Impact of processing on odour-active compounds of a mixed tomato-onion puree

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Highlights

- Dipropyl disulfide is the most potent odourant of the processed tomato-onion puree.
- Aroma generation and endogenous enzymes are strongly interrelated.
- Blanching prior to processing leads to lower volatiles levels of in the puree.
- All-in-one and split-stream process have distinct impact on aroma of mixed systems.

Abstract

Gas chromatography-olfactometry revealed thirty-two odour-active compounds in a heat-processed tomato-onion puree, among which twenty-seven were identified by gas chromatography-olfactometry-mass spectrometry (GC–O-MS) and comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC–TOF MS). Based on the results of two olfactometric methods, i.e. detection frequency and aroma extract dilution analysis, the most potent aroma components include: dipropyl disulfide, *S*-propyl thioacetate, dimethyl trisulfide, 1-octen-3-one, methional, dipropyl trisulfide, 4,5-dimethyl thiazole, 2-phenylacetaldehyde and sotolone. Processing of mixed vegetable systems can add complexity in their aroma profiles due to (bio)chemical interactions between the

components. Therefore, the impact of different processing steps (i.e. thermal blanching, all-in-one and split-stream processes) on the volatile profile and aroma of a mixed tomato-onion puree has been investigated using a GC-MS fingerprinting approach. Results showed the potential to control the aroma in a mixed tomato-onion system through process-induced enzymatic modulations for producing tomato-onion food products with distinct aroma characteristics.

Keywords

Gas chromatography-olfactometry; aroma; comprehensive two-dimensional gas chromatography; mixed vegetable system; endogenous enzymes; fingerprinting.

Chemical compounds studied in this article

Dipropyl disulfide (PubChem CID: 12377); *S*-Propyl thioacetate (PubChem CID: 61295); Dimethyl trisulfide (PubChem CID: 19310); 1-Octen-3-one (PubChem CID: 61346); Methional (PubChem CID: 18635); Dipropyl trisulfide (PubChem CID: 22383); 4,5-Dimethyl thiazole (PubChem CID: 62510); 2-Phenylacetaldehyde (PubChem CID: 998); Sotolone (PubChem CID: 62835).

1. Introduction

Aroma is a key determinant of the quality of fruits and vegetables and it is greatly affected by processing. The impact of processing conditions on the quality attributes of single vegetables systems has been widely investigated. Although most of the commercial vegetable-based food products are mixed ingredient systems, a limited number of studies has been performed on multi-ingredient systems. Tomato and onion are frequently used together in processed food

products such as sauces and soups. To produce such food products thermal and mechanical treatments are usually applied. In shelf-life studies, thermal processing is used to inactivate pathogen microorganisms and enzymes in foods in order to increase the product safety and shelf-stability. However, heat processing induces several chemical and physical changes which, in turn, affect the nutritional and sensory attributes of foods (Awuah, Ramaswamy, & Economides, 2007). In addition, tissue disruption during pre-processing steps such as mixing, homogenizing, maceration etc. increases the permeability and mediates the contact of endogenous enzymes with their substrates, thereby characteristic flavour compounds are formed (Lamikanra, 2002).

The flavour of tomato-based products has been subject of several studies. More than 400 volatile compounds have been so far identified as volatile substances of fresh tomato and tomato-based products (Petro-Turza, 1986; Krumbein & Auerswald, 1998), of which only a limited number play an important role in tomato aroma. In fresh tomato, the aroma is formed during ripening as well as upon tissue disruption. Lipoxygenase (LOX), hydroperoxide lyase (HPL), and alcohol dehydrogenase (ADH) are enzymes involved in the synthesis of volatile compounds with green and fresh notes (Gardner, 1995; Baldwin, Scott, Shewmaker, & Schuch, 2000). Endogenous LOX is responsible for the formation of saturated and unsaturated C_6- and C_9-alcohols and aldehydes, contributing to the fresh tomato aroma. The thermal process of tomato principally modifies saturated and unsaturated C_6-aldehydes, ketones, esters and carotenoid derivatives (Servili, Selvaggini, Taticchi, Begliomini, & Montedoro, 2000). During processing, a part of the volatile constituents evaporates (mainly those providing "green" notes) and at the same time new volatiles are generated by LOX activity, break-down of sugars, carotenoid degradation and Maillard reaction providing the characteristic "cooked" aroma of the tomato products (Petro-Turza, 1986; Servili et al., 2000). Moreover, endogenic glycosidases

can generate terpene and carotenoid derivatives from their odourless glycosidic compounds (Marlatt, Ho, & Chien, 1992).

Onion is widely used as a key savoury ingredient due to its characteristic culinary flavour which is dominated by sulfur-containing compounds. These volatile compounds are produced by enzyme catalyzed breakdown of non-volatile precursors, upon tissue disruption of the onion cells due to cutting, chopping, mastication, maceration etc. These processes enable alliinase (present in vacuoles) to get in contact with sulfur containing amino acids, alk(en)yl cysteine sulfoxides (present in cytoplasm), forming sulfenic acids and then thiosulfinates. These highly unstable molecules give rise to further rearrangements resulting in a mixture of sulfur-containing compounds which are responsible for the distinctive fresh onion aroma. Thiopropanal sulfoxide, the lachrymatory or tear factor, is also formed from *S*-trans-prop-1-enyl cysteine sulfoxide (isoalliin) precursor, but quickly degrades into other compounds, such as propanal, because of its instability. Thermal processing of onions gives rise to the formation of additional volatile compounds through various routes like non-enzymatic decomposition of non-volatile flavour precursors, thermal interaction of flavour precursors with carbonyl compounds and lipid-oxidation products and the initiation of Maillard reactions (Wang, 2008). The major flavour compounds found in thermally treated onions include a mixture of sulfur compounds such as mono-, di-, tri-, tetra-sulfides, thiophenes and thiols (Boelens, Valois, Wobben, & Gen, 1971; Colina-Coca, González-Peña, Vega, de Ancos, & Sánchez-Moreno, 2013; Villière et al., 2015). Although many studies have been performed on the onion volatile compounds, the real contribution of these volatiles to the aroma of processed onions is not so thoroughly investigated (Villière et al., 2015).

By the use of gas chromatography–olfactometry (GC–O), odour-active compounds can be detected in a complex mixture of hundreds of volatile compounds. The odour-active compounds

in fresh tomato have been investigated using GC–O in many studies (Langlois, Etievant, Pierron, & Jorrot, 1996; Krumbein et al., 1998; Mayer, Takeoka, Buttery, Whitehand, Naim, & Rabinowitch, 2008; Selli, Kelebek, Ayseli, & Tokbas, 2014). However, few studies have been performed to assess the odour-active compounds of cooked tomatoes (Landy et al., 2002). Moreover, studies have been performed to evaluate the volatile profile of fresh and processed onions (Boelens et al., 1971; Lanzotti, 2006; Colina-Coca et al., 2013). Nevertheless, assessing the contribution of these volatiles to the perceivable aroma has not been documented in much detail (Villière et al., 2015).So clearly few attempts have been made to identify the key aroma volatiles in cooked tomato and cooked onion individually, but to the best of our knowledge there is no prior literature on identification of key aroma volatiles in a mixed tomato-onion system as a function of different process conditions. Considering, mixed vegetables like tomato-onion as one of important ingredients for savoury food products, these studies should provide in-depth understanding of flavour generation in such complex system.

Processing of mixed vegetable systems can add more complexity in their volatile profile due to the interactions between the components. Both chemical and enzymatic interactions can potentially occur between compounds of the respective vegetables when they are processed together, which can affect the aroma profile of the mixed vegetables (Koutidou, Grauwet, & Acharya, 2016). Thus, it would be worthwhile to determine the relative role of endogenous enzymes in flavour generation of a mixed vegetables system. The enzymatic modulation can happen due to inactivation of the endogenous enzymes by blanching or by allowing them to act on the individual matrices (i.e. when vegetables were processed separately and mixed after the process, termed split-stream processed) and/or to the combined matrices (i.e. when vegetables were processed together, termed all-in-one processed) of the mixed vegetable system. In this context, the main objective of this study is (i) to determine the odour-active compounds of a mixed tomato-onion puree and (ii) to investigate the impact of processing on its qualitative

aroma profile. Different processing steps are known to alter the endogenous enzymes and should consequently affect the aroma generation in the mixed tomato-onion puree. The odour-active constituents of the mixed tomato-onion puree were detected and identified by using GC–O coupled with mass spectrometry (GC–O-MS). To achieve enhanced separation, resolve co-eluting compounds, and improve the identification of the odour-active compounds, comprehensive two-dimensional gas chromatography, coupled to time-of-flight mass spectrometry (GC × GC–TOF MS) has been implemented. The impact of the processing steps on the volatile and aroma compounds was studied using a SPME-GC-MS based fingerprinting approach along with multivariate data analysis (MVDA), which should include any unexpected changes in the tomato-onion volatile profile due to the range of varied process conditions studied.

2. Materials and methods

2.1 Samples and chemicals

A single batch of fresh tomato (*Solanum lycopersicum*, var. *Roma*) and fresh onion *(Allium cepa L*, var. *Jaune d'Espagne*) were purchased from a local market (Vlaardingen, Netherlands). They were carefully washed, cut into standardized slices (approximately 0.6-0.7 cm thickness) and packed in low-density polyethylene bags. A part of these non-blanched vegetables was immediately frozen with liquid nitrogen and stored at -20 °C. The rest was blanched at 95 °C for 6 min in an oven with steam (Leventi Combimat Mastermind, Levens Cooking & Baking Systems B.V, Netherlands), in order to inactivate the enzymes (Servili et al., 2000). The blanching conditions used were established before (data not shown). Then, they cooled in ice water for 10 min, frozen in liquid nitrogen at stored at -20 °C until processing. Prior to sample processing, the vegetables were thawed overnight at +4 °C.

158 Standard analytical reagents were used as follows: pentanal, *(E)*-2-pentenal, 1-pentanol,
159 hexanal, *(E)*-2-hexenal, *(Z)*-3-hexen-1-ol, 1-heptanol, *(E)*-2-heptenal, *(E,E)*-2,4-heptadienal,
160 octanal, *(E)*-2-octenal, 1-octen-3-one, 1-octanol, nonanal, *(E)*-2-nonenal, *(E,Z)*-2,4-decadienal,
161 *(E,E)*-2,4-decadienal, *(E)*-2-decenal, *trans*-4,5-epoxy-*(E)*-2-decenal, *(E)*-2-undecenal, 2-
162 phenylacetaldehyde, propenyl propyl disulfide, methyl propyl trisulfide, dipropyl trisulfide and
163 dimethyl trisulfide, alkane series from C_5 to C_{24} were obtained from Sigma Aldrich (Sigma
164 Aldrich, Netherlands). Analytical grade sodium chloride (NaCl) was obtained from VWR
165 chemicals (VWR International bvba, Leuven, Belgium), dichloromethane >99.9% purity from
166 Sigma Aldrich (Sigma-Aldrich Chemie Gmbh, Steinheim, Germany) and anhydrous
167 magnesium sulfide from Merck Chemical Co. (Merck KGaA, Darmstadt, Germany).

168

169 **2.2 Sample preparation**

170 The different tomato-onion puree samples prepared for this study are schematically
171 represented in Figure 1.

172 In order to prepare a non-blanched all-in-one tomato-onion puree, same amounts of non-
173 blanched tomato and non-blanched onion (1:1) were blended together (non-blanched all-in-one
174 puree) at high speed (8000 rpm) for a total duration of 20 min (consisting of five times 1 min
175 blending with 3 min interval). Then the obtained puree was heated up for 10 min until the
176 temperature of 90 °C was reached. The heating continued for 10 min at the same temperature.
177 To promote uniform heat transfer, the samples were stirred gently (40 rpm) during the heating
178 step, while the temperature was monitored externally by a digital sensor. The sample
179 preparation was entirely performed in a closed system using a Thermomix (Vorwek, Wuppertal,
180 Germany). For the preparation of a non-blanched split-stream tomato-onion puree, same
181 amounts of non-blanched tomato and non-blanched onion (1:1) were processed separately at

182 the same time using two thermomixers and immediately after the process, the single vegetable
183 purees were combined. In addition, by using all-in-one and split-stream process, a blanched all-
184 in-one and a blanched split-stream puree were prepared, respectively, by using blanched
185 vegetables.

186 After preparation, the samples were directly placed into 125mL aseptic polypropylene
187 containers (VWR International, Amsterdam, Netherlands), cooled in ice water for 10 min,
188 frozen in liquid nitrogen and stored at -20 °C until analysis. The sample preparation was
189 repeated three times (i.e. at three different days) to include the error of sample preparation in
190 the data analysis.

191

192 **2.3 Determination of odour-active compounds**

193 ***2.3.1 Extraction of the volatile compounds***

194 Liquid-liquid extraction is a widely used technique for the isolation of volatile compounds
195 in fruits and vegetables (Kelebek & Selli, 2011). Extraction of aroma compounds was
196 performed in dichloromethane, which was selected as the most representative extract according
197 to literature (Kelebek et al., 2011; Selli et al., 2014) and confirmed by in vivo evaluation (data
198 not shown).

199 An amount of 300 g of non-blanched all-in-one tomato-onion puree, 200 ml of saturated
200 $CaCl_2$ solution and 120 ml of dichloromethane were stirred at room temperature for 30 min.
201 The mixture was centrifuged at 10 °C (9000 × g, 30 min). The organic phase was recovered and
202 two subsequent extractions were performed on the aqueous phases in order to extract as many
203 aroma compounds as possible. The multiple extracts were combined and the organic phase was
204 dried with anhydrous magnesium sulfate ($MgSO_4$). After the dehydration, the collected extract

was further treated to separate the volatiles from the non-volatiles by solvent-assisted flavour evaporation (SAFE) (Bahr, Manching, Germany) at 40 °C under vacuum (10^{-3} Pa) (Pfeiffer Vacuum, Benelux BV) according to the method proposed by Engel (Engel, Bahr, & Schieberle, 1999) and the distillate was concentrated to 3 mL at 45 °C using a Vigreux column (Supelco, St Quentin, France). The resulting extract was subsequently stored at -20 °C in glass vials equipped with Teflon-lined cap until the analysis. The extraction of the aroma compounds was performed in duplicate.

2.3.2 Gas chromatography–olfactometry-mass spectrometry (GC–O-MS)

The gas chromatography–olfactometry-mass spectrometry (GC–O-MS) system consisted of an Agilent 6850 gas chromatograph coupled with an Agilent 5975 mass selective detector (Agilent, Santa Clara, CA, USA), an Olfactory Detection Port (ODP-2) and a MPS-2 as autosampler (Gerstel, Mulheim, Germany).

Chromatographic separations were conducted on two different columns: Rxi-5-Sil MS capillary column (30 m × 0.25 mm × 0.25 μm, Restek Corporation, Bellefonte, PA) and DB-WAX capillary column (20 m × 0.18 mm i.d., 0.3 μm film thickness; Agilent Technologies J&W, Santa Clara, CA, USA), with helium as carrier gas at 1.25 mL/min flow.

The liquid extracts (1μL) were injected in splitless mode. The injector was equipped with a Cooled Injection System (CIS). The GC effluent was split 1:1 into the MS and the sniffing port (ODP-2). The oven temperature was programmed starting from a temperature of 35 °C, which was retained for 2 min, following increase of 5 °C/min to 230 °C.

Mass spectra in the electron impact mode (MS-EI) were generated at 70 eV and they were taken over the m/z range 33 and 300 m/z with a speed of 2.7 scan/s and with a solvent delay of

5 min. The temperatures of the source and the quadrupole were respectively 230 °C and 150 °C.

MassHunter Qualitative Analysis (Version B.07.00, Agilent Technologies, Colorado, USA) was used to correlate the chromatograms with their aromagrams. Automated Mass spectra Deconvolution Identification System (AMDIS) (Ver. 2.70, 2011, NIST, Gaithersburg, MD, USA) and NIST (NIST/EPA/NIH 2011, National Institute of Standards and Technology, Gaithersburg, MD, USA) were used to deconvolute and identify the odour-active molecules, respectively.

In order to determine the odour-active molecules, a GC–O analysis was performed on the tomato-onion extract by using a detection frequency method (section 2.3.3) and an aroma extract dilution analysis (AEDA) (section 2.3.4).

2.3.3 Detection frequency method

The detection frequency method was applied to estimate the relative contribution of the odour-active compounds according to their odour potencies (Pollien, Ott, Montignon, Baumgartner, Munos-Box, & Chaintreau, 1997). The original aroma extracts were analyzed by GC–MS–O using a panel of ten experienced assessors (seven men and three women, aged 29–60), with previous experience in sensory analysis. Prior to smelling sessions, the assessors were trained to memorize fifty-six reference smells (descriptors) which were selected to cover a wide range of aroma. Bouillon, roasty, fruity, alliaceous or sweaty are examples of descriptors in the Common Flavour Language (CFL) developed by Unilever. Each panelist was asked to sniff the eluted compounds and describe their aroma. Aroma description and retention time of each compound detected was recorded. Samples were analyzed in duplicate

by all assessors. The sniffing procedure was carried out in a neutral and temperature-controlled room (22 °C). Aroma peaks were considered as relevant only if they were smelled by at least five assessors. The total number of panelists who could positively detect an odourant at a specific retention time was described by the Nasal Impact Frequency (NIF). NIF values were summarized yielding the final aromagram.

2.3.4 Aroma extract dilution analysis (AEDA)

The concentrated aromatic extract of the non-blanched all-in-one tomato-onion puree was stepwise diluted using dichloromethane to obtain different dilutions (1:10, 1:20, 1:30) of the original extract. The individual dilutions were sniffed by five panelists (three men and two women, aged 29–60) who were selected based on their performance during the detection frequency method. Sniffing of the series of dilutions proceeded until no odourant could be detected by the assessors. Each odourant was assigned with a flavour dilution factor (FD factor) representing the last dilution in which the odourants were still detectable (Schieberle & Grosch, 1987).

2.4 Comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry (GC × GC–TOF MS) analysis

To achieve enhanced separation, resolve co-eluting compounds, and improve the identification of the odour-active compounds which were undetermined after the first chromatographic separation (section 2.3.2), comprehensive two-dimensional gas chromatography, coupled to time-of-flight mass spectrometry (GC × GC–TOF MS, LECO Pegasus 4D Leco Corp St. Joseph, MI, USA) was used. The GC was equipped with a Rxi-5-Sil

MS column (30 m × 0.25 mm × 0.25 μm, Restek Corporation, Bellefonte, PA) and BPX-50 (1.15 m × 0.1 mm × 0.1 μm, Restek Corporation, Bellefonte, PA) as a second column.

Helium was used as a carrier gas at a flow rate of 1 mL min^{-1}. The main oven temperature program was as follows: 2 min at 40 °C, then increased to 250 °C at 3 °C/min. The temperature of the secondary oven was set 10 °C higher than the first one for the entire run. The modulator temperature was set 15 °C higher than the one in the second oven. Modulation time was optimized and set at 3 s. The MS transfer line temperature was 280 °C. Ions in the mass range of 33-300 m/z were acquired at a scan rate of 150 Hz after a solvent delay of 5 min. The TOF MS was operated with electron ionization (70 eV), a source temperature of 200 °C, and detector voltage of 1800 V. Time and temperature conditions for extraction and injection were similar to those described above. The injector was equipped with a MPS autosampler (Gerstel, Mulheim, Germany). Data processing was performed by Leco ChromaTOF software optimized for Pegasus (version 4.50.8.0). Peak identification was made using TOF MS with electron ionisation. Mass spectra were compared against the NIST mass spectral library (NIST/EPA/NIH 2011, National Institute of Standards and Technology, Gaithersburg, MD, USA) and against the FFNSC 2 library **(**Shimadzu Europa GmbH), an in-house flavour library (Unilever R&D, Vlaardingen, Netherlands) containing approximately 1000 spectra and Kovats indices from the literature.

2.5 HS-SPME-GC-MS based fingerprinting

In order to study the impact of the processing steps on the volatile profile of a mixed tomato-onion puree, all the purees (Figure 1) were analyzed using headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography-mass spectrometric (GC-MS) analysis.

2 g of thawed tomato-onion puree and 2 mL of saturated NaCl solution were homogenized in a 10 mL amber glass vial with PTFE/silicon septum seal screw-caps (Supelco, Bellefonte, PA, USA) and placed in the auto sampler at 5 °C.

GC–MS analysis was conducted on a GC-MS (model 7890A-5975C) (Agilent, Santa Clara, CA, USA) equipped with a MPS autosampler (Gerstel, Mülheim, Germany). Compounds separation was performed on a HP-5MS column, 30 m x 0.25 mm i.d., 0.25 µm film thickness (Agilent Technologies J&W, Santa Clara, CA, USA). SPME extraction of the volatile compounds was carried out using a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre (StableFlex, Supelco, Bellefonte, PA, USA). The GC-MS program as well as the extracting conditions used in this study were similar to our previous work (Koutidou et al., 2016; Koutidou, Grauwet, Van Loey, & Acharya, 2017). All the purees were analyzed in triplicate. Since the sample preparation per puree type was performed three times as well, nine GC-MS fingerprints were obtained per puree type. In the context of quality control of the GC-MS fingerprinting analysis all sample replicates were analyzed randomly in the same sequence. In addition, a mixture of standard compounds with known concentrations was analysed at the beginning and at the end of the GC-MS sequence.

2.6 Multivariate data analysis of HS-SPME-GC-MS chromatograms

An untargeted fingerprint GC-MS approach combined with multivariate data analysis (MVDA) was applied in order to detect differences between the samples according to the procedure described in our previous study (Koutidou et al., 2016). As a first step, all fingerprints were pre-processed using Automated Mass Spectral Deconvolution and Identification System (AMDIS) (Version 2.70, 2011, NIST, Gaithersburg, MD, USA) (aiming deconvolution) and Mass Profiler Professional (MPP) (Version 12.5, 2012, Agilent Technologies, Colorado, USA)

(aiming peak alignment and filtering). Multivariate statistical data analysis of the pre-processed data was performed in Solo (Version 6.5, 2011, Eigenvector Research, Wenatchee, WA, USA). Principal Component analysis (PCA) was carried out to evaluate each data set and to detect outliers. In order to compare the impact of different sample preparations, Partial Least Square Discriminant Analysis (PLS-DA) was performed, determining as *X*-variables the detected volatiles and as categorical *Y*-variables the differently processed purees. Considering the complexity of the multivariate model, the lowest number of latent variables (LVs) resulting in class separation was selected for each comparison. To verify the goodness of fit of the model, R2 values were calculated and root-mean-square error of cross-validation (RMSECV) was taken into account Bi-plots based on scores and correlation loadings were plotted to qualitatively investigate impact differences among the classes using OriginPro 8 (OriginLab Corporation, Northampton, MA, USA). Duncan's multiple comparison test was used to test for significant differences between the mean-peak areas ($p < 0.05$) of the discriminant volatiles.

Variable IDentification (VID) coefficients were calculated in order to select discriminant compounds and to evaluate their significance for class differentiation. They can be used as a tool for ranking the volatiles based on their discriminative power and they correspond to the correlation coefficient between each original *X*-variable and the *Y*-variable(s) as estimated by the PLS-DA model (Vervoort et al., 2012). Only variables with an absolute VID value higher than 0.700 were selected as fingerprint markers, plotted individually for each comparison and identified.

Identification was performed by comparing the deconvoluted mass spectra with the mass spectra from the NIST spectral library (NIST/EPA/NIH 2011, National Institute of Standards and Technology, Gaithersburg, MD, USA), the FFNSC 2 library **(**Shimadzu Europa GmbH) and an in-house flavour library (Unilever R&D, Vlaardingen, Netherlands) containing

approximately 1000 spectra. A threshold and a reverse match above 80%, in accordance with visual inspection, were implemented. Additionally, retention indices (RI) were calculated and used for compound identification along with mass spectral data (fragmentation pattern m/z). Moreover, pure chemicals standards were analyzed to further confirm the identifications.

3. Results and discussion

In the first part of the present study, the aim was to identify the odour-active volatiles in a mixed tomato-onion puree by using GC-O-MS. To further confirm the identification of the detected odour-active compounds, GC × GC–TOF MS was also applied. In the second part, the aim was to investigate the impact of different processing steps and sequences of these steps (i.e. blanching, all-in-one and split-stream processes) on the odour-active volatiles of a mixed tomato-onion puree.

3.1 Odour-active compounds of all-in-one tomato-onion puree

After screening a range of extraction methods, liquid-liquid extraction in dichloromethane was chosen and a representative to the puree extract was obtained. Two olfactometric methods, i.e. detection frequency and aroma extract dilution analysis, were applied in order to identify the most potent odour-active compounds of an all-in-one tomato-onion puree. The results of the olfactometric analysis are summarized in Table 1.

Thirty-two odour-active volatiles were detected in the tomato-onion extract via olfactometric methods. The identification of these compounds was based on the odour description given by the panelists, mass spectra identified by MS or TOF MS and the comparison of retention indices (RI) of the detected compounds with those reported in

literature. In order to further support the identification of the peaks, pure chemicals standards were analyzed as well. Due to the complexity of the matrix, among the thirty-two odour-active compounds detected, only eighteen were identified by one-dimension GC-MS. In order to achieve enhanced separation and resolve co-eluting compounds, comprehensive two-dimensional gas chromatography, coupled to time-of-flight mass spectrometry (GC × GC–TOF MS) was used, which resulted in an increase in the number of identified compounds to twenty-seven. Even after the application of GC × GC–TOF MS, five compounds could still not be identified (Table 1, compounds No 11, 24, 28), as these compounds either co-eluted on the secondary column or did fall below the detection limits of the instrument. Identified compounds corresponded to different chemical classes with sulfur compounds and aldehydes representing the most abundant groups of volatile compounds. Sulfur-containing compounds are considered to be derived from the degradation of sulfur-containing amino-acids largely present in onion and imparted alliaceous, sulfury, sweaty, onion and cabbage notes. Aldehydes are mainly generated by lipid-oxidation and derived mostly from tomato, which contributed to green, fruity, vegetable, fatty and spicy notes of the extract.

3.1.1 Detection Frequency method

A total of thirty-two volatiles were considered odour-active and their odour descriptions were evaluated by ten assessors (Figure 2a). On the basis of the NIF values detected on Rxi-5Sil MS column, the potentially most important volatiles with a NIF value 90 were *S*-propyl thioacetate (alliaceous), 4,5-dimethyl thiazole (nutty, burnt savoury), dimethyl trisulfide (alliaceous, sulfury, cabbage), 1-octen-3-one (mushroom), 2-phenylacetaldehyde (floral, honey) and dipropyl disulfide (fried onion, sweaty, green). Further important compounds with NIF equal to or greater than 70 were hexanal (green), methional (potato), unknown No. 11

(alliaceous, bouillon, plastic), furaneol (candy, caramellic), nitro-2-phenylethane (spicy, vegetable and sulfury, herbal), *(E,E)*-2,4-decadienal (fatty, fried), dipropyl trisulfide (sulfury, alliaceous), unknown No. 28 (bouillon, savoury, honey, spicy) and 3,5-diethyl-1,2,4-trithiolane (cabbage, onion, vegetable). The rest of the identified odourants showed a lower NIF value (≤ 60), indicating that equal or less than six panelists out of ten could detect them. These odourants were closely related to alliaceous, onion, sweaty, green, fruity, vegetable, fatty, phenolic, spicy, meaty and caramellic descriptors.

3.1.2 AEDA

In order to further categorize the odour-active compounds, the flavour dilution factors (FD) of the most potent odourants were determined by the five most sensitive panelists using the AEDA method (Figure 2b). The application of AEDA on the tomato-onion puree extract revealed eleven odour-active compounds sniffed by more than two assessors including five sulfur-containing compounds, two aldehydes, one ketone, two furanones and one phenolic compound (Table 1). The highest FD factor (FD = 30) was found for dipropyl disulfide which was associated with a fried onion, sweaty, green aroma, meaning that this compound was still detectable after the last dilution (1:30) of the extract. Other compounds with significant FD values (FD = 20) were *S*-propyl thioacetate (alliaceous), methional (potato),dimethyl trisulfide (alliaceous, sulfury, cabbage), 1-octen-3-one (mushroom), sotolone (spicy, maple) and dipropyl trisulfide (sulfury, alliaceous). Among them, sotolone had a low NIF value (NIF = 50), probably due to its low odour threshold (0.001 ppb in the water) (Kobayashi, 1989). Despite being two of the most characteristic odour-active compounds in tomato-onion puree extract and having been detected by nine out of the ten panelists, 2-phenylacetaldehyde and 4,5-dimethyl thiazole did not show up in the AEDA. This can be due to the high odour threshold, in case of 4,5-

417 dimethyl thiazole (470 ppb in the water) (Fors, 1983). Hence, assessors can detect it in the
418 concentrated extract because its concentration highly exceeds the threshold. On the other hand,
419 2-phenylacetaldehyde has low odour threshold (4 ppb in the water) (Buttery, 1993), indicating
420 that it was probably in very low concentration in the concentrated extract.

421 Based on the results of the two olfactometric methods, i.e. detection frequency (NIF ≥ 70)
422 and/or AEDA (FD ≥ 20), the aroma of the mixed tomato-onion puree is primarily related to
423 dipropyl disulfide (fried onion, sweaty, green), *S*-propyl thioacetate (alliaceous), dimethyl
424 trisulfide (alliaceous, sulfury), 1-octen-3-one (mushroom), methional (potato), dipropyl
425 trisulfide (sulfury, alliaceous), 4,5-dimethyl thiazole (nutty, burnt savoury), 2-
426 phenylacetaldehyde (floral, honey) and sotolone (spicy, maple).

427 *S*-Propyl thioacetate, dipropyl disulfide and dipropyl trisulfide add alliaceous, fried onion,
428 sulfury notes to the mixed tomato-onion puree and have been characterized as three of the most
429 prominent aroma compounds in onion (Wang, 2008). Dipropyl disulfide and dipropyl trisulfide
430 are major breakdown products of thermal degradation of *S*-propylcysteine sulfoxide and *S*-
431 propylcysteine, respectively (Kubec, Drhová, & Velíšek, 1999). Dimethyl trisulfide is a
432 breakdown product of thermal degradation of methyl-*L*-cysteine sulfoxide (Kubec et al., 1999).
433 It has been identified in the volatile profile of onions (Colina-Coca et al., 2013) and imparts
434 alliaceous, sulfury notes. Presence of dimethyl trisulfide has also been reported in tomato paste
435 (Miers, 1966) and fresh tomato (Du, Song, Baldwin, & Rouseff, 2015). Methional and 1-octen-
436 3-one contribute significantly to the aroma of the tomato-onion puree giving potato and
437 mushroom notes, respectively. Both compounds have been reported as odour-active volatiles
438 in tomato using AEDA (Krumbein et al., 1998; Tandon, Jordan, Goodner, & Baldwin, 2001).
439 1-Octen-3-one is a fatty acid degradation product, while methional is coming from methionine
440 through an enzyme-catalyzed process (Mayer et al., 2008; Vervoort et al., 2012; Koutidou et

al., 2016). 4,5-Dimethyl thiazole is a Maillard reaction product. Sotolone and 2-phenylacetaldehyde are amino acid-derived flavour compounds. 2-Phenylacetaldehyde is formed enzymatically in tomato from phenylalanine by a decarboxylase (Tieman, Taylor, Schauer, Fernie, Hanson, & Klee, 2006). Sotolone is probably formed from 4-hydroxy-*L*-isoleucine (Slaughter, 1999). It can also be thermally produced from intermediates generated from the Maillard reaction (Guerra & Yaylayan, 2011).

3.2 Impact of processing on the aroma generation of a tomato-onion puree

This section intends to summarize the results based on the impact of different processing steps (i.e. blanching, all-in-one versus split-stream processing) on the volatile profile and odour-active compounds of a mixed tomato-onion puree. It is known, that the biosynthesis of vegetable aroma during processing is complex. In addition, interactions between different reaction pathways may take place and generate unknown compounds. In order to include unexpected changes in the volatile fraction of the tomato-onion purees due to processing, an untargeted GC-MS fingerprinting approach along with a multivariate data analysis was applied. Different processing steps alter the endogenous enzymes and consequently affect the aroma generation in the mixed tomato-onion puree. The enzymatic modulation can happen in two ways: (i) Modulation of the enzymes in an all-in-one processed vegetable puree by inactivation during blanching. Thus, the aim was to evaluate the effect of the enzymatic activity on aroma generation by comparing a blanched all-in-one puree with a non-blanched all-in-one puree; (ii) Allow enzymes to act to individual matrices of the mixed vegetable system. Therefore, the objective was to investigate the interaction (if any) between enzymes from one vegetable and substrate of the other vegetable exists when vegetable are processed together (non-blanched all-

464 in-one processed), vis-a-vis processed separately and mixed after the process (non-blanched
465 split-stream processed).

466

467 ***3.2.1 Impact of blanching on volatile profile and aroma of a tomato-onion puree***

468 Thermal blanching is the most common way to completely inactivate enzymes in vegetables
469 prior to processing. In order to study the effect of blanching on the volatile profile of a tomato-
470 onion puree, two all-in-one purees were prepared, one from blanched and one from non-
471 blanched raw materials and their volatile fingerprints were compared. A PLS-DA bi-plot for
472 the first two latent variables for the selected model was constructed to investigate the effect of
473 blanching (Figure 3a).

474 If a volatile compound is located between the inner and outer ellipses of the bi-plot it is
475 considered to change prominently due to processing. The significance of the individual volatiles
476 for the discrimination of differently processed samples increases as a function of their distance
477 from the center. On Figure 3a, if one volatile is detected in higher concentration in the blanched
478 puree, then it will be projected close to the blanched all-in-one represented objects. On the
479 contrary, if a volatile compound has lower concertation in the blanched all-in-one puree and
480 consequently higher concentration in the all-in-one (non-blanched) puree, then it will be
481 projected close to the all-in-one (non-blanched) represented objects. For a detailed description
482 of the interpretation of the bi-plots, the reader is referred to previous studies (Vervoort et al.,
483 2012; Koutidou et al., 2016).

484 To quantitatively rank the importance of volatiles, Variable IDentification (VID)
485 coefficients were calculated. Only compounds with a VID equal or larger to 0.700 were selected
486 and termed as ‘discriminant markers’ (Table 2). Positive VID value implies higher

concentration in the indicated class, in this case, in the blanched all-in-one puree as compared to the other (non-blanched all-in-one), and a negative VID value indicates lower concentration in the blanched sample in contrast with the non-blanched sample. Markers in bold have been characterized as odour-active compounds in the tomato-onion puree extract according to the results from the GC–O (Table 1).

On average eighty-eight volatile compounds were detected in the headspace of all tomato-onion puree samples. The comparison of the volatile fingerprints of blanched all-in-one puree and non-blanched all-in-one puree, revealed fourty-six markers ($|VID| \geq 0.700$) which were all identified. The markers represent 42% of the total peak area of all the compounds detected in the headspace fraction. As expected, most of them (thirty-nine), were in higher concentration in the non-blanched all-in-one puree. It is clear that enzymes are strongly related to volatile generation, since only few markers were detected in the blanched puree. LOX and HPL are the main endogenous enzyme of tomato which are primarily involved in the synthesis of volatile compounds of tomato (Gardner, 1995; Baldwin et al., 2000). LOX is rapidly inactivated at 60 °C and reduced to less than 10% of its original level in less than 1 min. HPL shows a rapid loss of about 50% of its total activity at temperatures below 50 °C and it is completely inactivated after 1 min of heating at 65 °C (Anthon & Barrett, 2003). Alliinase is the main endogenous enzyme of onion. The optimum temperature of alliinase is 37 °C. Its activity decreases rapidly above this optimum temperature and it is completely inactivated at temperatures above 50 °C (Joslyn M.A. & Sano T., 1956).

Through the identification of these markers it is possible to link them with particular enzymatic and chemical reactions and gain further insight into the aroma generation during processing. The discriminant volatile markers selected in the non-blanched all-in-one puree are

510 comprised of diverse chemical classes including aldehydes, alcohols, sulfides, ketones and
511 furans, with aldehydes and alcohols being dominant over the others.

512 Aldehydes and alcohols are volatile components mainly derived from tomato. Most of the
513 aldehydes and their corresponding alcohols are formed through the LOX pathway, the ADH
514 pathway and the autoxidation of fatty acids. These reactions yield a hydroperoxide followed by
515 selective lyase cleavage to generate C_6-aldehydes from the 13-hydroperoxides of linoleic and
516 linolenic acids (Boukobza, Dunphy, & Taylor, 2001).

517 In the blanched tomato-onion puree, only seven markers with a positive VID were detected.
518 Since these vegetables were blanched, the above enzyme-related pathways were not expected
519 to play a key role in the formation of fatty acid degradation related volatiles. The identification
520 of these markers confirmed this, since only sulfuric compounds were detected. It is known that
521 sulfur-containing compounds are responsible for the flavour and pungency of *Allium* species
522 (Wang, 2008). The volatile compounds in the onion are formed from their non-volatile
523 precursors (*S*-alk(en)ylcysteine sulfoxides), by an alliinase-catalyzed process which occurs
524 directly upon tissue disruption. Given that onions were blanched, alliinase should be inactive
525 during the blending. However, it should be noticed that the first stage of enzyme-catalyzed
526 reactions might already have been initiated during the cutting of onions prior to blanching.
527 Moreover, throughout the thermal processing of onions, flavour compounds can be generated
528 mainly by two chemical pathways: non-enzymatic thermal degradation of flavour precursors
529 and thermal interaction of flavour precursors with carbonyl compounds or reducing sugars
530 (Wang, 2008).

531 Although a large number of chemical compounds were detected and identified by the
532 comparison of the blanched and the non-blanched all-in-one puree, only thirteen of them had a
533 real contribution to the aromatic quality of the puree, based on the results from the olfactometric

analysis. Among them, nine volatiles ((*E*,*E*)-2,4-heptadienal, (*E*)-2-octenal, dimethyl trisulfide, hexanal, (*E*,*E*)-2,4-decadienal, methyl propyl trisulfide, 1-octen-3-one, octanal and 2-phenylacetaldehyde), were in higher concentration in the non-blanched all-in-one puree, adding green, vegetable, fruity, floral, mushroom, alliaceous and fatty notes (marked as bold in Table 2). In the blanched all-in-one puree, four odour-active compounds (dipropyl trisulfide, *S*-propyl thioacetate, *cis*-3,4-dimethyl-2,3-dihydrothiophene-2-thiol and propenyl propyl disulfide), were identified in higher concertation as compared to the non-blanched all-in-one puree, imparting sulfury, alliaceous, fried onion and meaty notes (marked as bold in Table 2).

3.2.2 Impact of all-in-one versus split-stream processing on the volatile profile and aroma of a tomato-onion puree

In a mixed vegetable system, enzyme-catalyzed reactions can potentially occur crosswise (i.e. enzymes of tomato can act on onion and vice-versa) during processing when the respective vegetable components are processed together (Koutidou et al., 2016). For this reason, one non-blanched all-in-one and one non-blanched split-stream puree were prepared and their headspace fingerprints were compared to investigate the effect of cross-enzyme activity on the volatile profile. In the all-in-one process, tomato and onion slices were mixed and co-processed, whereas in the split-stream process they were blended and heated separately and mixed only after the thermal treatment.

Comparison between the non-blanched all-in-one and non-blanched split-stream puree, revealed twenty-nine markers ($|VID| \geq 0.700$), representing 43% of the total peak area of all the volatiles detected in the headspace fraction. Among them, seventeen were detected in greater amount in the all-in-one as opposed to the split-stream puree with four out of them being characterized as odour-active volatiles. In the split-stream puree, twelve markers were detected

558 in abundance as compared to the all-in-one puree and four out of them were odour-active
559 volatile compounds.

560 Based on the markers' identification, they are divided in two different groups. In the all-in-
561 one puree, most of the markers with positive VID are fatty acid degradation products, consisting
562 of various aldehydes and alcohols, whereas all the markers closely projected to the split-stream
563 puree are sulfur-containing compounds. The differences might be attributed to (i) enzymatic
564 activity; (ii) impact of pH change (due to the mixing) on the activity of the enzymes; (iii)
565 chemical interactions between the precursors originating from the different matrices; and (iv)
566 Maillard reaction−lipid oxidation interactions. These four contributing factors will be further
567 discussed below.

568 Fatty acid-degradation related aldehydes and alcohols are mainly derived from tomato and
569 they are formed by the LOX pathway during maceration. LOX and HPL, two of the most
570 important enzymes of tomato, use linoleic and linolenic acids as substrates to produce the fatty
571 acid degradation products. Sulfur-containing volatiles are responsible for the characteristic
572 flavour and aroma of *Allium* species. Their release is catalyzed by alliinase, the main
573 endogenous enzyme of onion, when the plant tissue is disrupted.

574 pH might be another important factor which affects the activity of the enzymes, and
575 consequently the flavour formation. In our previous study (Koutidou et al., 2016), a change of
576 pH due to mixing in a mixed vegetable system comprised of tomato and carrot seemed to impact
577 the rate of the volatile generating reactions. In the current study, the all-in-one puree, in which
578 the vegetables were co-processed, was characterized by a pH of 4.4-4.5. However, in the split-
579 stream puree, when single vegetables were processed separately, the pH of the onion and tomato
580 during the process (blending and heating) was 5.1 and 4.1, respectively and only after their
581 combination, the pH of the mixed vegetable puree was 4.4-4.5. The higher onion pH during the

582 split-stream process can explain the increased level of sulfuric compounds in the final puree as
583 its pH was closer to the optimum pH for alliinase which has been reported to be 6-7, while it is
584 completely inactivated at pH <3.5 (Lancaster, Shaw, Joyce, McCallum, & McManus, 2000).
585 Similarly, the all-in-one puree is dominated by fatty acid degradation products because the pH
586 of the mix during the process was closer to the optimal LOX pH (pH 6-7) in comparison with
587 the more acidic pH of the tomato in the split-stream process. As the pH during the all-in-one
588 process was more acidic, alliinase activity will have been reduced yielding lower concentrations
589 in sulfur-containing volatiles in the all-in-one puree.

590 Apart from enzymatic reactions, when individual vegetable components are processed
591 together, chemical reactions between the different vegetable matrices can potentially induce a
592 change in the volatile profile. To evaluate the relative contribution of chemical reactions
593 between the vegetable components in comparison to enzymatic reactions, blanched all-in-one
594 and blanched split-stream purees were also prepared and their fingerprints were compared with
595 the fingerprints of non-blanched all-in-one and non-blanched split-stream puree samples. The
596 bi-plot based on the selected PLS-DA model for these samples (Figure 4) indicated a large
597 difference between the volatile profiles of the non-blanched samples but a similar volatile
598 profile for the blanched samples. In a bi-plot classes which are projected close to each other are
599 considered to have similar volatile profile whereas classes which are projected far from each
600 other are considered as different (Koutidou et al., 2016). Consequently, despite the fact that
601 chemical reactions may occur between the vegetable components in a mixed vegetable system,
602 the impact of enzymatic reactions on the volatile profile is by far predominant.

603 Maillard reaction−lipid oxidation interactions are possible during processing. Both
604 reactions influence each other by either enhancing or suppressing effects or by reacting with
605 some intermediates and producing new compounds (Zamora & Hidalgo, 2011; Cerny, 2015).

606 The GC–O analysis has shown that among the volatile markers which showed to be different
607 between the all-in-one and the split-stream puree, only few have a real contribution to their
608 overall aroma. Dimethyl and dipropyl trisulfide, methyl propyl trisulfide as well as propenyl
609 propyl disulfide were the discriminant odour-active markers, detected in higher concentration
610 in the split-stream puree. These markers imparted alliaceous, sulfury and meaty aroma to the
611 tomato-onion puree. The discriminant odour-active markers of the all-in-one puree were
612 hexanal, *(E)*-2-octenal, *(E,E)*-2,4-heptadienal and *(E,E)*-2,4-decadienal. These volatiles
613 contribute to the all-in-one puree with green, fatty, sweet, fried and vegetable notes.

614

615 **4. Conclusions**

616 The most potent odour-active of a mixed heat-treated tomato-onion puree were identified
617 using Detection Frequency method and AEDA. Results obtained from both olfactometric
618 techniques showed that the overall aroma of tomato-onion puree is primarily related to dipropyl
619 disulfide (fried onion, sweaty, green), *S*-propyl thioacetate (alliaceous), dimethyl trisulfide
620 (alliaceous, sulfury), 1-octen-3-one (mushroom), methional (potato), dipropyl trisulfide
621 (sulfury, alliaceous), 4,5-dimethyl thiazole (nutty, burnt savoury), 2-phenylacetaldehyde
622 (floral, honey) and sotolone (spicy, maple).

623 Processing steps (i.e. blanching, all-in-on/split-stream processes) have a great impact on the
624 volatile profile and aroma of a mixed tomato-onion puree. Thermal blanching prior to blending
625 aiming to inactivation of the enzymes resulted in lower levels of volatiles as compared to puree
626 made from non-blanched vegetables. All-in-one processed puree revealed higher levels of fatty
627 acids degradation products such as aldehydes and alcohols as compared to split-stream puree.
628 On the other hand, split-stream puree resulted in higher levels of surfur-containing compounds
629 as compared to the all-in-one puree. Differences might be attributed to (i) enzymatic activity;

(ii) impact of pH change on the enzymatic activity due to mixing; (iii) chemical interactions between the precursors originating from the different matrices; and (iv) Maillard reaction−lipid oxidation interactions. Nevertheless, the impact of the chemical interactions between the flavour precursors on the volatile profile is assumed to be of lower importance compared to the enzymatic reactions, concluding that aroma formation and enzymatic activity are strongly interrelated in a mixed tomato-onion puree.

In conclusion, this work shows that processes tested which resulted in modulation of the enzymatic activity could lead to distinct aroma profiles in a mixed tomato-onion system. For instance, an all-in-one process or a split-stream process resulted in the expression of more tomato or more onion notes, respectively, affecting the aromatic quality of the tomato-onion puree. This knowledge provides us with the ability to start controlling the aroma in mixed vegetable systems through process-induced enzymatic modulations. Further understanding of the complexity of mixed vegetable systems in the aroma generation can be an eye-opener for more targeted tailoring of vegetable flavour in the near future.

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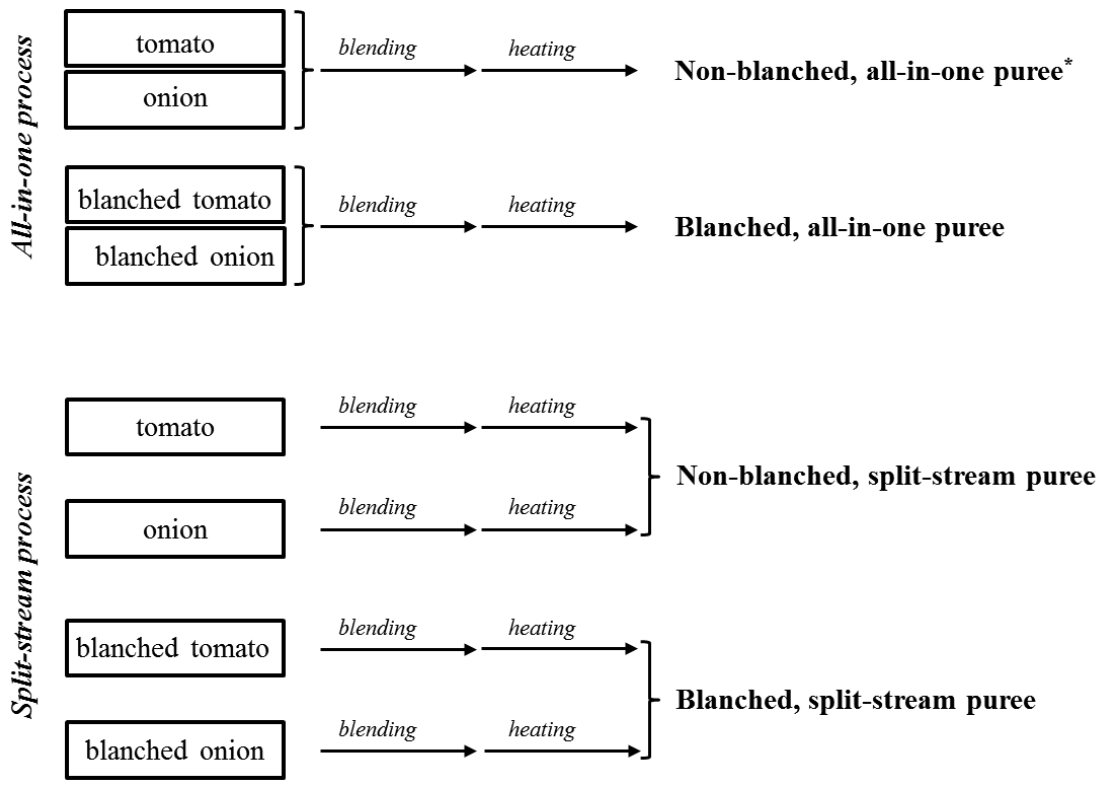


765

766 **Fig 1. Schematic overview of the sample preparation. In an all-in-one process, vegetables were mixed and**
767 **heated together. In split-stream process, single vegetables were blended and heated individually and**
768 **afterwards mixed together.**

769 *** Puree sample used for the GC-O analysis.**

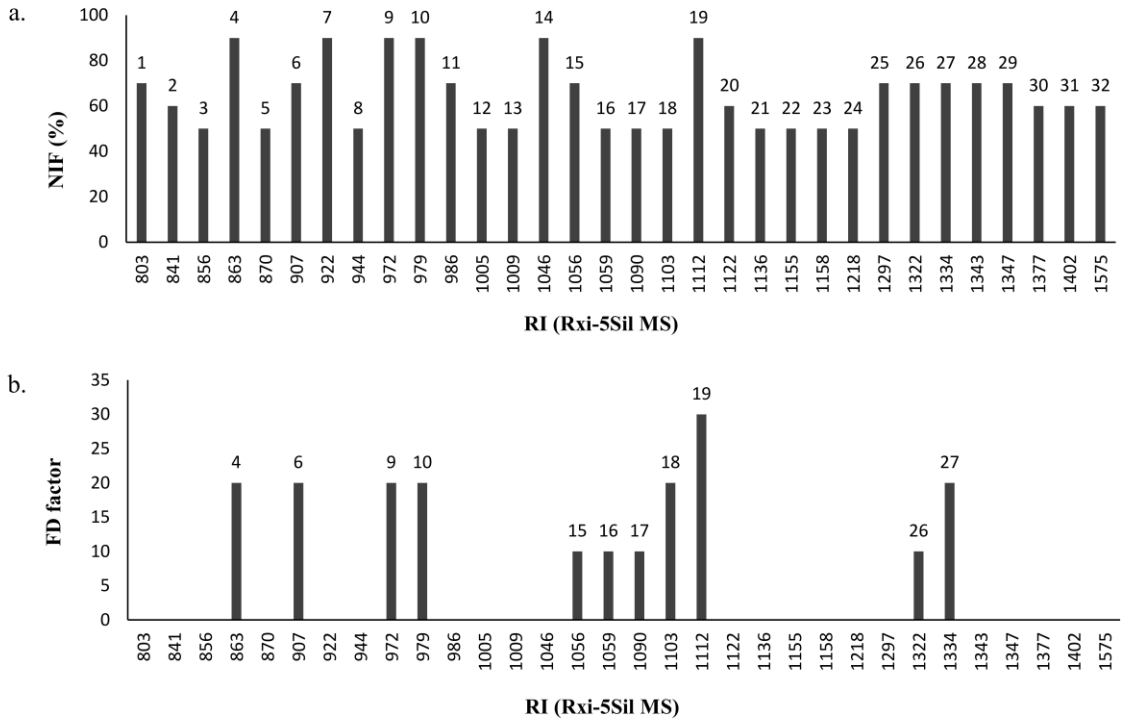


Fig 2. (a) Aromagram of odour-active compounds in tomato-onion puree obtained by detection frequency method. Numbers refer to odour-active compounds in Table 1. NIF: Nasal Impact factor; (b) Aroma extract dilution analysis aromagram of odour active compounds. . Numbers refer to odour-active compounds in Table 1. FD: Flavour dilution.

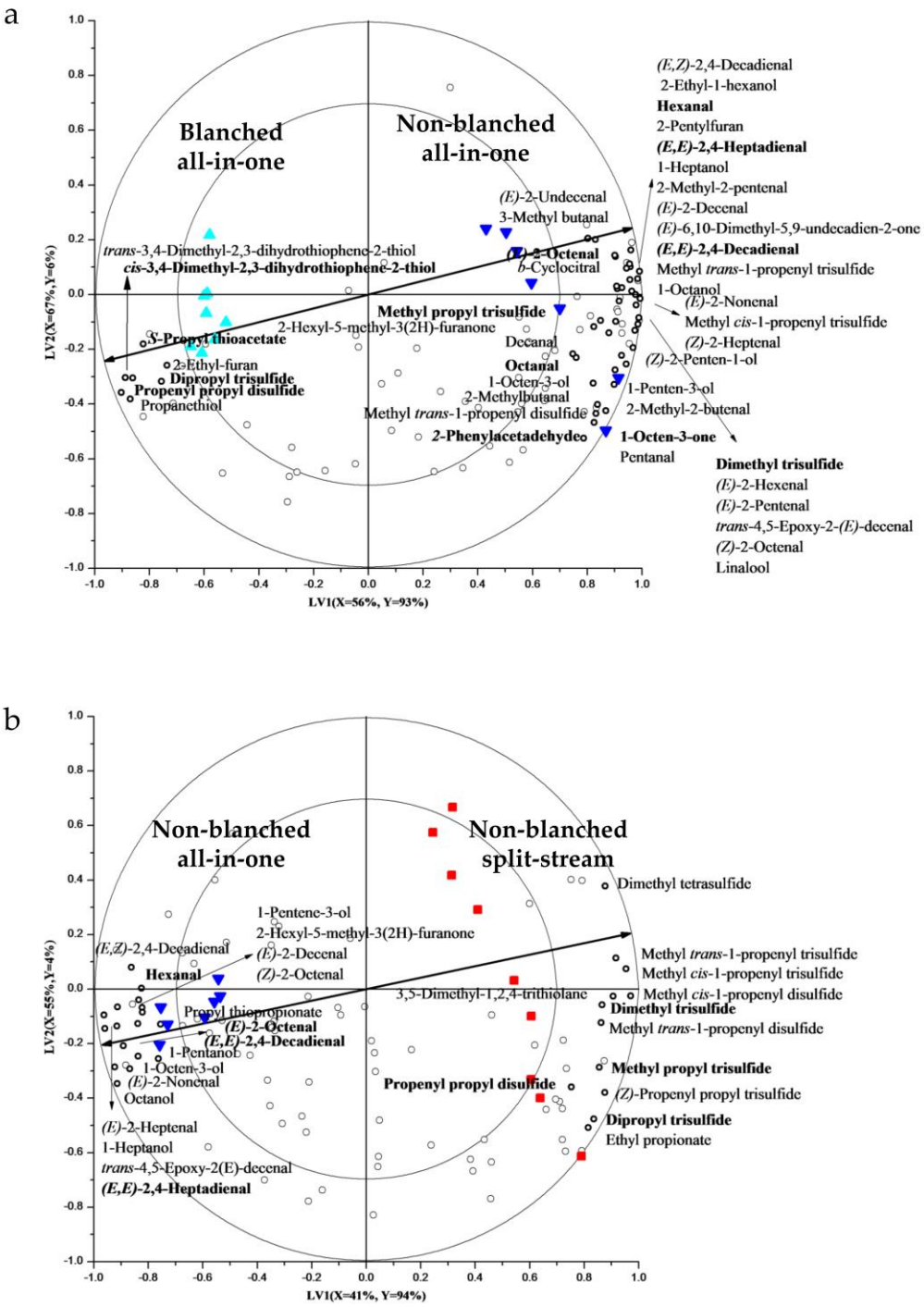
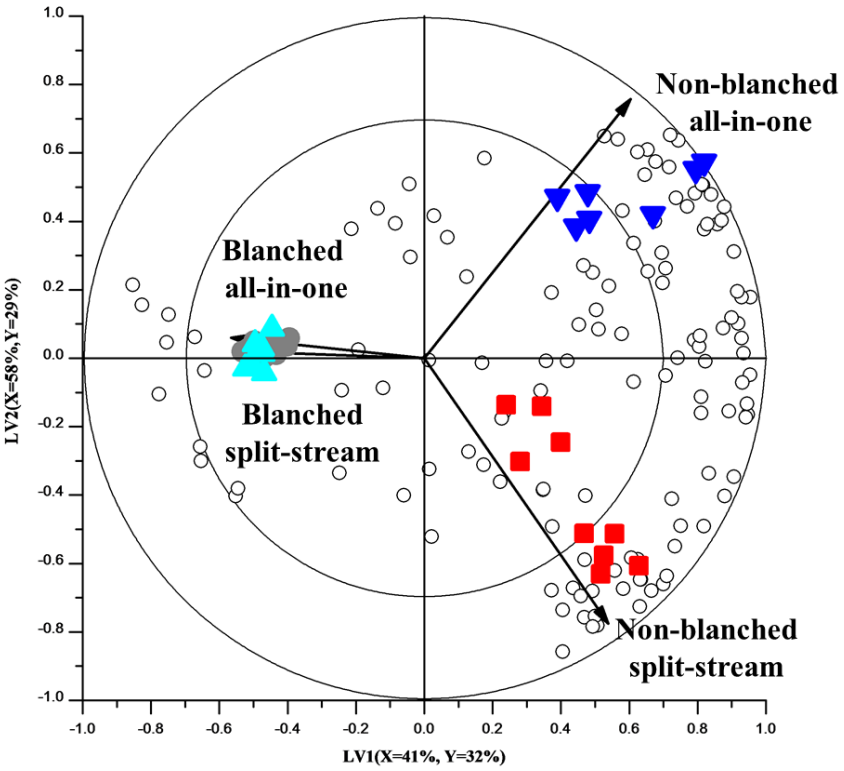


Fig 3. PLS-DA bi-plots visualizing impact differences between different purees. The open circles represent volatile compounds, of which selected discriminant compounds according to the established VID procedure (bold open circles) are identified only (Table 2). Discriminant compounds in bold are selected as odour-active compounds in tomato-onion puree (Table 1). Inner and outer ellipses on the bi-plots indicate a correlation coefficient of 70 and 100% respectively. The vectors represent the correlation loading for the categorical Y-variables. The X- and Y- variance explained by each LV is indicated in the respective axes. To verify the goodness of fit of the model R^2 values were calculated. (a) blanched all-in-one (▲) versus non-blanched all-in-one puree (▼), R^2 = 0.888; (b) non-blanched all-in-one (▼) versus non-blanched split-stream puree (■), R^2 = 0.885.



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786 **Fig 4. Bi-plot based on PLS-DA model selected to describe the different headspace fingerprints detected for non-**
787 **blanched all-in-one (▼),non-blanched split-stream (■), blanched all-in-one (▲) and blanched split-stream (♦) purees.**
788 **The open circles represent the detected headspace components. The vectors represent the correlation loading for the**
789 **categorical *Y*-variables. The *X*- and *Y*-variance explained by each LV is indicated in the respective axes.**

Table 1: Odour-active volatile compounds detected in tomato-onion puree extract

No	RI[a] (Rxi-5Sil MS)	RI[b] (DB-WAX)	Odour description	Compound	Identification[c]	NIF[d]	FD[e]
1	803	1080	green, grass	Hexanal	O, RI, MS, STD, TOFMS	70	
2	841		sweaty, meaty	2-(Methylthio)ethanol	O, RI, TOFMS	60	
3	856		green	*(Z)*-3-Hexen-1-ol	O, RI, MS, STD, TOFMS	50	
4	863		alliaceous	*S*-Propyl thioacetate	O, RI, TOFMS	90	20
5	870		alliaceous, burnt , rubbery	2,5-Dimethyl thiophene	O, RI, MS, TOFMS	50	
6	907	1471	potato	Methional	O, RI, MS, TOFMS	70	20
7	922	1354	nutty, burnt savoury, earthy	4,5-Dimethyl thiazole	O, RI, TOFMS	90	
8	944		alliaceous, onion	Methyl propyl disulfide	O, RI, TOFMS	50	
9	972	1398	alliaceous, sulfury, cabbage	Dimethyl trisulfide	O, RI, MS, STD, TOFMS	90	20
10	979	1314	mushroom	1-Octen-3-one	O, RI, MS, STD, TOFMS	90	20
11	986		alliaceous, bouillon, plastic, phenolic	Unknown		70	
12	1005	1302	fruity, sweet, green	Octanal	O, RI, MS, STD, TOFMS	50	
13	1009		vegetable, fatty	*(E,E)*-2,4-Heptadienal	O, RI, MS, STD, TOFMS	50	
14	1046	1663	floral, honey	2-Phenylacetaldehyde	O, RI, MS, STD, TOFMS	90	
15	1056	2058	candy, caramellic	Furaneol	O, RI, TOFMS	70	10
16	1059		fatty, sweet	*(E)*-2-Octenal	O, RI, MS, STD, TOFMS	50	10
17	1090	1884	phenolic, smoky	Guaiacol	O, RI, MS, TOFMS	50	10
18	1103		spicy, maple	Sotolone	O, RI, TOFMS	50	20
19	1112	1388	fried onion, sweaty, green	Dipropyl disulfide	O, RI, MS, TOFMS	90	30
20	1122	1432	meaty, fried onion, herbal	Propenyl propyl disulfide	O, RI, MS, TOFMS	60	
21	1136	1619	fried onion, bouillon	*cis*-3,4-Dimethyl-2,3-dihydrothiophene-2-thiol	O, RI, MS, TOFMS	50	20
22	1155	1604	green,cucumber	*(E,Z)*-2,6-Nonadienal	O, RI, MS, TOFMS	50	20
23	1158	1550	alliaceous, onion, sulfury	Methyl propyl trisulfide	O, RI, MS, STD, TOFMS	50	
24	1218		spicy, bouillon, vegetable	Unknown		50	

25	1297		spicy, vegetable and sulfury, herbal	Nitro-2-phenylethane	O, RI, TOFMS	70	
26	1322		fatty, fried	*(E,E)*-2,4-Decadienal	O, RI, MS, STD, TOFMS	70	10
27	1334	1694	sulfury, alliaceous	Dipropyl trisulfide	O, RI, MS, STD, TOFMS	70	20
28	1343		bouillon, savoury, honey, spicy	Unknown		70	
29	1347		cabbage, onion, vegetable	3,5-Diethyl-1,2,4-trithiolane	O, RI, TOFMS	70	
30	1377		alliaceous, sulfury, musty, vegetable	Unknown		60	
31	1402		caramellic, sweet, vanilla	Vanillin	O, RI, TOFMS	60	
32	1575		fried onion, vegetable, plastic	Unknown		60	

[a, b] Retention indices determined on Rxi-5Sil MS and DB-WAX column, respectively.

[c] Methods of identification; O, identified by aroma description; RI, identified by retention index via comparison with those reported in the literature; MS, identified by MS; STD, identified by retention time of chemical standard; TOFMS, identified by TOFMS.

[d] Detection frequency determined on Rxi-5Sil MS column.

[e] FD factor determined by aroma extract dilution analysis on Rxi-5Sil MS column.

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Table 2: Discriminant headspace compounds, selected in mixed tomato-onion puree, based on the VID procedure. The compounds are listed in increasing order of VID, where positive value indicates a higher concentration of a compound for the indicated class compared to other and negative value lower concentration compared to other class. The compounds in bold have been characterized by GC−O as odour-active volatiles in tomato-onion puree extract.

blanched all-in-one versus non blanched all-in-one			**non blanched all-in-one versus non blanched split-stream**		
VID (blanched)	**Compound**	**RI**	**VID** (all-in-one)	**Compound**	**RI**
-0.994	***(E,E)*-2,4-Heptadienal**$^+$	1009	-0.961	Methyl *cis*-1-propenyl disulfide	927
-0.986	*(E)*-2-Nonenal$^+$	1161	-0.955	Methyl *trans*-1-propenyl trisulfide	1164
-0.984	1-Heptanol$^+$	971	-0.923	Methyl *cis*-1-propenyl trisulfide	1158
-0.974	Methyl *cis*-1-propenyl trisulfide	1158	-0.916	Dimethyl tetrasulfide	1213
-0.971	***(E)*-2-Octenal**$^+$	1057	-0.878	3,5-Dimethyl-1,2,4-trithiolane	1134
-0.970	1-Octanol$^+$	1071	-0.852	**Dimethyl trisulfide**$^+$	964
-0.968	**Dimethyl trisulfide**$^+$	964	-0.841	Methyl *trans*-1-propenyl disulfide	936
-0.968	*(Z)*-2-Heptenal	955	-0.824	*(Z)*-Propenyl propyl trisulfide	1335
-0.963	**Hexanal**$^+$	800	-0.816	**Methyl propyl trisulfide**$^+$	1150
-0.961	2-Ethyl-1-hexanol	1028	-0.772	**Dipropyl trisulfide**$^+$	1330
-0.958	Methyl *trans*-1-propenyl trisulfide	1164	-0.748	Ethyl propionate	712
-0.956	2-Methyl-2-pentenal	829	-0.705	**Propenyl propyl disulfide**	1113
-0.953	*(E)*-2-Hexenal$^+$	850			
-0.948	2-Pentylfuran	991	0.765	Propyl thiopropionate	961
-0.940	*(E)*-2-Pentenal$^+$	751	0.788	1-Pentanol$^+$	764
-0.924	*(E)*-6,10-Dimethyl-5,9-undecadien-2-one	1445	0.819	**Hexanal**$^+$	800
-0.913	*(E,Z)*-2,4-Decadienal$^+$	1291	0.825	2-Hexyl-5-methyl-3(2H)-furanone	1432
-0.911	*β*-Cyclocitral	1220	0.826	*(E)*-2-Decenal$^+$	1261
-0.909	1-Penten-3-one	685	0.834	1-Penten-3-ol	680
-0.909	*trans* 4,5-Epoxy-2*(E)*-decenal$^+$	1375	0.846	*(E,Z)*-2,4-Decadienal$^+$	1291
-0.906	***(E,E)*-2,4-Decadienal**$^+$	1313	0.848	*(Z)*-2-Octenal	1046
-0.905	*(E)*-2-Decenal$^+$	1261	0.861	***(E,E)*-2,4-Decadienal**$^+$	1313
-0.896	*(Z)*-2-Penten-1-ol	764	0.897	1-Octen-3-ol	980
-0.888	*(Z)*-2-Octenal	1046	0.910	***(E)*-2-Octenal**$^+$	1057
-0.866	1-Penten-3-ol	680	0.914	*(E)*-2-Heptenal$^+$	955
-0.859	Linalool	1099	0.925	*trans* 4,5-Epoxy-2*(E)*-decenal$^+$	1375
-0.857	2-Methyl-2-butenal	738	0.950	1-Octanol$^+$	1071
-0.847	*(E)*-2-Undecenal$^+$	1360	0.951	*(E)*-2-Nonenal$^+$	1161
-0.834	**Methyl propyl trisulfide**$^+$	1148	0.967	1-Heptanol$^+$	971
-0.820	3-Methyl butanal	645	0.970	***(E,E)*-2,4-Heptadienal**$^+$	1009
-0.816	Pentanal$^+$	698			

-0.806	2-Hexyl-5-methyl-3(2H)-furanone	1432
-0.788	**1-Octen-3-one**[+]	978
-0.781	1-Octen-3-ol	980
-0.779	2-Methylbutanal	658
-0.769	Methyl *trans*-1-propenyl disulfide	935
-0.730	**Octanal**[+]	1003
-0.724	**2-Phenylacetaldehyde**[+]	1041
-0.719	Decanal	1208
0.760	2-Ethyl-furan	701
0.786	**Dipropyl trisulfide**[+]	1344
0.837	***S*-Propyl thioacetate**	866
0.890	***cis* 3,4-Dimethyl-2,3-dihydrothiophene-2-thiol**	1127
0.907	Propanethiol	608
0,916	*trans*-3,4-Dimethyl-2,3-dihydrothiophene-2-thiol	1171
0,937	**Propenyl propyl disulfide**	1116

Identification of the compounds was based on their retention index (RI) on HP-5MS column and their mass spectrum obtained from MS (by comparison with mass spectra stored in NIST and FFNSC library).

[+] **Identification of these compounds was further supported by the comparison of spectra and retention time with commercial standards.**