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Volatile profile of dehydrated cherry tomato: Influence of osmotic pre-treatment and microwave power

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Abstract

Vegetable flavour is a quality characteristic for consumer acceptability. Sun and air are traditionally used for drying tomatoes; however the optimal combination of techniques such as osmotic dehydration or microwave assisted air drying could lead to high quality self-stable products. The aim of this paper was to study the influence of different process variables on the volatile profile of dehydrated cherry tomato halves. The analyzed variables were: air drying temperature (40 and 55 ºC), microwave power (0 and 1 W/g) and previous osmotic dehydration with a 55 Brix binary sucrose solution at 30ºC for 120 min (OD1) or ternary solution of 27.5 % sucrose + 10 % NaCl (w/w) at 40 ºC for 60 min (OD2). Twenty major volatile compounds were identified in fresh tomatoes. Among them, 2-Isobutylthiazole and 6-Methyl-5-hepten-2-one stand out as impact volatile compounds. Dehydration modified the volatile profile, mainly due to the changes induced in some typical fresh-like tomato compounds, but also due to the generation of 5 new compounds: 1-Butanol, 2-Methyl-2-butenal, 3-Hydroxy-2-butanone, Furfural, Acetonitrile, related to Maillard reactions, and the catabolism of carotenoids and polyunsaturated fatty acids. Principal component analysis showed the possibility of obtaining dried cherry tomatoes with different volatile profiles depending on drying conditions.

Keywords: Cherry tomato; GC–MS; Volatile compounds; Osmotic dehydration; Microwave assisted hot-air drying.

1. Introduction

In recent years interest in dried tomatoes has increased due to both their multiple possibilities in cuisine and their functionality. Traditional methods such as sun-drying or hot-air convective drying are still the most widely used technique to obtain dried tomatoes. However, long processing times at high temperature and the presence of oxygen can cause oxidative heat damage, shown by both a marked loss of ascorbic acid and an increase in the 5-Hydroxymethyl-2-furfural (HMF) content, resulting in undesirable colour and appearance changes in the final product (Zanoni, Peri, Nani & Lavelli, 1999). Moreover, the quality in terms of taste and aroma is reduced due to loss of the most important volatile compounds that make up the aroma producing the development of an overly strong flavour (Lewicki, Vu Le, & Pomaranska-Lazuka, 2002).

An adequate combination of dehydration techniques would be interesting in order to reduce undesirable changes occurring as a result of the drying operation and improve moreover, the efficiency of the process. An example might be the implementation of an osmotic dehydration step followed by hot air drying with and without the application of microwave energy. By combining these techniques, the whole process is favoured by the numerous advantages provided by each of them.

Different studies show that osmotic dehydration before the hot air drying stage prevents the loss of volatile compounds which are responsible for the flavour (Dermesonlouoglou, Giannakourou & Taoukis, 2007) and additionally it permits an increase in the capacity of the dryers as the product has lower moisture content than the raw material. This fact, together with the application of microwave energy reduces drying times as a consequence of a more volumetric and uniform heating and in this way saves energy and increases the yield of the operation (Mudgett, 1989; Maskan, 2001; Andrés, Bilbao and Fito, 2004).

Flavour is one of the most important quality indicators of a fruit and a decisive factor in consumer purchasing choice. Aromatic compounds of tomato have been extensively studied, and although more than 400 volatile compounds have been identified, it is well known that only a small number are essential and really contribute to tomato flavour (Petro-Turza, 1987). Several papers dealing with the composition of the volatile phase from tomatoes responsible for the flavour of fresh fruits can be found (Buttery, Teranishi, Ling, Flath & Stern, 1988; Baldwin, Scott, Einstein, Malundo, Carr, Shewfelt & Tandon, 1998; Tandon, Baldwin & Shewfelt, 2000), although some authors also

63 acknowledge that not all the volatile compounds detected by analytical methods can be
64 detected by olfactory methods, as reported by Gocmen, Gurbuz, Rousef, Smoot &
65 Dagdelen (2004).
66 As a result of research the volatile compounds which have a major influence on the
67 fresh tomato flavour are well known. A combination of cis-3-Hexenal, cis-3-Hexenol,
68 Hexanal, 1- Penten-3-one, 3-Methylbutanal, trans-2-Hexenal, 6-Methyl-5-Hepten-2-
69 one, Methyl salicylate, 2-Isobutylthiazole, and β-Ionone at appropriate concentrations is
70 related to the aroma of fresh ripe tomatoes (Buttery, 1993). 2- Isobutylthiazole and cis-
71 3-Hexenal have been described as "flavour impact compounds" (Kazeniac & Hall,
72 1970). The most odour-active aroma volatiles which contribute to the fresh tomato
73 flavour have been determined as (Z)-3-Hexenal, Hexanal, 1-Octen-3-one, Methional, 1-
74 Penten-3-one and 3-Methylbutanal by Krumbein and Auerswald (2000). Tandon *et al*.
75 (2000) confirmed these compounds too, although they did not include 1-Octen-3-one
76 and Methional in their odour threshold measurements.
77 In recent years, the flavour of commercialized tomatoes has received extensive
78 complaints by consumers. This poor organoleptic quality is partly due to harvesting the
79 tomatoes at a green-ripened stage so that the fruit is suitable for transport (Kader,
80 Stevens, Albright-Holton, Morris & Algazi, 1977). Thereby, various papers related to
81 tomato post-harvest treatment have been published (Boukobza & Taylor, 2002;
82 Krumbein, Peters & Brückner, 2004) but very few reports focus on the changes in the
83 aroma profile of tomato occurring during the drying stage. Nevertheless, relevant
84 modifications take place.
85 Dried tomato aroma is characteristic and different to the fresh tomato aroma which is
86 also what the consumer expects, but these changes in the aroma must not be
87 accompanied by undesirable odours/flavours. For this reason, it is important to identify
88 the aromatic compounds in dried tomato and thereby to be able to explain how the
89 different drying techniques affect the aromatic profile, decreasing or increasing existing
90 compounds or even generating new ones.
91 Traditional varieties of large tomatoes have been used to obtain dried tomatoes but the
92 new small-sized varieties (cherry and plum tomatoes) present a higher dry matter and
93 soluble solids fraction, mainly due to the higher levels of sugars and organic acids
94 (Muratore, Licciardello & Maccarone, 2005). In addition, cherry tomatoes have a high
95 content of sensorily important aroma volatiles (Krumbein & Auerswald, 2000). Taking

into account these aspects, the optimal drying of cherry tomatoes may lead to a product with a stronger and more attractive flavour for consumers and greater value as a condiment in many dishes.

The aim of this paper was to study the influence of different process variables (air drying temperature, microwave power and previous osmotic dehydration) on the volatile profile of dehydrated cherry tomato halves.

2. Materials and Methods

2.1. Sample preparation

Cherry Tomatoes (*L. esculentum Mill* var. cerasiform cv. Cocktail) were used as raw material in this study. They were acquired in a local supermarket but always from the same supplier, which has three controlled production areas in different regions of Spain. They were visually selected by colour, size and absence of physical damage in order to ensure maximum homogeneity. Then, the tomatoes were cleaned and cut in halves along the equatorial zone to encourage flow of the material during the dehydration operations. The treatment of whole tomatoes was not convenient due the epidermis' waxy nature, which is impermeable to any exchange of material with the environment, unless a pretreatment that increases its permeability is performed (physical or chemical peeling) (Shi, Le Maguer, Kakuda & Lipaty, 1999). On the other hand, as the greatest lycopene content is found located in the skin (Shi *et al.*, 1999), peeling the fruit was not deemed suitable. Treatments were carried out on seven different batches of fresh cherry tomatoes. All samples were characterized in terms of moisture, soluble solids, water activity and volatile fraction.

2.2. Experimental methodology

Fresh cherry tomatoes cut into halves were subjected to osmotic treatment followed by microwave assisted air drying. Two different osmotic treatments were tested: 55 Brix binary solution of sucrose at 30ºC, 120 min (OD1) and ternary solution of 27.5% sucrose + 10 % NaCl (w/w), 40 ºC, 60 min (OD2). These osmotic treatment conditions were selected according to a prior study on the influence of the osmotic dehydration variables on the lycopene and β-carotene content in tomato cherry halves (Heredia, Peinado, Barrera & Andrés, 2009).

The following drying of fresh and osmopretreated cherry tomatoes were performed at two different air drying temperatures (40 and 55 ºC), and microwave power levels (0 and 1 W/g). The drying process was carried out in a specially designed convective hot-air dryer assisted by microwave energy and entirely monitored by a computer (Andrés *et al.,* 2004). This equipment allows process variables such as microwave energy, temperature and speed of the drying air, to be controlled. In addition, the equipment has sensors connected to measure the atmospheric air's relative humidity and temperature.

Drying of the samples was performed until they reached a final moisture content within the range found, 0.50-0.70 g of water/ g, in Spanish commercial products of tinned dry tomatoes in oil. The drying time to achieve the required final water content in the tomato samples was previously established from the drying curve obtained for each combination of drying conditions (Heredia, Barrera and Andrés, 2007).

2.3. Physicochemical analyses

All the physicochemical analyses were carried out on fresh tomatoes, after the osmotic treatment, and after the drying stage.

Moisture content was determined gravimetrically by drying to constant weight in a vacuum oven at 60 ºC (method 20.103 AOAC, 1980). Soluble solids content (Brix) was measured in previously homogenized samples with a refractometer at 20 ºC (ATAGO 3 T). In dried samples, dilution was necessary at a ratio of 4 g of water per gram of sample. Water activity (a_w) was determined with a dew point hygrometer ((FA-st lab, GBX). NaCl determinations were carried by using an automatic chloride analyzer (Sherwood, model 926). All determinations were carried out in triplicate in fresh and processed samples.

2.4. Volatile compound analysis

Aromatic compounds were extracted by purge and trap thermal desorption; 20 g of fresh and reconstituted (to a moisture equal to that of the fresh product) samples spiked with 200 µL 2-pentanol (10 µg/mL as an internal standard), were placed in a purging vessel flask and left in a water bath at 45 ºC for 20 min. During this time, purified nitrogen (200 mL min^{-1}) was forced through a porous frit placed at the bottom of the vessel, producing a stream of bubbles which passed through the sample, then the volatile compounds were collected. These were trapped in a 100 mg porous polymer (Tenax

TA, 20–35 mesh) packed into a glass tube placed at the end of the system. A total of 3 extracts were obtained for each sample. The volatile compounds were subsequently thermally desorbed using a direct thermal desorber (TurboMatrix TD, Perkin ElmerTM, CT-USA). Desorption was performed under a 10 mL min^{-1} helium flow at 240 ºC for 10 min. The volatiles were then cryofocused in a cold trap at -30 ºC and transferred directly onto the head of the capillary column by heating the cold trap to 250 ºC (at a rate of 99 ºC/s).

GC–MS analyses were performed using a Finnigan TRACETM MS (TermoQuest, Austin, USA). Volatile compounds were separated using a BP-20 capillary column (SGE, Australia) (60 m length, 0.32 mm i.d., 1.0 lm film thickness). Helium at a constant flow rate of 1 mL min^{-1} was used as a carrier gas. The temperature was programmed to increase from 40 ºC (2 min hold time) to 190 ºC at 4 ºC min^{-1} and finally to 230 ºC at 10 ºC min^{-1}. The MS interface and source temperatures were 250 ºC and 200 ºC, respectively. Electron impact mass spectra were recorded in impact ionization mode at 70 eV and with a mass range of m/z 33–433. A total of three extracts were obtained for each sample.

The identification of isolated volatile compounds was performed by comparing their mass spectra, retention times and linear retention indices (Kovats retention indices; KI) against those obtained from authentic standards when they were available: acetic acid, 1-Hexanol , 3-Hexen-1-ol, 2-Methyl 1-butanol, 2-Methyl-1- propanol, 1-Pentanol, 1-Penten-3-ol, Benzaldehyde, 2-Hexenal, 2-Octenal, 6-Methyl-5-hepten-2-one, 3-Pentanona, 1-Penten-3-one, 1-Butanol, 2-Methyl-2-butenal, Furfural, 3-Hydroxy-2-butanone and Acetonitrile (Sigma-Aldrich, San Louis, Missouri; Acros Organics, Geel, Belgium and Fluka Buchs,Schwiez, Switzerland). Otherwise they were tentatively identified by comparing their mass spectra (*m/z* values of the most important ions) with spectral data from the National Institute of Standards and Technology 2002 library as well as retention indices published in the literature (Tatsuka, Suekane, Sakai & Sumitanis, 1990; Kondjoyan & Berdague, 1996; Alves & Franco, 2003; Mayer, Takeoka, Buttery, Whitehand, Naim & Rabinowitch, 2008; Barrios, Sinuco & Morales, 2010). Kovats retention indices were determined by injection into the Tenax of a solution containing the homogenous series of normal alkanes (C_8 –$C_{20;}$ by Fluka Buchs, Schwiez, Switzerland) in the same temperature-programmed run, as described above. Semiquantitative analyses were carried out (Soria, Martínez-Castro & Sanz, 2008),

since not all the standards were available and as on the other hand the objective of this study was to evaluate the differences between different treatments. Therefore it was considered that quantification was not necessary.

The data (μg/100 g of fresh tomato) were expressed by using the amount of internal standard and the relative area between the peak areas of each compound and the peak area of the internal standard, assuming a response factor equal to one.

2.5. Statistical analysis

The statistical analyses of the variance (factorial ANOVA) with a confidence level of 95% (p-value ≤ 0.05) were carried out using of the software package Statgraphics Plus 5.1 to estimate the significant effect of the different variables of the process (drying air temperature, power of incident microwaves, and osmotic treatment). Furthermore, a Principal Components Analysis, PCA, (Martens & Næs, 1989) (Unscrambler version 9.7; CAMO Process AS, Oslo, Norway) was applied to describe the relationships among the changes on the profile of volatile compounds and process variables.

3. Results and Discussion

3.1. Physicochemical changes induced by processing in cherry tomato

Table 1 shows the mass fraction of water (x^w), total soluble solids (x^{ss}) and NaCl (x^{NaCl}) of processed cherry tomato samples for each treatment as well as their water activity (a_w). Fresh samples showed $x^w = 0.91(0.007)$, $x^{ss} = 0.080(0.002)$ and $a_w = 0.988(0.002)$. Results show a reduction of moisture content and an increase in soluble solids, NaCl or/and sucrose, in the samples after osmotic treatment, resulting in a reduction in water activity. Nevertheless, differences can be appreciated regarding osmotic agents. Therefore, the gain in soluble solids was higher in samples dehydrated with the ternary solution of water, NaCl and sucrose (OD2) than in samples dehydrated with a binary sucrose one (OD1). This phenomenon is related to the lower molecular weight of NaCl allowing easier penetration in the vegetal tissue (Heredia et al., 2009).

After the hot air-microwave drying stage, the moisture content of the samples varied between 0.50 and 0.67 g of water/ g, thus falling within the range of commercial products. Regarding soluble solids content, untreated samples presented the least content whereas pretreated ones achieved superior values, the samples processed with

the ternary solution (OD2) being the most stable due to their lower water activity values as a consequence of NaCl intake.

3.2. Volatile profile changes in cherry tomatoes induced by drying

Table 2 shows the 20 major volatile compounds (µg/ 100g, as well as the standard deviation (SD)) of the identified compounds in the seven different batches of fresh cherry tomatoes used in this work. These compounds include different chemical classes such as free acids, alcohols, ketones, aldehydes and one heterocyclic compound. Concretely, the identified volatile compounds were: Acetic acid, 12 aroma volatiles derived from the lipid oxidation-derived pathway (1-Hexanol, 3-Hexen-1-ol, 1-Octanol, 1-Octen-3-ol, 1-Pentanol, 1-Pecten-3-ol, 2,4 Decadienal, (Z)-2-Heptenal, 3-Hexanal, E-2-Hexenal, E-2-Octenal, 1-Pecten-3-one) (Yilmaz, 2000), Benzaldehyde from shikimic acid pathway, two volatile compounds related to lycopene catabolism (6-Methyl-5-hepten-2-one and 6-Methyl-5-hepten-2-ol) (Buttery *et al.*, 1988), two volatiles related to amino acid metabolism (2-Methyl-1-butanol, 2-Methyl-1-propanol, 2-Isobutylthiazole) (Richard & Multon, 1992) and 2-Pentanone from carbohydrate degradation. Those volatiles produced by the lipid-oxidation pathway and auto-oxidation are typically volatile aldehydes and alcohols responsible for fresh and green sensorial notes. E-2-Hexenal, 1-Hexanol, 3-Hexen-1-ol, 2-Isobutylthiazole, 6-Methyl-5-hepten-2-one and 1-Penten-3-one, are considered to be the most characteristic volatile compounds in fresh tomato aroma (Buttery, 1993).

Among the 20 identified compounds, five of them represented 77.3 % of the volatile fraction in fresh cherry tomatoes. Concretely, 3-Hexanal and E-2-Hexenal presented the high concentration values, followed by 2-Methyl-1-butanol, 1-Hexanol and 3-Hexen-1-ol, respectively. Hence, it could be said that fresh cherry tomatoes were characterized by high contents of the sensorially important volatiles such as 3-Hexanal and (E)-2-Hexenal. (E)-2-Hexenal were associated with the attributes sweetness and fruitiness (Krumbein *et al.*, 2004). Some authors also established a good correlation between high aldehydes contents and the appreciated aroma of fresh tomato (Petró-Turza, 1987).

On the other hand, other volatile compounds such as 2-Isobutylthiazole and 6-Methyl-5-hepten-2-one, have been described as impact volatile compounds; even though they have a low concentration their contribution to whole tomato aroma is important (Kazeniac & Hall, 1970). Among them, 2-Isobutylthiazole, a heterocyclic compound,

has been described as tomato-like flavour (Richard & Multon, 1992). When it is added to processed tomato products in very low concentrations such as 25-50 ppb, it increases "fresh tomato-like" aroma. 6-Methyl-5-hepten-2-one has been also characterized as having a fruit-like aroma (Kazeniac & Hall, 1970).

Cherry tomato has important differences in volatile fraction when compared with other varieties, in particular the presence of 2-Methyl-1-butanol, an isoamylic alcohol, related to the acetification process. In acetic fermentation, this alcohol would be gradually consumed to produce acetic acid, increasing the representation of this acid in the volatile profile. In fact, optimal commercialization and the consumption of fresh cherry tomatoes are strongly conditioned by the predisposition of this fruit to acetification in comparison with other varieties. 1-Hexanol and 3-Hexen-1-ol have been related to a powerful, fresh, green grass odour and are responsible for delicate floral fragrances such as muguet or lilac. 1-Hexanol is a volatile derived from lipids via oxidation when cells are disrupted imparting mint tones in tomatoes. Berna, Lammertyn, Buysens, Di Natale & Nicolaï (2005) analyzed the relationship between aroma and tomato varieties considering consumers' preferences. A strongly preference for the tomato varieties whose aroma was a result of a complex interaction of 3-Methyl-1-butanol, Hexanal and 1-Hexanol was found.

Variability of fresh fruit was low as reflected by the ANOVA carried out for each volatile component by considering the batch factor (Table 2). Nevertheless, 4 compounds showed statistically significant differences (at 95% or 99% confidence level). Among them, 2-Isobutilthiazol, one of the considered flavour impact compounds as mentioned previously. For this reason, in order to accurately identify changes induced by processing on the volatile profile of cherry tomatoes the batch used in each treatment was taking into account. The effect of osmotic treatment and microwave assisted hot-air drying on the volatile profile of cherry tomato was evaluated through the relative changes induced by the treatment in each compound. Consequently, for each compound, concentration (C) after treatment was divided by the value for fresh cherry tomatoes (C_0) taking into account the corresponding batch (table 3). For those compounds generated during processing, table 4 shows the mean concentration (μg/100g) value for each one. A total of 25 volatile compounds were identified in dehydrated cherry tomatoes (tables 3 and 4) due to the appearance of 5 new compounds (table 4). Both osmotic treatments and further drying stage strongly modify the aroma

294 profile of cherry tomatoes as can be deduced from the ratio values (C/C_0) different to 1
295 for all compounds originally present in fresh. Table 3 reports a strong decrease ($C/C_0 <$
296 1) of many of the volatiles components, including 3-Hexanal, E-2-Hexenal, 2-Methyl-1-
297 butanol, 1-Hexanol and 3-Hexen-1-ol. As can be observed, osmotic treatment with the
298 ternary solution sucrose-NaCl-water (OD2) and shorter time than OD1 promoted an
299 increase of some volatile compounds (6-Methyl-5-hepen-2-ol, 1-Octanol, 1-Octen-3-ol,
300 1-Pentanol). This fact is consistent with the results obtained by Torres, Talens, Carot,
301 Chiral & Escriche (2007) who reported that in general; a high osmodehydration level in
302 the samples induced losses of volatiles, but the use of diluted solutions and shorter
303 treatment times could give rise to the enhancement of volatile production. It might
304 however be pointed out that OD2 promoted higher losses of 2-Isobutylthiazole. Osmotic
305 pretreatment just generated one new compound, 2-Methyl-butenal (data not available in
306 tables 4) its concentration being 0.33 (0.04) and 0.24 (0.02) mg/ 100 g under ODI and
307 OD2 conditions, respectively.
308 Regarding the changes induced by drying stage in considered flavour impact
309 compounds in fresh tomato, important losses of 2-Isobutilthiazol, between 80-90%, and
310 a similar trend for 6-Methyl-5-hepten-1-one except for samples dehydrated under the
311 mild condition (hot-air drying at 40 ºC without microwave heating and osmotic
312 pretreatment application) were registered. Nevertheless, Methyl-5-hepten-1-ol, another
313 volatile compound related to degradation of carotenoids, and more precisely from the
314 oxidative cleavage of lycopene, experimented an increase as a consequence of the
315 thermal treatment carried out in absence of microwave heating. This fact could be
316 related to the longer processing times associated with these conditions. In summary, it
317 could be said that during thermal treatments the impact compounds of the fresh tomato
318 flavour originated by the endogenous pathways in fresh tomato decreased (Buttery *et*
319 *al.*, 1990); whereas the Maillard reactions activation, carotenoids and polyunsaturated
320 fatty acids catabolism, promote the genesis of volatile compounds related to the cooked
321 flavor (Servili, Selvaggini, Taticchi, Begliomini & Montedoro, 2000). As such, Furfural
322 is the most representative generated aroma from Maillard reactions not involving
323 sulphur amino acids. Foods achieved higher temperatures under an electromagnetic
324 energy field than under convective hot-air drying stimulated by a fast nearly
325 instantaneous volumetric heating. This phenomenon is related to the energy of water
326 molecules converted into kinetic energy and then into heat, when the water molecules

realign in the changing electrical field and interact with the surrounding molecules (friction) (Khraisheh, Cooper & Magee, 1997).

The other new compounds are 1-Butanol, 2-Methyl-2-butenal, 3-Hydroxy-2-butanone and Acetonitrile. Results confirmed the role of microwave heating as Furfural precursor whereas drying carried out exclusively under hot-air did not imply any Furfural generation (table 4).

A PCA was conducted to evaluate the global effect of the treatments on the volatile fraction, from a descriptive point of view. Figure 1 shows the biplot of sample scores and compound loadings obtained by means of PCA analysis. Samples were codified as (microwave power level / air drying temperature / osmotic pretreatment).

The first two components explain the 73% of total variance (PC1 53% and PC2 20%). For the scores, proximity between samples indicates similar behaviour in terms of the volatile profile. For loadings, proximity between variables demonstrates some similarity in the levels of their concentrations. It shows that the first principal component clearly differentiates fresh batches located in the right quadrant near typical volatile compounds of fresh tomato like 1-Penten-3-ol, 2-Isobuthylthiazole, 2-Mehyl-1-propanol, Benzaldehyde, etc., from treated samples located on the left one. Osmodehydrated samples appear in the middle of the biplot. This fact indicates the presence of higher fresh typical volatile compound concentration in these samples than in samples submitted to drying, especially in samples exclusively osmodehydrated with sucrose (OD1). Some differences could be also appreciated between drying samples. Thus, samples exclusively dried by convective hot-air drying mainly at low temperature are located closer to fresh batches (Buttery & Ling, 1993); whereas the samples submitted to microwave and stronger conditions are located further away. This indicates that thermal treatments, especially microwave application, strongly modified volatile profile of tomato samples which is coincident with the results founded by other authors such as Servili *et al.* (2000). In fact, most of the typical compounds in fresh tomato decreased or disappeared while some other new compounds like furfural, which has been reported to appear under heat treatments (Servili *et al.*, 2000*)* were found.

4. Conclusions

The volatile profile of dry cherry tomatoes was affected in a different ways depending on processing conditions. Microwave energy modified the volatile fraction of fresh

tomato developing a new profile, mainly due to both furfural generation and activation of Maillard Reactions. Osmotic pretreatment in a sucrose solution followed by hot-air drying was the combination of drying techniques that mainly preserved the typical aroma of fresh cherry tomato. Finally, Principal Component Analysis (PCA) confirmed the possibility of obtaining dried cherry tomatoes with different volatile profiles either by microwave power or the application of hot-air drying.

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References

Alves, G.L. & Franco M.R.B. (2003) Headspace gas chromatography–mass spectrometry of volatile compounds in murici (Byrsonima crassifolia L. Rich). *Journal of Chromatography, 985*, 297–301.

Andrés, A., Bilbao, C. & Fito, P. (2004). Drying kinetics of apple cylinders under combined hot air–microwave dehydration. *Journal of Food Engineering, 63 (1),* 71-78.Baldwin, E.A., Scott, J.W., Einstein, M.A., Malundo, T.M.M., Carr, B.T., Shewfelt, R.L., & Tandon, K.S. (1998)**.** Relationship between sensory and instrumental analysis of tomato flavour. *Journal of American Society for Horticultural Science, 125*, 906–915.

Barrios, J. C.; Sinuco, D. C. & Morales A. L. (2010) Compuestos volátiles libres y enlazados glicosídicamente en la pulpa de la uva Caimarona (Pourouma cecropiifolia Mart.). *Acta Amazónica, 40 (1),*189-198.

Berna, A. Z., Lammertyn, J., Buysens, S., Di Natale, C. & Nicolaï, B. M. (2005). Mapping consumer liking of tomatoes with fast aroma profiling techniques. *Postharvest Biology and Technology, 38(2),* 115-127.

Boukobza, F. & Taylor, A.J. (2002). Effect of postharvest treatment on flavour volatiles of tomatoes. *Postharvest Biology and Technology, 25*, 321–331.

391 Buttery, R.G., Teranishi, R., Ling, L.C., Flath, R.A., & Stern, D.J. (1988). Quantitative
392 studies on origins of fresh tomato aroma volatiles. *Journal of Agricultural and Food*
393 *Chemistry, 36*, 1247–1250.
394 Buttery, R. G., Takeoka, G., Teranishi, R., & Ling, L. C. (1990). Tomato aroma
395 components: identification of glycoside hydrolysis volatiles. Journal *of Agricultural*
396 *and Food Chemistry, 38*, 2050-2053.
397 Buttery, R.G. (1993). Quantitative and sensory aspects of flavor of tomato and other
398 vegetables and fruits. In: Flavor Science: Sensible Principles and Techniques. (Eds.:
399 T. Acree and R. Teranishi), ACS, Washington, D.C., pp. 259-286.
400 Dermesonlouoglou, E.K., Giannakourou, M.C., & Taoukis, P. (2007). Stability of
401 dehydrofrozen tomatoes pretreated with alternative osmotic solutes. *Journal of Food*
402 *Engineering, 78(1),* 272-280.
403 Gocmen, D., Gurbuz, O., Rousef, R.L., Smoot, J.M., & Dagdelen, A.F. (2004). Gas
404 chromatographic-olfactometric characterization of aroma active compounds in sun-
405 dried and vacuum-dried tarhana. *European Food Research and Technology, 218,*
406 573–578.
407 Heredia, A., Barrera, C. & Andrés, A. (2007). Drying of cherry tomato by a
408 combination of different dehydration techniques. Comparison of kinetics and other
409 related properties. *Journal of Food Engineering, 80(1),* 111-118Heredia, A.,
410 Peinado, I., Barrera, C., & Andrés. A. (2009). Influence of process variables on
411 colour changes, carotenoids retention and cellular tissue alteration of cherry tomato
412 during osmotic dehydration. *Journal of Food Composition and Analysis, 22,* 285-
413 294.
414 Kazeniac, S.J., & Hall, R.M. (1970). Flavor chemistry of tomato volatiles. *Journal of*
415 *Food Science, 35*, 519-530.
416 Kader, A.A., Stevens, M.A., Albright-Holton, M., Morris, L.L., & Algazi, M., (1977).
417 Effect of fruit ripeness when picked on flavor and composition in fresh market
418 tomatoes. *Journal of American Society for Horticultural Science, 102,* 724–731.
419 Khraisheh, M.A.M., Cooper T.J.R., & Magee, T.R.A, (1997). Microwave and air drying
420 I. Fundamental considerations and assumptions for the simplified thermal
421 calculations of volumetric power absorption. *Journal of Food Engineering, 33(1),*
422 207–219.

423 Kondjoyan, N., & Berdague, J. L. (1996). A compilation of relative retention indices for
424 the analysis of aromatics compounds (1st ed.). France: Edition du Laboratoire
425 Flaveur.
426 Krumbein, A., & Auerswald, H. (2000). Aroma volatiles in tomato varieties-
427 instrumental, sniffing and quantitative descriptive analysis. In: Schieberle, P., Engel,
428 K.H. (Eds.), Frontiers of Flavour Science. Deutsche Forschungsanstalt für
429 Lebensmittelchemie, Garching, pp. 51–55.
430 Krumbein A., Peters P., & Brückner B. (2004). Flavour compounds and a quantitative
431 descriptive analysis of tomatoes (Lycopersicon esculentum Mill.) of different
432 cultivars in short-term storage. *Postharvest Biology and Technology, 32,* 15–28.
433 Lewicki, P. P., Vu Le, H. & Pomaranska-Lazuka, W. (2002). Effect of pre-treatment on
434 convective drying of tomatoes. *Journal of Food Engineering, 54,* 141–146.
435 Martens, H., & Næs, T. (1989). Multivariate calibration. Chichester, UK: Wiley.
436 Maskan, M. (2000). Microwave/air and microwave finish drying of banana. *Journal of*
437 *Food Engineering,* 44, 71–78.
438 Mayer, F.; Takeoka, G. R.; Buttery, R.G.; Whitehand, L.C.; Naim, M. & Rabinowitch,
439 H. D. (2008). Studies on the Aroma of Five Fresh Tomato Cultivars and the
440 Precursors of cis- and trans-4,5-Epoxy-(E)-2-Decenals and Methional. *Journal of*
441 *Agricultural and Food Chemistry, 56,* 3749–3757.
442 Mudgett, R.E. (1989). Microwave food processing. *Food Technology, 43,* 117–126
443 Muratore, G., Licciardello, F., & Maccarone, E. (2005). Evaluation of the chemical
444 quality of a new type of small-sized tomato cultivar, the plum tomato (Lycopersicon
445 lycopersicum). *Italian Journal of Food Science, 17(1),* 75–81.
446 Petro-Turza, M. (1987). Flavor of tomato and tomato products. *Food Review*
447 *International, 2,* 309–351.
448 Richard, H. & Multon, J.L. (1992). Les arômes alimentaires, TEC & DOC Lavoisier Ed.
449 Paris, France.
450 Servili, M., Selvaggini, R., Taticchi, A., Begliomini A.L., & Montedoro, G.F. (2000).
451 Relationships between the volatile compounds evaluated by solid phase
452 microextraction and the thermal treatment of tomato juice: optimization of the
453 blanching parameters. *Food Chemistry, 71,* 407-415.
454 Shi, J., Le Maguer, M., Kakuda, Y., & Lipaty, A. (1999). Lycopene degradation and
455 isomerization in tomato dehydration. *Food Research International, 32(1),* 15-21.

Soria, A. C., Martínez-Castro, I. & Sanz, I. (2008). Some aspects of dynamic headspace analysis of volatile components in honey. *Food Research International*, *41*, 838-848.

Tandon K.S., Baldwin E.A., & Shewfelt R.L. (2000). Aroma perception of individual volatile compounds in fresh tomatoes (Lycopersicon esculentum, Mill.) as affected by the medium of evaluation. *Postharvest biology and technology, 20*, 261–268.

Tatsuka, K.; Suekane, S.; Sakai, Y. & Sumitanis, H. (1990). Volatile Constituents of Kiwi fruit Flowers: Simultaneous Distillation and Extraction versus Headspace Sampling. *Journal of Agricultural and Food Chemistry*, *38*, 2176-2180.

Torres, J.D., Talens, P., Carot, J.M., Chiral, A., & Escriche. I. (2007). Volatile profile of mango (Mangifera indica L.), as affected by osmotic dehydration. *Food Chemistry*, *101*, 219-228.

Yilmaz, E. (2010). Oxylipin Pathway in the Biosynthesis of Fresh Tomato Volatiles. *Turk Journal of Biology*, *25*, 351-360.

Zanoni, B., Peri, C., Nani, R., & Lavelli, V. (1999). Oxidative heat damage of tomato halves as affected by drying. *Food Research International*, *31(5)*, 395–401.

Table captions:

Table 1. Physicochemical characteristics of samples after osmotic treatment and drying (under different temperature/ microwave power conditions) in terms of mass fraction of water (x^w (g w/g product)), soluble solids (x^{ss} (g ss/g product)), NaCl (x^{NaCl} (g NaCl/g product)) and water activity (a_w) (n=3).

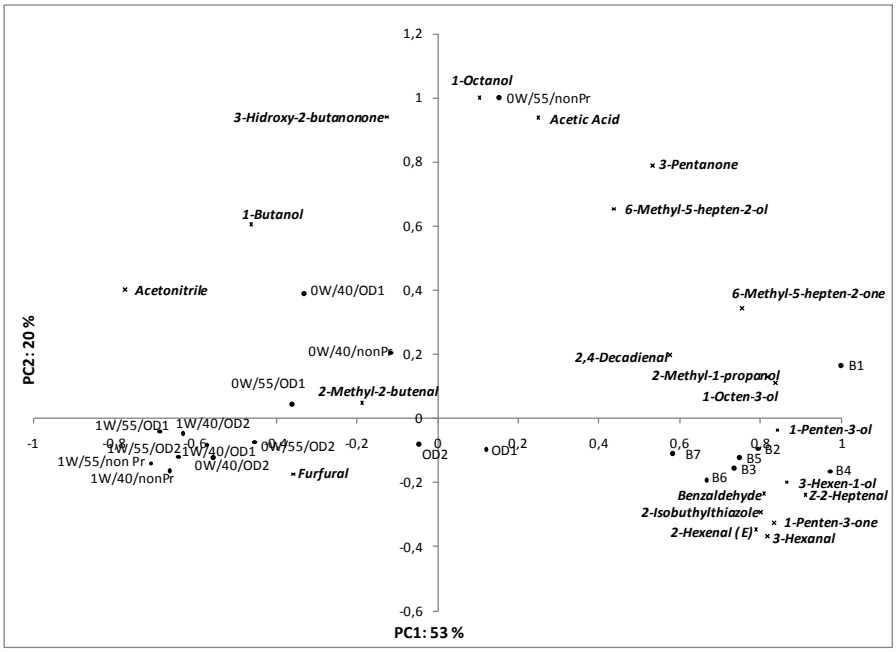
Table 2. Volatile compounds (μg/100g, expressed by using the amount of internal standard and the relative area between the peak areas of each compound and the peak area of the internal standard, assuming a response factor equal to one) in fresh cherry tomato batches (n=3).

Table 3. Changes of volatile compounds in dried cherry tomato under different process conditions expressed as concentration ratio (C/C_0) (n=3).

Table 4. Concentration (μg/100g, expressed using the amount of internal standard and the relative area between the peak areas of each compound and the peak area of the internal standard, assuming a response factor equal to one) of generated compounds during drying of cherry tomatoes under different process conditions (n=3).

Figure captions:

Figure 1. Biplot for dehydrated cherry tomatoes under different conditions (microwave power level/ drying temperature/osmotic pretreatment) and volatile compounds (PC1 and PC2) obtained by means of the PCA. Data from fresh batches (B1-B7) and osmodehydrated (OD1 and OD2) tomatoes have been also performed.



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510 **Table 1.** Physicochemical characteristics of samples after osmotic treatment and drying (under different
511 temperature/ microwave power conditions) in terms of mass fraction of water (x^w (g w/g product)),
512 soluble solids (x^{ss} (g ss/g product)), NaCl (x^{NaCl} (g NaCl/g product)) and water activity (a_w) (n=3).

OD	T (º C)	Power (W/g)	x^w	x^{ss}	x^{NaCl}	a_w
OD1	-	-	0.855(0.006)	0.129(0.002)	-	0.9810(0.0012)
OD2	-	-	0.842(0.002)	0.162(0.003)	0.0243(0.0012)	0.9650(0.0012)
nonPr			0.57(0.07)	0.295(0.017)	-	0.911(0.009)
OD1	**40**		0.620(0.008)	0.31(0.03)	-	0.926(0.013)
OD2		**0**	0.58 (0.02)	0.362 (0.004)	0.063(0.005)	0.87(0.06)
nonPr			0.67(0.07)	0.32(0.07)	-	0.941(0.006)
OD1	**55**		0.536(0.012)	0.458(0.007)	-	0.865(0.003)
OD2			0.50(0.03)	0.467(0.017)	0.0662(0.0115)	0.802(0.003)
nonPr			0.60 (0.02)	0.35(0.03)	-	0.919(0.012)
OD1	**40**		0.542(0.022)	0.433(0.005)	-	0.910(0.012)
DO2		**1**	0.55(0.03)	0.422(0.012)	0.06(0.01)	0.858(0.009)
nonPr			0.51(0.05)	0.30(0.02)	-	0.927(0.016)
OD1	**55**		0.58(0.04)	0.36(0.03)	-	0.92(0.07)
OD2			0.57(0.05)	0.374(0.005)	0.069 (0.003)	0.867(0.017)

513 Mean (standard deviation)
514 Osmotic pretreatment: nonPr (without osmotic pretreatment); OD1 (55 Brix, 30 ºC, 120 minutes); OD2 (27.5 % sucrose + 10 %
515 NaCl (w/w), 40 ºC, 60 minutes).
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520 **Table 2.** Volatile compounds (μg/100g, expressed by using the amount of internal standard and the
521 relative area between the peak areas of each compound and the peak area of the internal standard,
522 assuming a response factor equal to one) in fresh cherry tomatoes batches (n=3)

523

Volatile compounds	**KI cal**	**ID**	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Batch 7	Mean	***p-value***
Free acids											
Acetic acid	1519	MS;KI	2.3 (0.7)	0.54 (0.08)	0.8 (0.4)	0.2 (0.3)	1.4 (0.7)	0.9(0.5)	1.02 (0.14)	1.3 (0.7)	*
Alcohols											
1-Hexanol	1410	MS;KI	47 (10)	28 (4)	27 (5)	52 (3)	30 (1)	16 (4)	22 (2)	32 (13)	*
3-Hexen-1-ol	1447	MS;KI	50 (15)	39 (8)	29.9 (0.9)	36 (3)	33.9 (1.8)	18 (3)	32.24 (0.05)	34 (10)	*
2-Methyl-1-butanol	1232	MS;KI	40 (6)	46 (6)	56 (2)	69 (6)	66 (5)	78 (22)	49 (3)	58 (15)	*
6-Methyl-5-hepten-2-ol	1522	MS;KI	0.9 (0.3)	0.68 (0.19)	0.58 (0.03)	0.28 (0.04)	0.47 (0.04)	0.351 (0.015)	0.82 (0.02)	0.6 (0.2)	*
2-Methyl-1-propanol	1148	MS;KI	6.86 (0.15)	9.28 (0.02)	8.42 (0.02)	12.8 (1.4)	15 (3)	12.5 (1.6)	9.1 (1.4)	11 (3)	*
1-Octanol	1620	MS;KI	2.5 (0.3)	0.4 (0.2)	0.26 (0.02)	0.19 (0.04)	0.30 (0.02)	0.23 (0.03)	0.27 (0.03)	0.59 (0.08)	n.s.
1-Octen-3-ol	1507	MS;KI	1.2 (0.4)	0.8 (0.4)	0.71 (0.04)	0.85 (0.09)	0.925 (0.014)	0.777 (0.117)	0.90 (0.04)	0.9 (0.2)	n.s.
1-Pentanol	1253	MS;KI	19 (10)	14 (9)	13.8 (1.7)	14.4 (1.7)	13.9 (0.9)	7.414 (0.013)	10.8 (0.3)	13 (5)	n.s.
1-Penten-3-ol	1208	MS;KI	10 (3)	11 (4)	15 (3)	9.8 (0.4)	17 (8)	11.2 (0.5)	15.7 (1.3)	13 (4)	n.s.
Aldehydes											
Benzaldehyde	1613	MS;KI	2.6 (0.2)	2.5 (0.9)	2.43 (0.12)	2.07 (0.08)	1.96 (0.18)	1.5 (0.3)	1.94 (0.03)	2.1 (0.5)	n.s.
2,4-Decadienal	1846	MS;KI	0.82 (0.02)	0.69 (0.02)	1.0 (0.4)	1.7 (0.4)	0.94 (0.09)	0.47 (0.18)	0.47 (0.09)	0.9 (0.5)	*
Z-2-Heptenal	1297	MS;KI	7 (2)	7 (2)	5.9 (0.2)	8.0 (1.6)	5.52 (0.16)	7 (3)	4.7 (0.9)	6 (2)	n.s.
Z-3-Hexenal	1136	MS;KI	77 (20)	133 (22)	124 (23)	136 (39)	135 (23)	175 (27)	115 (10)	132 (31)	n.s.
E-2-Hexenal	1242	MS;KI	48 (24)	83 (23)	126 (12)	105 (21)	115 (7)	126 (6)	130 (9)	103 (32)	*
E-2-Octenal	1502	MS;KI	3.53	3.45	4.9	9.1	3.3	2.3 (0.8)	2.7	4 (2)	**

			(0.02)	(0.02)	(1.8)	(0.5)	(0.5)		(0.5)		
Ketones											
6-Methyl-5-hepten-2-one	1301	MS;KI	12 (4)	12.08 (1.18)	17 (2)	19 (4)	13.78 (1.03)	17 (7)	16 (2)	15 (4)	n.s.
3-Pentanone	1029	MS;KI	16.2 (0.5)	14 (3)	11.08 (0.57)	17 (3)	8.3 (0.2)	13 (5)	8.73 (1.09)	13 (4)	n.s.
1-Penten-3-one	1076	MS;KI	10 (2)	15 (4)	14 (2)	18 (4)	12.0 (1.3)	23 (12)	10.3 (1.3)	15 (6)	n.s.
Heterocyclic compounds											
2-Isobutylthiazole	1478	MS;KI	3.6 (0.6)	3.67 (0.04)	6.3 (0.7)	7.8 (0.9)	4.1 (0.3)	2.4 (0.8)	2.83 (0.18)	4 (1)	**

KI cal: Kovats retention indices calculated.**ID**: Method of identification, MS (comparison with mass spectrum stored in NIST library), KI (comparison of Kovats indices with the literature).Mean (standard deviation)n.s.: not significantly different.* *p-value < 0.05; **p-value < 0.001*

524

525 **Table 3**. Changes of volatile compounds in dried cherry tomato under different process conditions
526 expressed as concentration ratio (C/C_0) (n=3).

Volatile compounds	Concentration Ratio (C/C_0)							
	OD1	**OD2**	**0/40/nonPr**	**0/40/OD1**	**0/40/OD2**	**0/55/ nonPr**	**0/55/OD1**	**0/55/OD2**
Free acids								
Acetic acid	0.14 (0.02)	---	0.83 (0.16)	1.1 (0.5)	0.9 (0.3)	2.2 (1.8)	0.39 (0.09)	0.6 (0.3)
Alcohols								
1-Hexanol	0.348 (0.03)	0.19 (0.02)	0.38 (0.06)	0.21 (0.04)	0,017 (0.002)	0.49 (0.09)	0.101 (0.004)	0.064 (0.009)
3-Hexen-1-ol	0.41 (0.05)	0.19 (0.03)	0.0692 (0.0102)	0.032 (0.005)	---	0.0143 (0.0002)	0.0092 (0.0006)	0.016 (0.002)
2-Methyl-1-butanol	0.93 (0.12)	0.503 (0.009)	0.574 (0.019)	0.34 (0.04)	0.0418 (0.0012)	1.5 (0.7)	0.441 (0.004)	0.143 (0.005)
6-Methyl-5-hepten-2-ol	0.71 (0.04)	2.57 (0.15)	0.82 (0.09)	1.902 (0.009)	0.10 (0.14)	1.3 (0.3)	1.44 (0.09)	1.09 (0.03)
2-Methyl-1-propanol	0.542 (0.07)	0.54 (0.04)	0.15 (0.03)	0.120 (0.015)	0.011 (0.015)	1.7 (0.8)	0.1554 (0.0102)	0.103 (0.009)
1-Octanol	0.16 (0.06)	1.54 (0.02)	3.4 (0.2)	3.1 (0.3)	1.7 (0.6)	2.84 (1.07)	1.45 (0.16)	1.171 (0.112)
1-Octen-3-ol	0.60 (0.14)	1.76 (0.05)	1.05 (0.06)	0.4375 (0.0015)	0.48 (0.05)	0.58 (0.13)	0.71 (0.06)	0.849 (0.104)
1-Pentanol	0.62 (0.09)	1.22 (0.04)	1.19 (0.09)	0.845 (0.103)	0.25 (0.04)	1.28 (0.15)	0.54 (0.04)	0.51 (0.12)
1-Penten-3-ol	---	0.44 (0.07)	0.345 (0.009)	0.39 (0.04)	0.041 (0.007)	0.592 (0.007)	0.107 (0.003)	0.071 (0.008)
Aldehydes								
Benzaldehyde	0.40 (0.08)	0.92 (0.03)	0.747 (0.002)	0.35 (0.03)	0.48 (0.163)	0.22 (0.17)	0.49 (0.03)	0.69 (0.04)
2,4-Decadienal	0.5 (0.2)	4.4 (0.8)	2.44 (0.16)	0.92 (0.07)	0.97 (0.08)	0.19 (0.06)	1.34 (0.17)	1.2 (0.2)
Z-2-Heptenal	0.59 (0.06)	0.36 (0.02)	0.33 (0.03)	0.11 (0.03)	0.13 (0.03)	0.09 (0.09)	0.25 (0.03)	0.135 (0.006)
3-Hexanal	1.02 (0.02)	0.109 (0.015)	0.082 (0.018)	0.0419 (0.0004)	0.081 (0.004)	0.04 (0.02)	0.092 (0.003)	0.059 (0.002)
E-2-Hexenal	0.89 (0.09)	0.099 (0.002)	0.048 (0.007)	0.0176 (0.0016)	0.0043 (0.0002)	0.035 (0.012)	---	0.0061 (0.0012)
E-2-Octenal	0.34 (0.09)	0.75 (0.02)	1.128 (0.016)	0.18 (0.04)	0.537 (0.104)	0.075 (0.105)	1.01 (0.14)	0.80 (0.05)
Ketones								
6-Methyl-5-hepten-2-one	0.42 (0.09)	0.29 (0.02)	0.83 (0.04)	0.175 (0.019)	0,168 (0.017)	1.79 (0.03)	0.257 (0.018)	0.165 (0.002)
3-Pentanone	0.36	0.44	0.98 (0.07)	1.26 (0.15)	0.51 (0.03)	1.7 (0.6)	0.714	0.41 (0.03)

	(0.03)	(0.03)					(0.005)	
1-Penten-3-one	0.84 (0.02)	0.36 (0.02)	0.29 (0.13)	0.13 (0.08)	0.165 (0.007)	0.02 (0.03)	0.341 (0.004)	0.123 (0.002)
Heterocyclic compounds								
2-Isobutylthiazole	0.57 (0.2)	0.142 (0.004)	0.1091 (0.0015)	0.094 (0.013)	0.047 (0.007)	0.11 (0.03)	0.1618 (0.0103)	0.1284 (0.0102)

Process variables: microwave-power/air temperature/osmotic pretreatment.Osmotic pretreatment: nonPr: without osmotic pretreatment; OD1: 55 Brix, 30 ºC, 120 minutes; OD2: 27.5 % sucrose + 10 %NaCl (w/w), 40 ºC, 60 minutes.C: Concentration of the volatile compound in treated samples (μg/100g).C_0: Concentration of the volatile compound in fresh sample (μg/100g).

Table 4. Concentration (μg/100g, expressed by using the amount of internal standard and the relative area between the peak areas of each compound and the peak area of the internal standard, assuming a response factor equal to one) of generated compounds during drying of cherry tomato under different process conditions (n=3).

Volatile Compounds	**0/40/nonPr**	**0/40/OD1**	**0/40/OD2**	**0/55/nonPr**	**0/55/OD1**	**0/55/OD2**	**1/40/nonPr**
Alcohols							
1-Butanol	3.14 (0.02)	1.65 (0.04)	0.11 (0.16)	2.6 (0.3)	2.33 (0.06)	0.83 (0.02)	0.47 (0.03)
Aldehydes							
2-Methyl-2-butenal	3.4 (0.2)	0.65 (0.04)	0.06 (0.08)	---	0.45 (0.02)	0.53 (0.06)	---
Furanes							
Furfural	---	---	---	---	---	---	0.62 (0.06)
Ketones							
3-Hydroxy-2-butanone	6.1 (0.9)	14.1 (0.2)	0.98 (0.02)	16 (6)	0.766 (0.003)	0.73 (0.08)	---
Nitriles							
Acetonitrile	0.43 (0.12)	0.54 (0.08)	0.558 (0.015)	0.9 (0.8)	0.754 (0.108)	0.548 (0.102)	0.655 (0.114)

Process variables: microwave-power/air temperature/osmotic pretreatment.

Osmotic pretreatment: nonPr (without osmotic pretreatment); OD1 (55 Brix, 30 ºC, 120 minutes); OD2 (27.5 % sucrose + 10 % NaCl (w/w), 40 ºC, 60 minutes).