increased to 150 MPa after which pressure was further built up to 600 MPa at a rate of 10 MPa/s. Assuming no effect of pressure on spore inactivation under HPHT conditions, HPHT processes were at least

thermally equivalent to the thermal treatment aiming a F_0-value of 3 min. The product temperature was recorded online and the holding time was adjusted to achieve the targeted F_0 value. The vessels were decompressed after the required holding time. On average, the total process time (t_p) was 20 min (**Fig. 2**).

2.2.3 Post treatment sample handling

Following treatments, samples were immediately transferred to ice water to stop any process-induced reaction. Consequently, treated samples were emptied in a cooling room and transferred to a small volume (10 ml) polyethylene terephthalate tubes with a polyethylene cap. Hereafter, the tubes were frozen in liquid nitrogen and stored at -40 °C until analysis.

2.3 Analysis of the volatile profile by headspace fingerprinting

2.3.1 Headspace SPME-GC-MS analysis

Targeting detection of a wide range of volatiles in a particular food extract, a headspace (HS) fingerprinting SPME-GC-MS method of analysis was optimized beforehand. The method includes incubation, extraction using an appropriate type of fiber coating and GC-MS parameters. Carrot puree (3 g) was weighed into 20 mL headspace vials (Supelco, Belfonte, USA). All headspace analyses were conducted on an Agilent 7890A gas chromatograph (GC) coupled to a 5975C mass selective detector (MS) (Agilent Technologies, Santa Clara, CA, USA) and equipped with a CombiPAL autosampler (CTC Analytics, Zwingen, Switzerland). Vials were equilibrated in the incubator at 40 °C for 10 min under agitation at 500 rpm. The carrot puree volatile compounds in the headspace of the vial were sampled for 30 min by means of a solid phase microextraction (SPME) fiber with 50/60 µm DVB/CAR/PDMS (divinylbenzene/carboxen/polydimethylsiloxane) sorptive coating (StableFlex; Supelco,

189 Bellefonte, PA). The fiber was preconditioned according to the manufacturer's
190 guidelines before its first use. Desorption was carried out for 10 min at 250 °C. The
191 volatiles were injected in splitless mode and subsequently separated on a capillary
192 column (30 m × 0.25 mm; 1.0 µm film thickness, HP-5MS; Agilent Technologies,
193 Santa Clara, CA), using helium as carrier gas at a constant flow rate of 1.2 mL/min. The
194 column oven was programmed at a starting temperature of 40 °C, which was retained
195 for 5 min, after which it was elevated to 170 °C at a rate of 5 °C/min and retained for 5
196 min, followed by a second ramp to 230 °C at 15 °C/min. After 5 min at the final
197 temperature, the oven was cooled again to the initial temperature. Mass spectra were
198 obtained by electron ionisation (EI; 70 eV), with a scanning range of *m/z* 29–250. MS
199 ion source and quadrupole temperatures were 250 and 150 °C, respectively.

200 ### 2.3.2 Data preprocessing and multivariate data analysis

201 As commonly observed in GC-MS analysis, co-eluting compounds were present in the
202 obtained chromatograms. Therefore, all chromatograms were analyzed with Automated
203 Mass Spectral Deconvolution and Identification System (AMDIS) (Version 2.66, 2008,
204 National Institute of Standards and Technology, Gaithersburg, MD, USA) to extract
205 "pure" component spectra from complex chromatograms. The deconvoluted spectra
206 were then analyzed with Mass Profiler Professional (MPP) (Version 12.0, 2012, Agilent
207 Technologies, Diegem, Belgium) aiming filtering and peak alignment. The MPP
208 obtained a spreadsheet containing peak areas, which was used as an input for the
209 statistics. The multivariate data were analyzed with a multivariate statistical data
210 analysis (MVDA) which was carried out in Solo (Version 6.5, 2011, Eigenvector
211 Research, Wenatchee, WA, USA). As a preprocessing step, all data were mean-centered
212 and the variables were weighed by their standard deviation to give them equal variance.
213 In a first approach, principal component analysis (PCA) was performed to evaluate each

data set and to detect potential outliers. In this work, the MVDA was performed at two parts. In a first part, the comparison of the impact of the different sterilization treatments per carrot puree type (orange versus yellow) was carried out applying principal least squares discriminant analysis (PLS-DA) to the data set, considering the volatiles as *X*-variables and the blanched, thermal treatment and HPHT treatment as the three categorical *Y*-variables. In a second part, PLS-DA was performed to study the influence of the carrot variety (orange versus yellow) per treatment type. In this case, the volatiles were considered as *X*-variables and the carrot varieties as two *Y*-variables. Determining the complexity of the model, the lowest number of latent variables (LVs) resulting in a class separation were used. In PLS-DA, to qualitatively investigate impact differences among the classes, bi-plots were plotted. To quantitatively select discriminant headspace components, Variable IDentification (VID) coefficients were calculated [20]. These values correspond to the correlation coefficient between each original X-variable and Y-variable (s). The volatile identification was carried out for compounds which had a VID coefficient higher than 0.800 in absolute value. Identification was performed by comparing the compound's mass spectra from the NIST spectral library (NIST08, version 2.0, National Institute of Standards and technology, Gaithersburg, MD, USA). A visual inspection of the spectral matching was carried out accepting a threshold match and reverse match of 80%. Retention time indices were calculated. All plots were made using OriginPro 8 (Origin Lab Corporation, Northampton, MA, USA). A Tukey's multiple comparison was used to test for significant differences between the mean peak areas ($p < 0.05$) of the discriminant headspace components.

2.4 Analysis of nutritional and structural characteristics

2.4.1 Determination of the carotenoid profile and content

In order to determine the carotenoid profile and content of the carrot purees, an extraction step was performed based on the procedure of Sadler et al. [29]. Briefly, carrot puree was mixed with $CaCl_2$ (ratio 1:1) and 50 ml extraction solution (containing 50 % hexane, 25 % ethanol, 25 % acetone and 0.1 % BHT) and the mixture was stirred for 20 minutes at 4 °C. After adding 15 ml reagent grade water, the mixture was stirred for another 10 minutes at 4 °C. The organic layer, which could be separated from the aqueous layer and which contains the carotenoids, was filtered (Chromafil PET filters, 0.20 µm pore size, 25 mm diameter; Macherey-nagel, Duren, Germany) and stored in dark vials before further analysis. The extraction procedure was carried out under dimmed red light.

The carotenoids in the extract were separated and quantified using an HPLC system (Agilent Technologies 1200 Series, Diegem, Belgium), equipped with a C_{30} column (5 µm x 250 mm x 4.6 mm, YMC Europe, Dinslaken, Germany) and a diode array detector. During the analysis, the temperatures of the autosampler and the column were kept at 4 °C and 25 °C, respectively. To separate the different carotenoid isomers, linear gradient elution was used. The gradient was built up in 20 min from 81% methanol, 15% methyl-t-butyl-ether and 4% reagent grade water to 41% methanol, 55% methyl-t-butyl-ether and 4% reagent grade water at a flow rate of 1 ml/min [30]. Identification and quantification of the carotenoids was performed at 450 nm. Calibration curves for all-*trans*-β-carotene, 15-*cis*-β-carotene, 13-*cis*-β-carotene, 9-*cis*-β-carotene and lutein (CaroteNature, Lupsingen, Switzerland) were used to quantify the β-carotene and lutein content of the orange and yellow carrot purees, respectively.

2.4.2 Objective colour measurement

Colour measurements (CIE *L*a*b** values) were conducted using a Hunterlab ColourQuest colorimeter (45°/0° geometry, Illuminant D65, Reston, VA, USA). The instrument was calibrated daily with a black and green ceramic tile. At a 10° angle, the CIE colour space coordinates were determined in triplicate, whereby *L** is indicating the lightness (varying from 0, black, to 100, white), *a** is a measure for the redness (varying from -60, green, to +60, red) and *b** is a measure for the yellowness (varying from -60, blue, to +60, yellow).

2.4.3 Determination of the particle size distribution

The particle size distribution of the carrot purees was measured by laser diffraction using a Malvern Mastersizer S long bench diffractor (Malvern Instruments Ltd., Great Malvern, UK). Laser light (HeNe Laser, wavelength 633 nm, diameter 18 mm) was sent through a suspension of carrot puree (±10 g) in water. The light that was scattered by particles between 0.06 and 880 μm was measured by a series of photodetectors (42 element composite solid state detector array). From the intensity distribution of the scattered light, the particle size distribution of the sample was calculated by the instrument software using the Mie theory. The parameter d (*v*, 0.5) was calculated which indicates the median diameter or the value of the particle diameter at 50 % in the cumulative distribution.

2.4.4 Analysis of the microstructure

The microstructure of the different carrot purees was visualised using light microscopy. To 1 mL of carrot puree, 4 mL water and 5 mL 0.01% toluidine blue solution were added and the mixture was incubated at room temperature for 10 min. The mixture was analysed using an Olympus BX-41 light microscope (Olympus, Optical Co. Ltd.,

Tokyo, Japan) at a magnification level of 10x. Micrographs were taken using image analysis software (AnalySIS pro 5.0 Soft Imaging System GmbH, Bensheim, Germany).

3 Results and discussion

In the following sections, the results will be discussed starting from impact comparison on the volatile profile of each carrot variety using a headspace fingerprinting method (untargeted approach, section 3.1) to impact on nutritional and structural quality aspects (targeted approach, section 3.2).

Oil enrichment of the puree directly affects the volatilizable food extract leading to a decrease in volatility of particular compounds at selected incubation and extraction conditions during the HS-SPME analysis. Consequently, comparing fingerprints of a particular carrot puree (e.g. yellow carrot) enriched with oil or not might lead to biased results not necessarily explaining the effect of oil in particular food reactions but more probably revealing the clear effect of oil on the volatility of particular compounds. Consequently, when comparing the volatile fraction with the headspace fingerprinting (section 3.1), the effect of oil was not studied.

3.1 Comparing impact of thermal and HPHT processes on volatile profile by headspace fingerprinting

In the present work, prior to treatment, vegetables were blanched (section 2.1). Therefore, enzymatic activities were not expected to have a significant impact on the formation of volatiles and consequently changes will be related to non-enzymatic process-induced chemical reactions. A representative total ion chromatogram of the headspace profile of both the blanched/reference orange and yellow carrot samples is depicted in **Fig. 3**. Over 100 distinct headspace components were detected in carrot purees, terpenes and aldehydes being the most abundant. As explained in section 2.3.2

and as schematically shown in **Fig. 1**, deconvoluted spectra were analyzed with Mass Profiler Professional (MPP) aiming filtering and peak alignment. The MPP yielded a spreadsheet containing peak areas per detected compound, which was used as an input for the multivariate data analysis (MVDA). In this work, the selected discriminative markers are particularly discussed for their consequences on flavour.

After using PCA as an exploratory technique, PLS-DA was applied as a supervised method to find differences among the three treated sample classes (blanched, HT, HPHT) taking into account the available knowledge on sample class. Considering the volatiles as *X*-variables and the different treatments as categorical *Y*-variables, biplots of scores and correlation loadings were constructed. This type of plot offers a tool to graphically summarise the analytical data to reveal relationships between samples and to determine volatiles characterising a certain group of samples (section 3.1.1). Variable IDentification coefficients (VID's) were calculated in a following step as a more quantitative tool to select discriminant markers (i.e. compounds which detected amount was different in one class compared to the other classes) (section 3.1.2).

3.1.1 Visualisation of impact differences

In **Fig. 4**, biplots, based on PLS-DA, are representing the process impact differences for orange and yellow carrot purees. From the figures, both the similarity of samples within one group (e.g. repetition of treatments) as well as the differences among the samples of different groups (e.g. thermal versus HPHT processing) can be derived. Fingerprints of samples from the same group clustered and showed clear resemblance. In general, a clear effect of sterilization on the headspace fraction could be observed: large distance was observed between samples from the blanched class and the thermal and HPHT treated sample class. In addition, equivalent sterilization treatments showed different volatile profiles. The separation between the three classes (blanched, HT, HPHT) in

orange carrot purees can be quantified by a variance in the *Y*-variables of 93% described by the first two latent variables. Similar value (88%) was determined in yellow carrot purees. Constructing ellipses representing correlation coefficients of 70 % and 100 % some idea about the importance of volatiles for a specific group/class can be obtained: all volatiles placed between the two ellipses explained the first two latent variables in more than 70 % of its variability. Graphically, if those volatiles are projected between those two ellipses close to a particular group, it means that they are important and are characterized by a higher concentration within that group, compared to the others. However, it is a challenge to deduce from the biplots information about variables which concentration is clearly different in a particular group compared to another group (i.e. discriminant markers), since these are the first interesting compounds to zoom further into in order to understand the observed difference in process impact.

3.1.2 Selection of discriminative markers

Variable IDentification (VID) coefficients as defined by Ooms (1996), serve a quantitative measure to select discriminative markers from the headspace fingerprints (section 2.3.2). In other words, it ranks the components based on their importance for a particular class compared to the other classes: high positive values demonstrating high concentration of a certain compound for that particular class compared to the other classes and low negative values demonstrating the opposite. In this work, for both orange and yellow carrot, volatiles with an absolute value of VID more than 0.800 were considered relevant, identified and further zoomed into (**Table 1**). These discriminant volatiles were plotted individually as a function of processing. To clearly show the most important of the selected discriminant components, those with VID's higher than 0.900 are represented in **Fig. 5** (for orange carrot) and to **Fig. 6** (for yellow carrot). In these plots, the mean areas and the standard errors calculated from the six replicates were

depicted. From these figures, the concentration of those compounds compared to the other groups can be deduced. Several trends could be observed. Terpenes showed relevant VID coefficients in both orange and yellow carrot purees. However, their identity was different depending on the matrix. As indicated in the introduction, terpenes are typical, naturally present flavour compounds. On the one hand, monoterpenes such as sabinene's concentration was the highest in the blanched class. Sterilization might have degraded this terpene. On the other hand, higher concentrations of p-cymenene and α-ionone were found after applying both sterilization treatments. The changes detected in degradation products from carotenoids such as α-ionone are in agreement with previous studies [32, 33], which showed that sterilization processes affected the total β-carotene concentration as well as its isomerization and bio-accessibility. Degradation products of limonene such as terpinolene and ϒ-terpinene, where significantly less detected after HPHT process compared to the other groups. The same effect was observed in the case of other monoterpenes such as o-cymene, sabinene, sesquithuijene. Sesquithujene and β-caryophyllene epoxide were even not detected after high pressure treatments (**Fig. 5**). Published reports demonstrated that terpinolene and caryophyllene contribute significantly to carrot flavour intensity [34]. These conclusions are in agreement with Trejo Araya et al. [7] who determined the headspace volatiles of carrot sticks pasteurized by high pressure processing (600 MPa, 2 min) and thermal treatments (90°C, 5 min). They showed that all monoterpenes and terpinolenes were still present after treatment, and, in some cases, even increased. Since correlations between carrot volatile changes after treatments (either 600 MPa, 2min or 90°C, 5min) and sensorial changes was observed before [7], sensorial studies of these purees could give interesting information about the relevance of the flavour profile

383 modification. In future, further research should be done to investigate a possible
384 relationship between the flavour changes and carotenoid profile in carrot purees.
385 The selected aldehydes as markers in carrot purees were characterized by a negative
386 VID in blanched purees and by a positive VID in the sterilized purees (**Table 1**). In
387 other words, their formation was clearly enhanced by processing and their presence
388 discriminated the blanched from the sterilized samples. This fact could be explained
389 because aliphatic aldehydes can be formed from unsaturated fatty acids due to thermal
390 oxidation [35]. In this study, aldehydes from the degradation of oleic, linoleic and
391 linolenic acid were identified as markers. Thus, aldehydes from oxidized oleic acid such
392 as heptanal, octanal, decanal and 2-decenal were detected in higher concentration after
393 the treatment in orange carrot purees (**Fig. 4**). This trend was also observed in previous
394 studies in carrot pieces where heptanal, octanal and *trans*-2-decenal were selected as
395 markers in the study of the impact of thermal and high pressure processing technologies
396 [25]. Other aldehydes which showed a relevant VID in these purees such as hexanal and
397 2-octenal could be formed by oxidation of linoleic acid [34]. In the present study, the
398 carrot variety had an influence on the determination of markers being only heptanal and
399 octanal markers in the case of yellow carrot purees (**Table 1**). Both aldehydes showed a
400 negative VID in blanched purees indicating that they were formed after treatment in
401 yellow carrot purees. Although both sterilization conditions were established targeting a
402 particular processing value, the temperature histories of the treatments where not the
403 same (**Fig. 2**): the temperature history of the HPHT treatment coming more close to the
404 High-Temperature-Short-Time principle. Taking this knowledge into account, particular
405 reactions (e.g. thermal oxidation of fatty acid) would be estimated to be less pronounced
406 after HPHT treatment (possibly explaining the higher concentration of heptanal after
407 conventional thermal treatment compared to HPHT treatment). However, particular

aldehydes such as 2-nonenal and 2-octenal were significantly more detected after HPHT treatment compared to its thermal equivalent (**Fig. 5**). This observation can be explained by the effect of pressure on the oxygen solubility. This is in line with reports in which oxidative chemical reactions were enhanced under increased pressure [26-28].

3.2 Comparing impact of thermal and HPHT processes on nutritional and structural quality aspects

3.2.1 Characterisation of blanched carrot purees

The blanched orange and yellow carrot purees were characterised in terms of microscopy and particle size (structural characteristics), carotenoid content and isomerisation (nutritional characteristics) and colour values as this might be related to changes in carotenoids. Both samples without and with the addition of olive oil were evaluated. An overview is given in this section. Based on the results, relevant samples for comparing the impact of thermal and HPHT process were selected.

In **Fig. 7**, typical light micrographs of blanched orange carrot purees (A = without oil addition; B = with oil addition) and blanched yellow carrot purees (C = without oil addition; D = with oil addition) are presented. It can be observed that due to mixing and high pressure homogenisation, the carrot tissue was broken down to cell fragments, individual cells and cell clusters. In **Fig. 7B** and **Fig. 7D**, the emulsified oil droplets can clearly be visualised. Comparing micrographs of orange and yellow carrot purees, it is hard to observe clear differences in cell shape and size. Based on the results of the particle size distribution measurements (**Fig. 8**), it can however be seen that in general the particles in the yellow carrot purees are somewhat larger than the particles in the orange carrot purees. Nevertheless, the differences are very limited. Additionally, it can be observed in **Fig. 8** that the particle size distribution curves for samples with and without oil addition are coinciding. This means that the addition of olive oil to the

samples does not have an impact on the carrot tissue particle size after homogenisation. For the samples where oil was added, the particle size distribution curves show a higher volume percentage at small particle size (around 10 µm), compared to the particle size distribution curves of the samples without oil addition. These small particles are a representation of the emulsified oil droplets.

Overall, from a structural point of view, it can be concluded that the blanched yellow and orange carrot purees, which were used as starting material for thermal and HPHT processes, are quite similar with regard to their microstructure and their particle size.

Table 2 summarises the characterisation of the yellow and orange carrot puree in terms of their carotenoid and carotenoid-isomer content and colour values. In orange carrot purees, all-*trans*-β-carotene and its *cis*-isomers were identified to be the main carotenoids. This is in agreement with previous studies on carrots (e.g. [36-38]). In the samples where oil was added, a slightly higher amount of all-*trans*-β-carotene was detected. This could be due to a slightly higher extraction yield as a result of the oil. In general, for all orange carrot purees, the *trans*-isomer accounts for at least 75 %. In the yellow carrot purees, lutein was shown to be the main carotenoid [14], although some smaller unidentified peaks were present in the chromatogram. From the quantitative results in **Table 2**, it can be observed that the concentration of lutein in the blanched yellow carrot purees was very low. Compared to the carotenoid content in the orange carrot puree, the lutein content in the yellow carrot puree was a factor 10 lower. Based on this observation, it was decided not to include the yellow carrot puree in the comparison study (thermal versus HPHT processing) as changes in the lutein content as a result of processing would be difficult to perceive. With regard to the colour values of the purees, the following observations could be made. The samples where oil was added were in both cases lighter (higher L^* value) and less red (lower a^* value) compared to

458 their counterparts without oil addition. For the orange carrot purees, the samples with
459 oil were more yellow (higher b^* value), whereas the opposite was observed for the
460 yellow carrot purees. When putting the yellow carrot purees next to the orange carrot
461 purees, the yellow carrot purees were shown to be lighter and less red. In general, the
462 trends in the experimentally determined L^*, a^* and b^* values were a good reflection of
463 the visual observations of the carrot purees.
464 As explained above, the remaining part of this work, i.e. the actual comparison of the
465 impact of thermal and HPHT processes on nutritional and structural quality aspects, has
466 been focussed on the orange carrot purees.

467 3.2.2 Impact on structural quality aspects

468 The sterilised orange carrot purees were analysed for their particle size distribution and
469 their microstructure. With regard to the particle size, no changes could be observed as a
470 consequence of processing (data not shown). This was independent on the technology
471 that was applied, i.e. thermal or HPHT processing and on the presence or absence of oil.
472 This result implies that the samples were stable during processing in terms of particle
473 size. On the micrographs, cell separation and cell wall swelling can be observed as a
474 result of the sterilisation processes. This has mainly been attributed to β-eliminative
475 pectin depolymerisation and consecutively pectin solubilisation, which are known to
476 occur during processes of high thermal intensity [39, 40]. However, when comparing
477 samples from thermal and HPHT processes, it was hard to differentiate between the two
478 groups of samples based on the degree of pectin solubilisation (data not shown). In the
479 present study, no clear statement can thus be made on the differential effect of thermal
480 and HPHT processes on the microstructure of the carrot samples.

3.2.3 Impact on nutritional quality aspects and colour

Fig. 9 shows the results for the carotenoids in the sterilised orange carrot purees. It should be noted that the results are presented as contributions instead of absolute concentrations. Carotenoid contributions are defined as the proportion of a particular carotenoid relative to the total carotenoid content. This transformation (from absolute carotenoid concentration to carotenoid contribution) has been performed, since as a result of processing, the carotenoid extractability might change **[38]** which can bias the results and conclusions. By expressing the data as contributions, the changing extractability effect is filtered out.

From **Fig. 9A**, it can be observed that for all plain orange carrot purees (without oil addition), whether or not processed, the contribution of all-*trans*-β-carotene was around 70 – 80 %, implying a total *cis*-isomer contribution of 20 – 30 %. The three different *cis*-isomers were present in similar amounts, however the contribution of 13-*cis*-β-carotene was always shown to be lower than the contribution of 9-*cis*- and 15-*cis*-β-carotene. The results show that the sterilisation processes applied in this study (F_0 = 3 min) did only result in a limited or no effect on the carotenoids for carrot samples where no oil was present. A differentiation between thermal and HPHT processing in terms of carotenoids can hardly be made for these samples.

For the carrot purees where oil was added during the preparation (**Fig. 9B**), a clear effect of the sterilisation processes on the carotenoid content can be observed. The all-*trans*-β-carotene contribution was decreased to around 50 %, indicating that in these samples, isomerisation took place during processing. Compared to the samples without oil, 13-*cis*-β-carotene was more important in this case. Difference might be explained by the presence of the oil. Comparing samples enriched with oil but processed with different technologies (**Fig. 9B**), a slightly higher degree of 13-*cis*-β-carotene was detected after traditional thermal processing compared to HPHT processing. The fact

507 that at temperatures above 100 °C, increased processing time results in a higher degree
508 of isomerization was already observed by Knockaert et al. [13] studying β-carotene
509 isomerization in an oil/carrot emulsion.
510 As a final point of comparison, the colour values of the orange carrot purees were
511 evaluated (**Table 3**). Similar to the observations for the carotenoids, orange carrot
512 purees where no oil was added showed no changes in L^*, a^* and b^* values upon the
513 different sterilisation processes. It can thus be deduced that there is a clear correlation
514 between colour and carotenoids and this confirms that the colour of the orange carrot
515 purees is largely determined by the carotenoids. Also in the study of Vervoort et al.
516 [13], correlations between the colour values and specific carotenoids have been
517 revealed. For the orange carrot purees where oil was added during preparation, some
518 changes can be observed as a result of sterilisation. **Table 3** indicates that the sterilised
519 samples had a similar lightness, but they were less red than the blanched sample,
520 independent on the processing technology that was applied. This observation could be
521 related to decrease in all-*trans*-β-carotene which has been noted (**Fig. 9B**). Interestingly,
522 the b^* value of the sterilised carrot purees did not change for HPHT treated samples
523 compared to blanched samples, whereas for HT treated sample, a limited increase of the
524 b^* value could be seen. A similar behavior has been reported by Vervoort et al. [13] in
525 their comparative study on carrot pieces.
526 In literature, other studies on carrots have been conducted comparing specific quality
527 attributes of high pressure and thermally processed carrots. However, in most cases, no
528 fair comparison between both technologies could be made, since the processing
529 conditions that were applied did not result in an equivalent microbial inactivation (e.g.
530 [41, 42]). More recently, this fact was taken into consideration in studies of Knockaert
531 et al. [8] and Vervoort et al. [13] on carrot pieces. By applying specific processing

conditions for both the thermal and high pressure process, the comparison between both technologies could occur on a fair basis. In the former study, focus was given to carotenoid concentration and bioaccessibility and carrot tissue microstructure, in the latter study, a whole range of quality attributes was considered, including enzymes, sugars, vitamins, carotenoids and colour. In common with our study is the analysis of the carotenoids. Some main differences and similarities are listed up. At first, the contribution of all-*trans*-β-carotene is higher in the studies performed on carrot pieces. In the present work, more isomerisation has probably been induced during the mixing and homogenisation process. In the studies on carrot pieces (no oil present), HPHT processing has been shown to result in a better retention of all-*trans*-β-carotene compared to thermal processing (a significantly lower all-*trans*-β-carotene contribution was detected in thermally sterilised carrot pieces). In the present work on orange carrot puree, thermal and HPHT processing affected the carotenoid contribution in a similar way, i.e. a limited or no effect in case of plain orange carrot puree and a distinct decrease in all-trans-β-carotene contribution in case of carrot puree with oil. The presence of oil clearly increased the sensitivity of β-carotene towards isomerisation, which is in line with literature (e.g. [43]). Overall, it thus seems that the state of the matrix (homogenised puree versus pieces) plays an important role in determining whether there is a different effect of thermal and high pressure high temperature sterilisation processes.

4 Conclusion

In this case study on orange and yellow carrots, the effect of thermal and high pressure high temperature (HPHT) processing on a range of important quality attributes was compared. The impact on the quality parameters was investigated from non-targeted and targeted approach. In the first part, a HS-SPME-GC-MS fingerprinting technique was

used to compare the volatile fraction of differently processed carrot puree. In a second part, a targeted approach to analyze specific quality related parameters (color, carotenoid profile, particle size distribution, microscopy) was performed.

From the first part, the research outputs clearly showed the potential headspace fingerprinting approach to zoom into discriminative markers which were clearly detected in other concentrations depending on the processing technology used. In this context, fingerprinting can be seen as a fast, data-based, hypothesis-free comparative starting point, but it is not a result on its own. In the end, the identity of the fingerprint marker should be studied in more detail, for example in the suggestion of the consequence of the difference detected for the consumer. In this work, the identity of the markers was specifically linked to flavour considerations. Given the fact that different sterilization technologies and also different colored carrot varieties seem to generate distinct headspace fingerprints suggests the potential that by particular mixing and selection of the sterilized purees designing targeted carrot flavor profile could become within reach. However, to establish models enabling process and product design, more quantitative insight will be indispensable. In this context, kinetic studies in which exact concentrations of selected markers are evaluated within a range of processing variables should be the first step to be taken [44].

For the second part, as yellow carrot purees were shown to contain a very low amount of carotenoids, focus was given to orange carrot purees for the actual comparative study. The addition of olive oil to the orange carrot purees was considered as well. HPHT processing and HT processing were shown to affect the particle size and the microstructure of the orange carrot purees in a similar way. From a nutritional point of view, in plain orange carrot puree, there was no or a limited effect of sterilisation processes on the β-carotene content and isomerisation, independent on the technology

582 that was applied. In case oil was added to the orange carrot purees, significant
583 isomerisation of all-*trans*-β-carotene occurred, both during HPHT processing and
584 during HT processing. Overall, the colour values were shown to be a good
585 representation of the changes in carotenoids as a result of processing. Overall, for this
586 type of product and for the selected conditions and the selected nutritional and structural
587 quality parameters, it can be stated that no clear distinction can be made between HPHT
588 and HT sterilisation.
589 In general, when comparing the impact of HPHT and thermal processing, HPHT
590 processing resulted in a clear different effect on the volatile profile of orange and yellow
591 carrots, whereas no clear distinction could be with respect to the effect on structural and
592 nutritional attributes. In future, further research should also be done to investigate a
593 flavour consequence of the change in the headspace fraction.

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601

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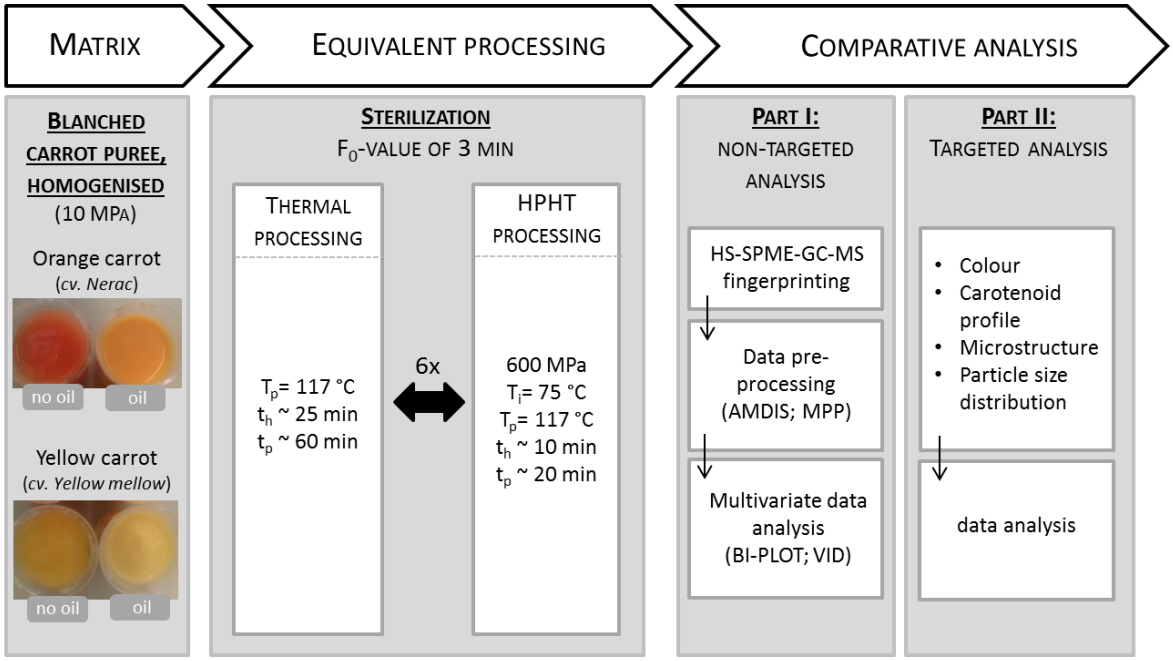


Fig. 1. Schematic overview of the general objective of this work. T_p: holding process temperature; t_h: holding time at process temperature; t_p: total process time including heating and cooling of the sample. T_i: initial product temperature at which the pressure build-up was initiated.

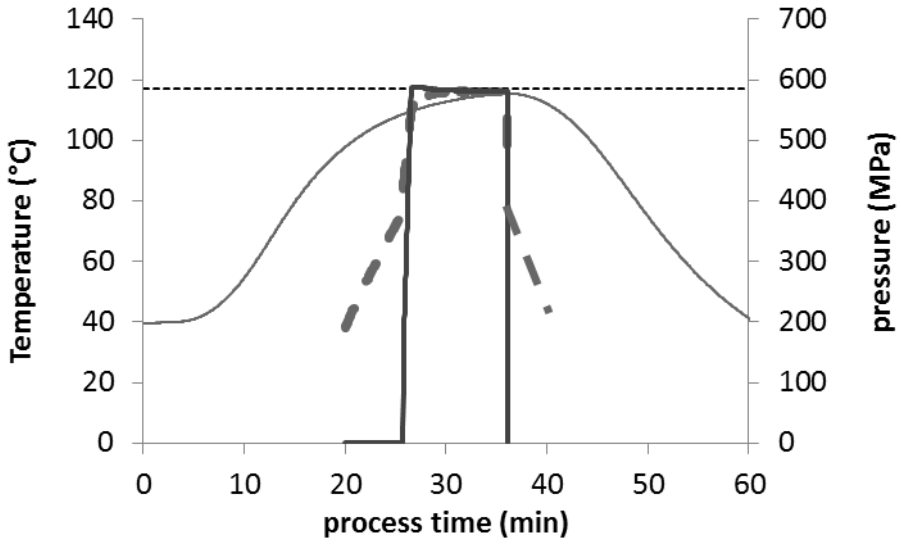


Fig 2. The profiles of thermal treatment with product temperature (thin grey solid line) and HPHT treatment with product temperature (dashed thick grey line) and pressure (thick dark solid line).

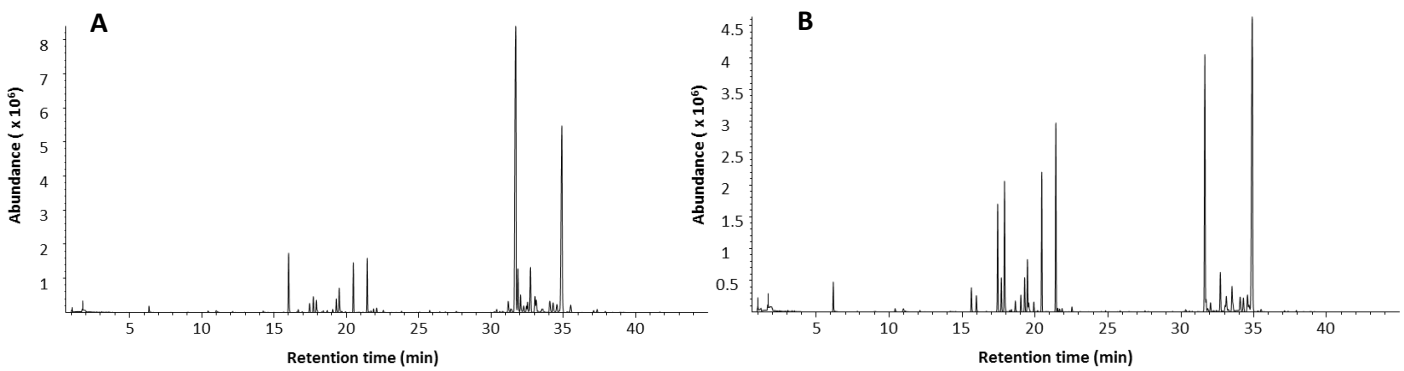


Fig 3. Total ion chromatogram of the headspace of blanched carrot purees obtained by SPME-GC-MS: (A) orange carrot; (B) yellow carrot.

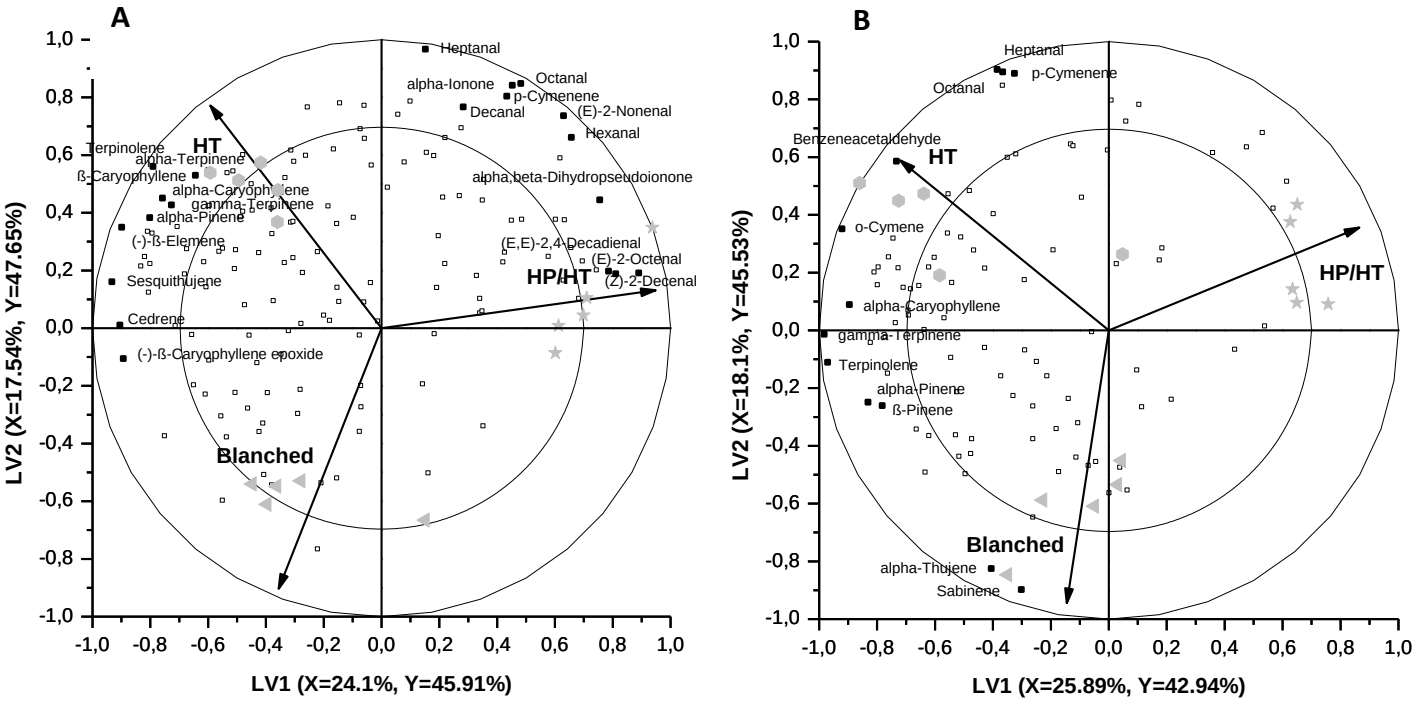
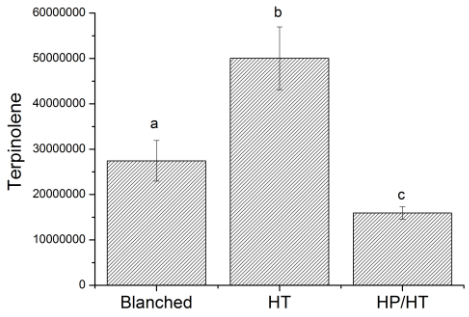


Fig. 4. PLS-DA biplots of the effect of the treatment on carrot headspace fraction in blanched purees (▲) and after applying thermal treatment (HT,●) or high pressure high temperature treatment (HPHT, ★). (**A**) Orange carrot purees; (**B**) yellow carrot purees. Different volatiles are represented by small, open squares. Volatiles with VID higher than 0.800 in absolute value are named and marked in bold (small filled squares). Vectors indicate the correlation loadings for the categorical *Y*-variables. The percentages of the variances in *X* and *Y* explained by each latent variable (LV1 and LV2) are indicated on the respective axes.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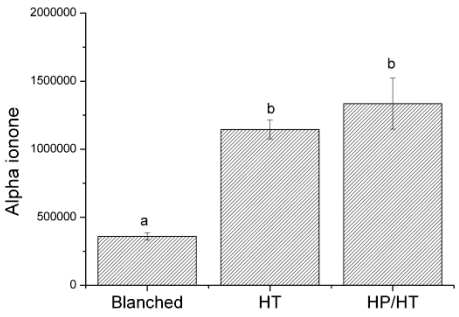
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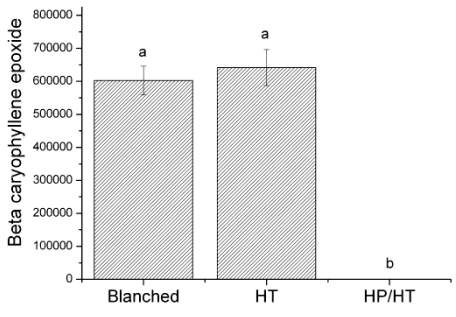
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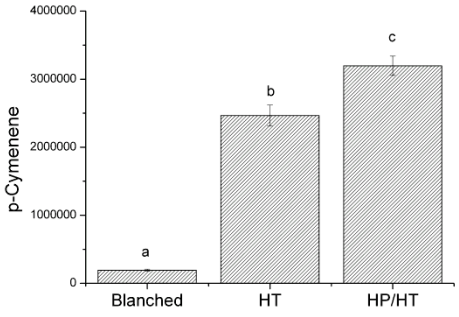
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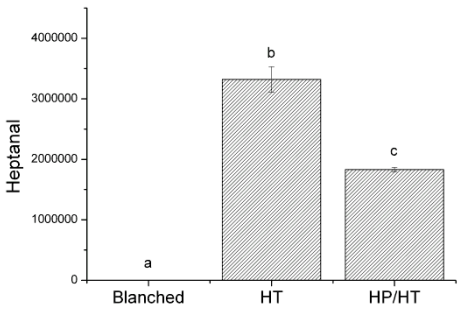
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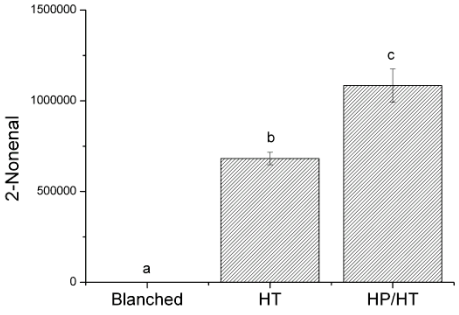
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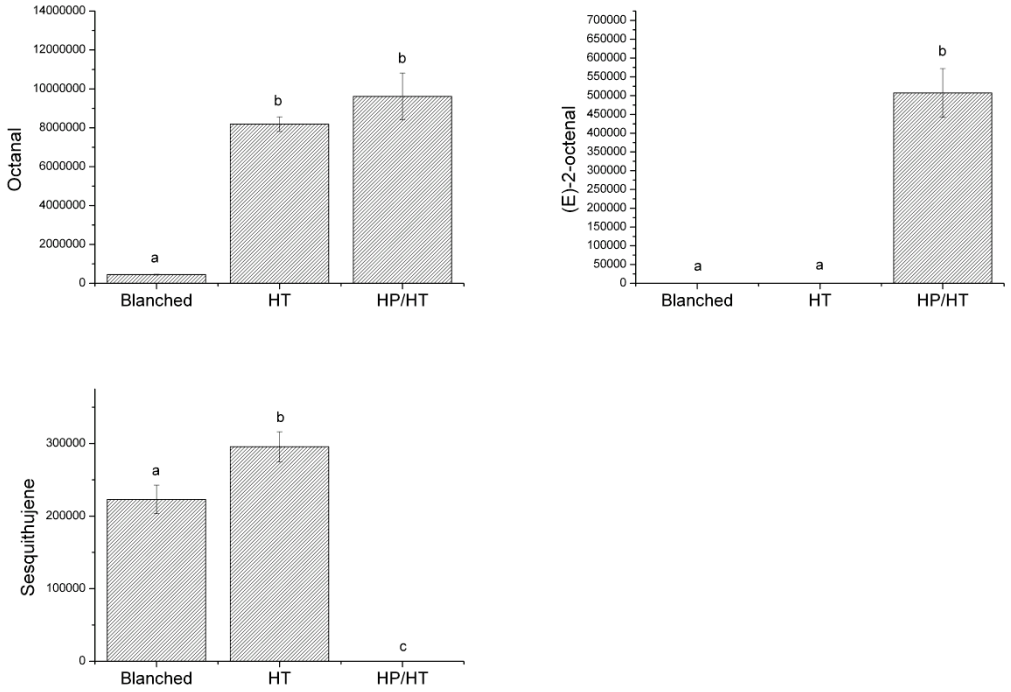

Fig. 5. Discriminative headspace components for comparison of treatment impact on orange carrot purees. Volatiles with VID higher than 0.900 in absolute value in **Table 1** are represented. The *Y*-axis indicates the peak area and error bars represent the standard error of the analysis (n = 6).

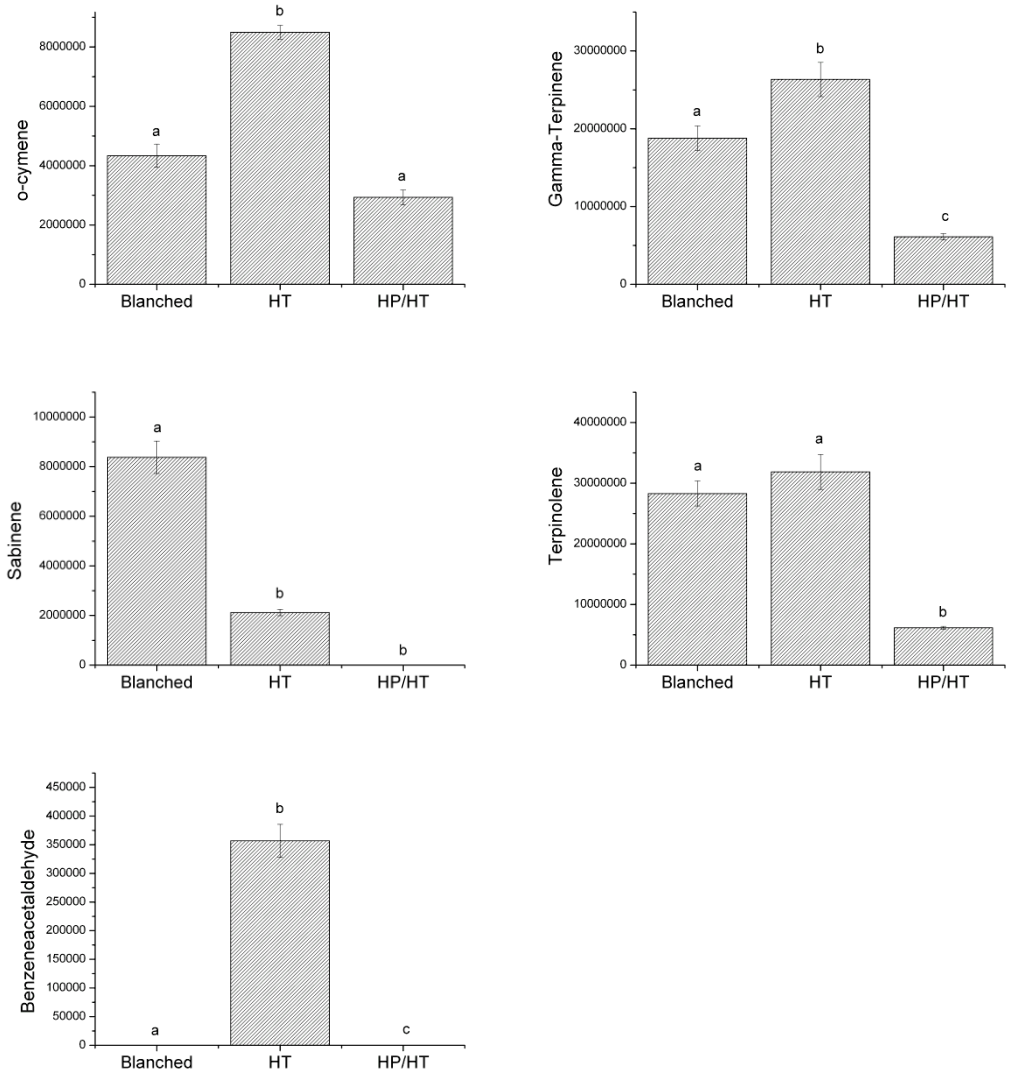

Fig. 6. Discriminative headspace components for comparison of treatment impact on yellow carrot purees. Volatiles with VID higher than 0.900 in absolute value in **Table 2** are represented. The *Y*-axis indicate the peak area and error bars represent the standard error of the analysis (n = 6).

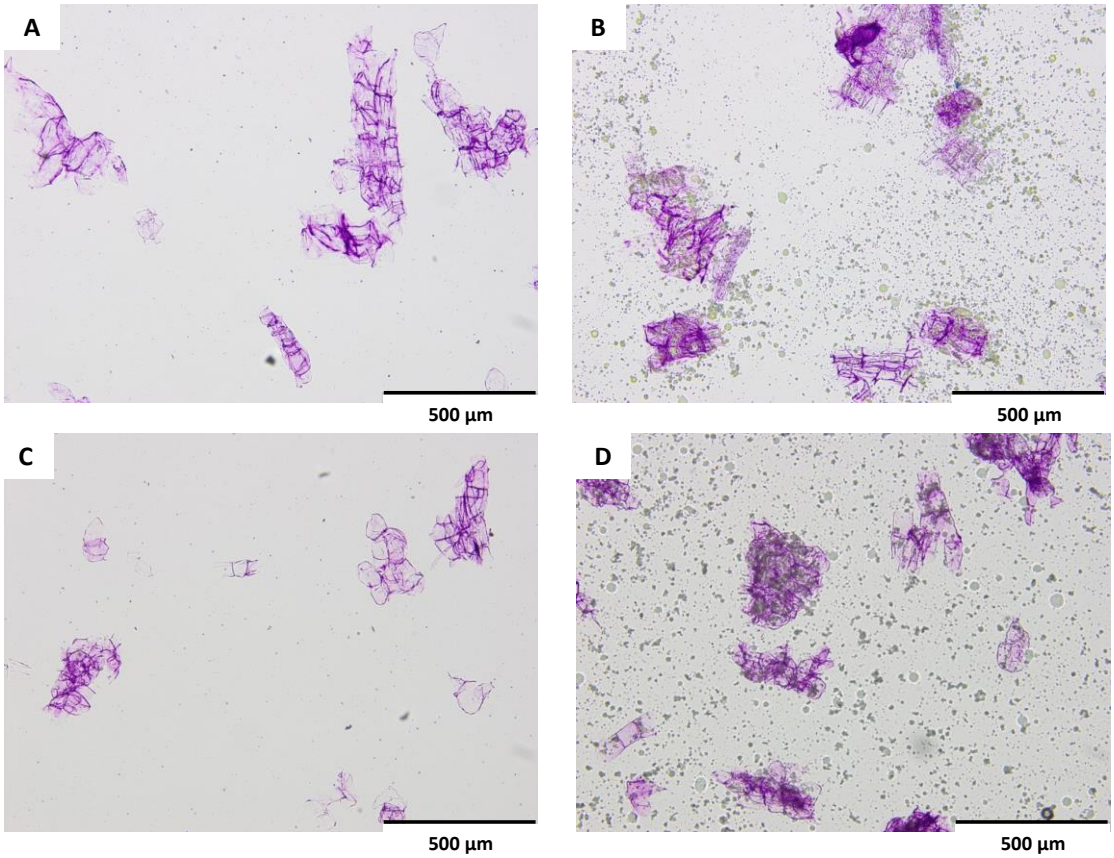

Fig. 7: Light micrographs of blanched orange carrot purees without oil addition (A) and with oil addition (B) and of blanched yellow carrot purees without oil addition (C) and with oil addition (D).

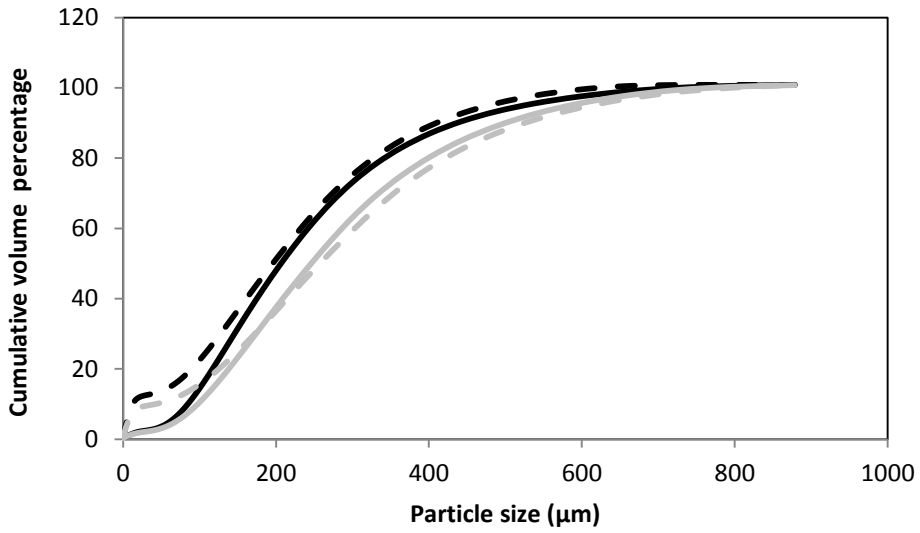

Fig. 8: Cumulative particle size distribution curves of blanched orange carrot purees (black curves) and of blanched yellow carrot purees (grey curves) without oil addition (full curves) and with oil addition (dashed curves).

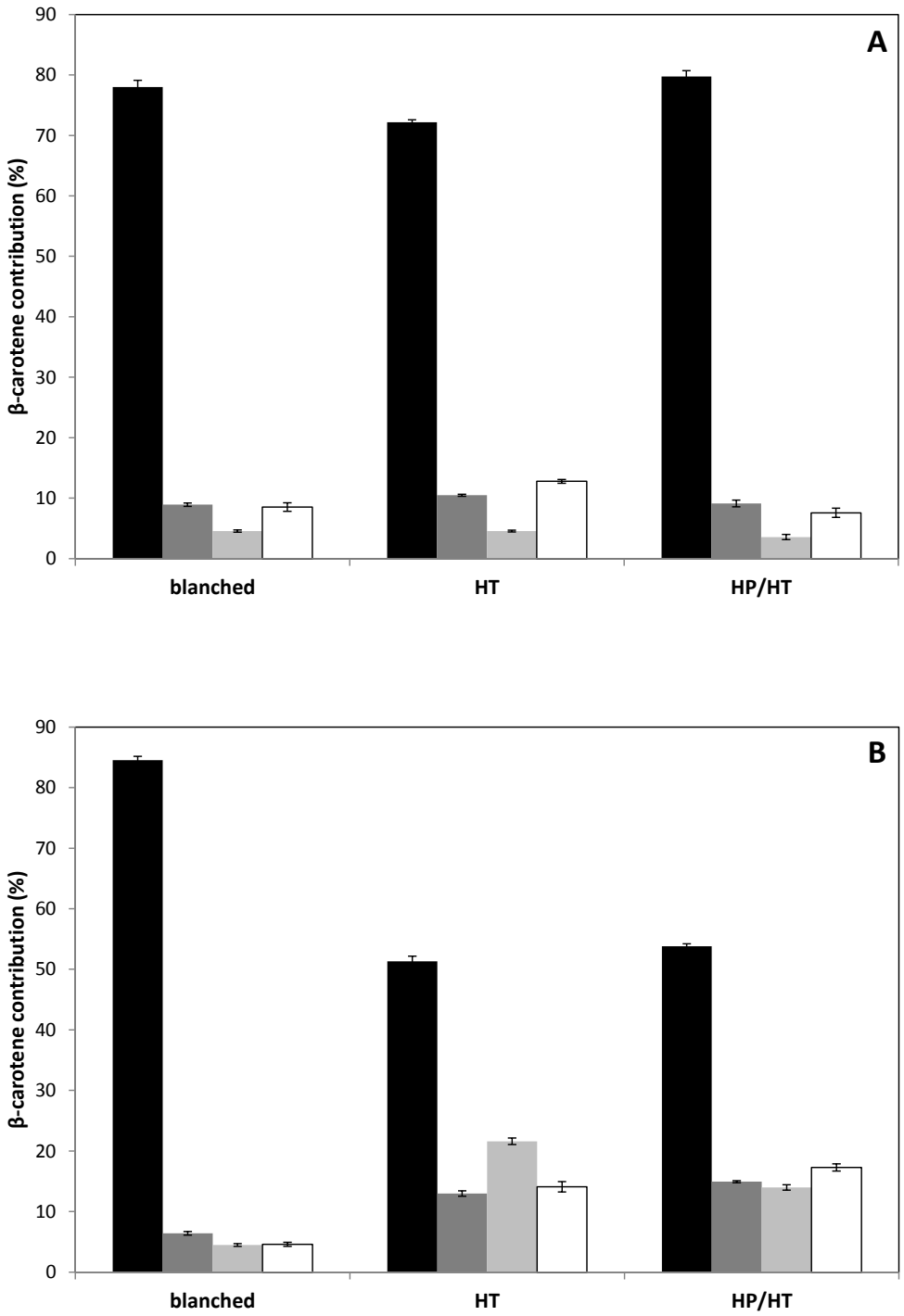

Fig. 9: *β*-Carotene contribution (mean ± standard error) in blanched orange carrot purees and in sterilized orange carrot purees using traditional thermal processing (HT) and high pressure high temperature processing (HPHT) without the addition of oil (A) and with the addition of oil (B). (■) all-*trans*-β-carotene contribution; (■) 9-*cis*-β-carotene contribution; (■) 13-*cis*-β-carotene contribution; (□) 15-*cis*-*β*-carotene contribution.

Table 1. Discriminative headspace components for each treatment in orange and yellow carrot purees, selected through the VID procedure (higher than 0.800 in absolute value), and listed in increasing order of VID coefficient. Positive/negative VID coefficients indicate an increase/decrease of its concentration respectively, after the corresponding treatment in comparison with the other treatments. The corresponding PLS-DA model contained two latent variables, explaining more than 93 and 88 % of *Y*-variance in orange and yellow carrot, respectively. The retention time index (RTI) per compound is listed.

	Blanched			**HT**			**HPHT**		
Orange carrot puree	Compound	RTI	VID	Compound	RTI	VID	Compound	RTI	VID
	Octanal	1153	-0.972	γ-Terpinene	1211	0.804	Sesquithujene	1537	-0.907
	α-Ionone	1558	-0.954	α-Terpinene	1171	0.806	β-Caryophyllene epoxide	1833	-0.899
	Heptanal	1048	-0.942	α-Pinene	1087	0.821	Cedrene	1587	-0.898
	2-Nonenal	1307	-0.933	β-Caryophyllene	1568	0.825	β-Elemene	1531	-0.852
	p-Cymenene	1244	-0.912	α-Caryophyllene	1604	0.842	β-Caryophyllene	1568	-0.814
	Hexanal	933	-0.876	β-Elemene	1531	0.861	α,β-Dihydropseudoionone	1574	0.802
	Decanal	1349	-0.815	Terpinolene	1240	0.945	2,4-Decadienal	1468	0.803
							2-Decenal	1401	0.827
							2-Octenal	1207	0.906
Yellow carrot puree	Heptanal	1048	-0.819	γ-Terpinene	1211	0.804	Terpinolene	1240	-0.953
	Octanal	1153	-0.816	p-Cymenene	1244	0.808	γ-Terpinene	1211	-0.934
	p-Cymenene	1244	-0.814	Heptanal	1048	0.828	α-Pinene	1087	-0.867
	α-Thujene	1077	0.885	Benzeneacetaldehyde	1195	0.936	β-Pinene	1134	-0.824
	Sabinene	1127	0.937	o-Cymene	1178	0.959	α-Caryophyllene	1604	-0.818

Table 2: Characterisation of the lipophilic extract of the blanched orange and yellow carrot purees without or with oil addition in terms of carotenoid content and colour values (L*, a*, b*).

orange carrot puree				**yellow carrot puree**		
	without oil	**with oil**			**without oil**	**with oil**
all-*trans*-β-carotene (µg/g carrot puree)	25.4 ± 2.4	33.7 ± 2.0		**lutein** (µg/g carrot puree)	3.1 ± 0.7	2.5 ± 0.2
9-*cis*-β-carotene (µg/g carrot puree)	2.9 ± 0.1	2.6 ± 0.3				
13-*cis*-β-carotene (µg/g carrot puree)	1.5 ± 0.1	1.8 ± 0.1				
15-*cis*-carotene (µg/g carrot puree)	2.8 ± 0.4	1.8 ± 0.2				
L*	47.5 ± 2.5	64.1 ± 0.1		**L***	58.3 ± 0.1	75.1 ± 0.1
a*	31.7 ± 2.7	24.6 ± 0.1		**a***	5.8 ± 0.1	1.5 ± 0.1
b*	43.3 ± 4.2	68.8 ± 0.3		**b***	61.0 ± 0.4	51.3 ± 0.2

Table 3: Colour values (L*, a*, b*) of the lipophilic extract of blanched orange carrot purees and of sterilised orange carrot purees using thermal (HT) and high pressure high temperature (HPHT) processing, without and with the addition of oil.

orange carrot puree without oil			
	Blanched	**HT**	**HPHT**
L*	47.5 ± 2.5	46.1 ± 0.1	48.0 ± 0.5
a*	31.7 ± 2.7	31.6 ± 0.2	31.9 ± 1.0
b*	43.3 ± 4.2	46.5 ± 0.2	39.5 ± 2.0

orange carrot puree with oil			
	Blanched	**HT**	**HPHT**
L*	64.1 ± 0.1	65.9 ± 0.3	66.2 ± 0.4
a*	24.6 ± 0.1	18.6 ± 0.2	18.8 ± 0.6
b*	68.8 ± 0.3	74.1 ± 1.2	66.7 ± 2.9