

Industrial scale microwave processing of tomato juice using a novel continuous microwave system

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1	Industrial Scale Microwave Processing of Tomato Juice using a novel Continuous
2	microwave system
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25 Abstract

This study evaluated the effect of an industrial scale continuous flow microwave volumetric 26 heating system in comparison to conventional commercial scale pasteurisation for the 27 processing of tomato juice in terms of physicochemical properties, microbial characteristics 28 and antioxidant capacity. The effect against oxidative stress in Caco-2 cells, after in vitro 29 digestion was also investigated. Physicochemical and colour characteristics of juices were 30 very similar between technologies and during storage. Both conventional and microwave 31 32 pasteurisation inactivated microorganisms and kept them in low levels throughout storage. ABTS.⁺ values, but not ORAC, were higher for the microwave pasteurised juice at day 0 33 however no significant differences between juices were observed during storage. Juice 34 processed with the microwave system showed an increased cytoprotective effect against H₂O₂ 35 induced oxidation in Caco-2 cells. Organoleptic analysis revealed that the two tomato juices 36 37 were very similar. The continuous microwave volumetric heating system appears to be a viable alternative to conventional pasteurisation. 38

39

40 Keywords

41 microwave, tomato juice, continuous, processing, antioxidant, in vitro digestion, Caco-2 cells

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47 **1. INTRODUCTION**

Tomato is one of the most popular and widely grown fruits in the world and a major 48 component of the Mediterranean diet. Tomato has high concentrations of compounds with 49 50 antioxidant potential such as vitamin C and carotenoids (Beecher, 1998). It is well accepted that the consumption of tomato and tomato products can result in the reduction of the risk of 51 chronic diseases such as cardiovascular disease and cancer (Willcox, Catignani & Lazarus 52 2003). Food antioxidants can scavenge the reactive oxygen species present in the human 53 body and thus lower the oxidative damage in tissues (Willcox et al. 2003; Cilla, Laparra, 54 Alegria, Barbera & Farre 2008). Therefore, ensuring the retention of high amounts of these 55 compounds after processing is important to maintain the health-giving properties of tomato 56 products. Thermal processing is the most commonly used method to inactivate 57 microorganisms and enzymes and prolong the shelf life of tomato juice. However, thermal 58 59 processing can adversely affect the organoleptic characteristics, the nutrient content and the antioxidant capacity of foods (Igual, García-Martínez, Camacho & Martínez-Navarrete 60 61 2011). Modern consumers demand products of high quality which are convenient, nutritious and minimally processed with fresh like characteristics (Hong & Wang 2014). Because of 62 these demands, the food industry is showing a greater interest in the adoption of novel food 63 processing technologies (Señorans, Ibáñez & Cifuentes, 2003). Food producers that want to 64 minimise thermal damage and thus maintain or increase nutrient content, can achieve this 65 mainly by improving the efficiency of heat delivery and temperature control. Contemporary 66 conventional heating systems aim to achieve this but heating based on convection and 67 68 conduction poses significant restrictions. Microwave heating is one of these novel thermal technologies that can be used as an alternative in order to achieve or possibly enhance tomato 69 70 juice shelf life, quality and nutrient content. The main feature of microwave heating is the unique ability to generate heat from within a food matrix which is not feasible by any other 71

72 conventional heating method (Fu 2004). In several cases, microwave processing has proven 73 to be not only much quicker, but also capable of better preserving quality and nutritional characteristics (e.g. vitamin retention) compared to conventional heating technologies 74 75 (Chandrasekaran, Ramanathan & Basak 2013). One of the most important concerns of microwave heating is the non-uniform temperature distribution which can have implications 76 in terms of safety as well as quality (Chandrasekaran et al. 2013). Volumetric and continuous 77 systems are quite new to the market and utilise a unique delivery method of microwave 78 energy to achieve a much greater penetration depth during processing (AMT, 2015). 79 80 Although they claim to offer a viable alternative by achieving heating uniformity, decreasing processing times and offering operational advantages to the processor, the exact effect on 81 82 product quality, safety and organoleptic properties has not been assessed properly in 83 comparison with existing practices.

The determination of the bioaccessibility of bioactive compounds appears to be a more relevant indicator of the nutritional value of foods compared to their concentration in the food matrix (Knockaert, De Roeck, Lemmens, Van Buggenhout, Hendrickx, Van Loey, 2011). Therefore, understanding how a novel processing technology affects the bioaccessibility of bioactives is important in assessing this technology and to that extend, no data exists in the literature to date.

In this study, we assessed the application of a novel continuous microwave volumetric heating (MVH) system to tomato juice, one of the most popular products that are processed worldwide, with conventional heating systems. The aim was to validate and compare the MVH system with conventional heat treatment with regards to operational characteristics, physicochemical, microbiological, nutritional and organoleptic characteristics both in situ and during storage.

97 2. MATERIALS AND METHODS

98 2.1. Sample preparation and preliminary trials

99 Fresh ripe tomatoes (*Dorothy* variety) were purchased from a local supplier (Down 100 Wholesale, U.K.). Tomatoes were washed, cut and pressed to obtain the juice using a packing 101 press (100 P2 Voran Maschinen GmbH, Austria) industrial equipment. Preliminary trials 102 were conducted in order to identify the appropriate pasteurisation conditions. The processing 103 conditions chosen (see 2.2) were able to reduce the total viable counts (TVC) below the 104 detection limit. All juice samples were stored at 4°C for a period of 56 days and analysed on 105 day 0, 7, 14, 28 and 56.

106

107 2.2. Conventional and novel processing of tomato juice

108 Conventional batch pasteurisation of tomato juice (CP) was performed with an industrial 109 steam jacket kettle (Culino kettle, Hackman, Finland). The kettle was filled with 30 L of raw 110 tomato juice and processed at a target temperature of 85°C for 5 min, under a turbulent flow 111 pattern with an overall processing time (including come-up time) of 20 min. An emptying 112 valve was used to collect samples which were immediately cooled down in ice. The product 113 temperature was registered using a thermocouple connected to a data logger.

114 Microwave volumetric heating (MVH) of tomato juice was performed with an industrial 115 continuous microwave system supplied by Advanced Microwave Technologies (AMT, 116 Edinburgh, UK). The system comprises of a process tank, pump, pressure and temperature 117 sensors, flow meter, rotation device and the MVH unit. The microwaves were produced by 118 six magnetrons (6 x 3 kW, total input = 18kW; 2450 MHz) placed in either side of the 119 microwave transparent processing tube which operated at $85 \pm 0.4^{\circ}$ C (Fig. 1). The feed pump 120 supplied the system with a flow of 100 L of tomato juice per hour. The overall residence time of the juice inside the processing tube was 81.8 ± 1.1 sec. The temperature was automatically recorded before and immediately after treatment, as soon as the product left the processing tube. The pasteurised samples were collected in sterile containers and cooled down in iced water.

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126 **2.3. Physicochemical analysis**

Moisture of the tomato juices was determined gravimetrically. Total soluble solids (°Brix) 127 were measured using a refractometer (Eclipse, Bellingham + Stanley Ltd, UK). 128 Measurements were performed at a stable temperature (20°C). Titratable acidity was 129 130 measured according to Adekunte et al. (2010). Results were reported in g citric acid/100 g sample. The pH of tomato juice samples was measured using a digital pH metre (Jenway 131 3510, U.K.). Serum cloudiness was evaluated according to Silva, Sato, Barbosa, Dacanal, 132 Ciro-Velásquez, & Cunha (2010). Briefly, the sample is centrifuged and the optical density of 133 the supernatant is determined at 660 nm. Colour measurements were performed with the use 134 of a reflectance colorimeter (Minolta Chroma 173 Meter CR-410, Konica-Minolta, Basildon, 135 U.K.) equipped with a CIE 1931 standard observer and D65 Illuminant. The juice was placed 136 in glass cell made of optical glass with a 60 mm diameter and 38 mm depth. The CIELab 137 system L*, a* and b* was followed. The chroma (C) parameter was also determined, 138 $C = (a^{*2} + b^{*2})^{1/2}$. 139

140

141 **2.4. Light microscopy**

The microstructure of the tomato juices after processing was assessed using a CX41 light microscope (Olympus, U.K.). The samples were stained with toluidine blue and observed on a glass slide and evaluated using different magnifications. Representative images were taken with a digital video camera (JVC TK C1480BE).

146 **2.5. ABTS and ORAC antioxidant capacity assays**

Tomato juice extract was obtained by vortexing 0.5 g freeze dried tomato juice in 10 ml 80% 147 ethanol at 2500 rpm for 20 min and centrifuged for 10 min at $2500 \times g$, prior to analysis. The 148 ABTS radical-scavenging assay is based on the discolouration of the radical cation 3-ethyl-149 benzothiazoline-6-sulfonic acid (ABTS+; Sigma, UK.). The procedure was performed 150 according to Miller et al. (1993) as improved by Re, Pellegrini, Proteggente, Pannala, Yang, 151 & Rice-Evans (1999). Absorbance was measured at 734 nm after 10 min incubation. The 152 results were expressed as µmol Trolox equivalents per g of dried weight using an appropriate 153 calibration curve. The oxygen radical absorbance capacity (ORAC) assay was performed 154 according to Huang et al. (2005) with some modifications. Fluorescence of the samples was 155 recorded for 100 min at 2 min intervals using a plate reader (Tecan, Safire 2190, UK). 156 Excitation wavelength was set at 485 nm and emission wavelength at 530 nm. ORAC values 157 158 were calculated using the areas under the fluorescein decay curves (AUC), between the blank and the sample. Results were expressed as µM Trolox equivalents (TE) per g of dried weight. 159

160

161 **2.6. Microbiological analysis**

At each sampling interval, juice samples were opened aseptically and a suitable dilution 162 series was prepared in maximum recovery diluent (MRD) (Oxoid code CM733, Oxoid, 163 Basingstoke, UK) and the appropriate dilutions were prepared. Total viable counts (TVC) 164 were enumerated by spread plating onto plate count agar (PCA) (Oxoid, Basingstoke, UK), 165 after aerobic incubation at 30 °C for 48 h. Lactic acid bacteria were enumerated on de Man 166 Rogosa and Sharp agar (MRS) (Oxoid, Basingstoke, UK) by pour plating and incubating at 167 30°C for 72 hours. Enterobacteriaceae were enumerated onto Violet Red Bile Glucose Agar 168 (VRBGA) (Oxoid, Basingstoke, UK) by pour plating and incubating at 37 °C for 72 hours. 169 Yeasts and moulds were enumerated on Rose-Bengal Chloramphenicol agar (Oxoid, 170

Basingstoke, UK) with incubation at 25°C for 72 and 120 hours. Each sample was plated in
duplicate and the results (the mean of the two plates) were expressed as log₁₀CFU/ml of juice.

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174 2.7. In vitro digestion model

In order to investigate the cytoprotective effect against H₂O₂-induced oxidative stress of the 175 bioaccessible fractions of the two types of juices on Caco-2 cells, tomato juice samples after 176 conventional and microwave pasteurisation (day 0) were subjected to a simulated in vitro 177 digestion coupled with Caco-2 cells. Juice samples were weighed in amber glass tubes and 178 179 subjected to a simulated human gastric and small intestinal digestion based on the method described by Hedrén et al. (2002) and Colle, Van Buggenhout, Van Loey & Hendrickx, 180 (2010) with modifications, in order to obtain the bioaccessible fraction of the tomato juices. 181 182 All steps were carried out under dimmed light. The digests were centrifuged at $5000 \times \text{g}$ for 60 min at 4°C to separate the soluble juice fraction, followed by filtration using 0.22 µm 183 membrane filters (Millipore, UK). Samples were stored in amber tubes at -80°C under 184 nitrogen until further analysis. In order to ensure the inactivation of enzymes, all digests were 185 heated in a water bath for 4 min at 100°C and then cooled before they were used for 186 incubation with the Caco-2 cells (Cilla et al. 2008). 187

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189 **2.8. Caco-2 cells culture**

Human intestinal Caco-2 cells (American Type Culture Collection (ATCC) were cultured in medium comprising Minimum Essential Medium (MEM; Life Technologies, U.K.). Cultures were maintained according to Cilla et al. (2008). For the assays, Caco-2 cells were seeded onto 24-well plates, at a density of 1×10^5 cells with 1 ml of MEM and the culture medium was changed every three days. Twenty one days after confluency, the culture medium was removed from the wells and the cell monolayers were washed with phosphate buffered saline heated to 37°C. The cells were pre-incubated (37°C/5% CO₂/95% RH) for 24 h with the bioaccessible fractions of the tomato juice samples, with a ratio of fraction to culture media of 1:1 (v/v) in order to preserve cell viability. Afterwards, the MEM was removed and the cells were washed with PBS. The induction of oxidative stress was carried out by exposure to a 5 mM H₂O₂ solution in MEM for 1 h (37°C/5% CO₂/95% RH).

201

202 **2.9. Cell viability assay**

The alamarBlue assay was used to determine cell viability of Caco-2 cells after pre-203 incubation with bioaccessible fractions of the tomato juices and also to establish the relative 204 cytotoxicity of different concentration of H₂O₂ on Caco-2 cells. Briefly, the medium in the 205 24-well plates was replaced with a 10% v/v alamarBlue® in media solution. 100 µL of the 206 medium was added to 4 wells of the 96-well plate for control measurement. 100µL of 207 208 alamarBlue® was added to every well of the 24-well plate. Both the 24 and 96-well plates were incubated at 37°C/5% CO₂ for 4 h. 100µL from each 24-well plate were transferred into 209 210 the 96 well plate. Absorption was measured at 570 and 600 nm using an automatic plate 211 reader (Tecan, Sufire², Reading, UK). Results were calculated according to the manufacturer's manual. 212

213

214 **2.10. Organoleptic analysis**

A hedonic test was conducted with 28 assessors in individual booths, aged between 21 and 60, who scored the acceptability of various tomato juice attributes using the following scoring system: 1 - dislike extremely to 9 - like extremely. Each assessor was asked the score the following attributes for each sample: sweetness, odour, flavour, acidity, appearance and overall acceptability. Prior to organoleptic panelling, all samples were tested for microbiological safety. Samples were served in transparent plastic glass, coded with three
digit random numbers. Organoleptic analysis took place in the sensory suite at College of
Agriculture Food and Rural Enterprise.

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224 **2.11. Data analysis**

The experiment was performed in two different occasions in order to obtain two independent trials. Differences between treatments were assessed with two way analysis of variance (ANOVA) followed by Tukey's post hoc test. One way analysis of variance was used to determine between treatments for the organoleptic analysis and alamarBlue assay. A significance level of p < 0.05 was used for comparisons between treatments and storage time.

230

231 **3. RESULTS AND DISCUSSION**

3.1. Characterisation of treated tomato juice after processing and during storage

233 The moisture content immediately after processing for the CP and MVH pasteurised juice was 96.10 \pm 0.20 % and 96.47 \pm 0.10 %, respectively. The soluble solids content was 2.25 234 °Brix for both juices and this remained stable during storage. There was no significant 235 difference in titratable acidity (0.35-0.44 g citric acid/100g) or pH values (4.20-4.26) between 236 the two processing technologies and storage time had no significant effect on these 237 parameters. Limited or no effects on pH and soluble solid values has also been reported in 238 similar studies with orange juice processed with high intensity pulsed electric fields and 239 conventional thermal treatments (Yeom, Streaker, Zhang & Min, 2000; Elez-Martinez, 240 Soliva-Fortuny, and Martin-Belloso, 2006). Both redness (a*; 1.96 ± 1.07 and 1.48 ± 0.30 241 for the CP and MVH pasteurised tomato juice, respectively) and chroma values (C; 7.44 \pm 242

2.24 and 7.31 \pm 0.48 for the CP and MVH pasteurised tomato juice, respectively) of the 243 tomato juices after processing (day 0) were quite low compared to commercial 244 conventionally pasteurised tomato juice (Sánchez-Moreno, Plaza, de Ancos and Cano 2006) 245 which is attributed to the specific tomato variety that was used to prepare the juice in this 246 study. All colour parameters studied did not differ significantly between the two processing 247 technologies (p > 0.05) and during storage. Cloudiness of the two types of juices was also 248 evaluated during storage (Fig. 2). Fruit juices are comprised of the pulp (insoluble phase) 249 dispersed in a viscous solution (i.e. the serum). Cloudiness is related to the suspension of 250 251 particles in the serum which are comprised of proteins, pectin, lipids, hemicellulose, cellulose and other minor components (Chou & Kokini, 1987). Cloudiness was found to be 252 253 significantly higher for MVH tomato juice (p < 0.05) (Fig. 2). Smaller suspended particles in 254 the serum of the CP juice allow more light to pass through, which results to lower absorbance values and cloudiness (Kubo, Augusto, Cristianini 2013). Cloudiness was gradually reduced 255 for both juices during storage until day 14. Subsequently, cloudiness was stabilised for MVH 256 257 juice and decreased further for the CP juice until day 28. The progressive reduction in cloudiness during storage was probably due to the precipitation of larger size pulp particles as 258 well as polymerisation of phenolic compounds and proteins (Cao, Bi, Huang, Wu, Hu & Liao 259 2012). The difference in the stabilisation and cloudiness values observed may indicate 260 differences in the microstructure of the two juices. Figure 3 illustrates the microstructures of 261 262 tomato juice by means of optical microscopy. Images of non-treated tomato juice presented intact cells containing carotenoid crystals within them. The images of CP and MVH 263 pasteurised samples presented broken cells with internal components within the broken cells 264 265 and also outside suspended on the juice serum. In general, a higher number of broken cells were observed in MVH samples which means more antioxidant compounds could be released 266 and are available for absorption. 267

268 **3.2. Radical scavenging capacity of tomato juice during storage**

The total antioxidant capacity of CP and MVH pasteurised tomato juice was determined by 269 means of the ABTS and ORAC assays (Table 1). The ABTS value for the MVH juice was 270 significantly higher compared to the CP one at day 0 of storage (p < 0.05). ORAC values 271 showed no statistically significant differences between the two processing technologies at day 272 0. An increased retention of antioxidant capacity during microwave processing has been 273 shown in other studies. The work of Kaur, Khurdiya, Pal & Kapoor, (1999) has shown that 274 microwave processed tomato juice had a higher retention of ascorbic acid, total carotenoids 275 and lycopene contents compared to conventionally processed juice. Igual, García-Martínez, 276 Camacho & Martínez-Navarrete, (2010) have also found a higher retention of ascorbic acid in 277 grapefruit juice pasteurised with the use of microwaves compared to a conventional heat 278 pasteurisation. However, microwave and conventional pasteurisation caused a similar 279 280 decrease of the total phenol content and DPPH values. Microwave processing of kiwifruit puree has also been found to result in significantly higher antioxidant activity compared to 281 282 conventional heat treatment (Benlloch-Tinoco, Igual, Salvador, Rodrigo & Martínez-Navarrete 2014). In this study, differences between ABTS and ORAC results were expected 283 because of the different nature of the two methods. ABTS is an electron transfer method 284 which measures the capacity of an antioxidant to reduce an oxidant, whereas ORAC is based 285 on hydrogen atom transfer in which antioxidant and substrate compete for thermally 286 generated peroxyl radicals. The higher antioxidant capacity observed here determined with 287 ORAC versus ABTS has also been found in other studies (Zulueta Esteve and Frígola 2009). 288 The total antioxidant capacity of the juices showed fluctuations throughout the entire period 289 of storage (Table 1). It is noteworthy that these fluctuations in both types of tomato juice 290 were quite similar. Both ABTS and ORAC values showed a significant increase in 291 antioxidant capacity at the end of the storage period for CP but not for MVH juice. These 292

differences are not usual. It has been shown that flavonoids, vitamins and total phenol
content, responsible for total antioxidant capacity can undergo fluctuations in fruit juices
during cold storage (Del Caro, Piga, Vacca, Agabbio, 2004; Klimczak, Malecka, Szlachta &
Gliszczynska-Swiglo, 2007).

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3.3. Effect of processing on the microbiological characteristics during storage

The effect of both types of processing on total viable counts (TVC), lactic acid bacteria 299 (LAB), Enterobacteriaceae and yeasts and moulds counts of tomato juice during storage at 300 301 4°C for 56 days, was investigated. Immediately after processing (day 0 of storage) both types of tomato juice had counts below the limit of detection for all the microorganisms tested. 302 303 Throughout storage, LAB, Enterobacteriaceae and yeasts and moulds counts remained below 304 the detection limit for both juices. Only TVC counts were detected on day 28 (2.13 \pm 0.33 and $2.00 \pm 0.33 \log$ CFU/ml for CP and MVH juices, respectively) which remained stable 305 until day 56 (2.16 \pm 0.25 and 2.05 \pm 0.12 log CFU/ml for CP and MVH juices, respectively), 306 307 with no significant differences between storage days (p > 0.05) or between processing technologies (p > 0.05). The results from the present study are in accordance with the results 308 of Hsu, Tan and Chi (2008) that showed LAB, Enterobacteriaceae and yeasts and moulds 309 counts remained below the detection limit in thermally pasteurised tomato juice for at least 28 310 days of refrigerated storage. The low microbial counts during storage are consistent with the 311 312 stable pH values observed for both types of tomato juice since a reduction in pH may be attributable to organic acid production as a result of microbial growth. Even though the 313 heating mechanism of the two technologies is different the results reveal a very similar effect 314 315 on the microbial stability during storage. Microwave volumetric heating appears to be equally as effective for microbial inactivation and the prolongation of the shelf life of tomato juice, as 316 317 the conventional technology.

318 **3.4.** Protective effect against induced oxidation after *in vitro* digestion

319 During digestion, antioxidant and other functional constituents, present in the food being digested, could be released and metabolised or remain within the food. Therefore, it is 320 321 important to quantify the fraction of the ingested antioxidants which are available for use by the body (Wootton-Beard, Moran & Ryan 2011). This is referred to as bioaccessibility and 322 represents the quantity of nutrients which are released from the food matrix and are 323 accessible for transport into the mucosa (Hedrén et al. 2002). Recently, several studies have 324 used in vitro digestion models to determine the bioaccessibility of several nutrients such as 325 lycopene (Colle et al. 2010), and β -carotene (Knockaert et al. 2011) after processing with 326 novel or conventional technologies. These in vitro models are usually coupled with 327 chromatographic or spectrophotometric methods. In this study we evaluated the antioxidant 328 effect of the two juices by combining an in vitro digestion model with an intestinal epithelia 329 330 model (i.e. Caco-2 cells) in order to offer a more realistic view on what is occurring during digestion. To the best of our knowledge this is the first study that determined the effect of 331 332 commercial scale processing technologies using an in vitro digestion/Caco-2 cells model. Figure 4 illustrates the effect that CP and MVH pasteurised tomato juice had against H₂O₂-333 induced oxidative stress in Caco-2 cells, after in vitro digestion. Incubation of Caco-2 cells 334 with a 5 mM solution of H_2O_2 resulted in a significant reduction in Caco-2 viability (79.77 \pm 335 1.67 % compared to the control) which is consistent with the study of Cilla et al. (2008) who 336 found a similar effect of H₂O₂ on Caco-2 cells. After H₂O₂ diffuses to mitochondria, it has 337 been found to cause a loss of mitochondrial integrity and function and ultimately cell death 338 (Mronga, Stahnke, Goldbaum, & Richter-Landsberg, 2004). In this study, for the Caco-2 cell 339 cultures that were pre-incubated with bioaccessible fractions of CP and MVH tomato juices 340 the AlamarBlue assay showed increased cell viability for both types of processed juices. 341 Tomato is considered a rich source of several antioxidants, such as ascorbic acid, vitamin E, 342

343 carotenoids, flavonoids and phenolic acids (George, Kaur, Khurdiya, & Kapoor, 2004). Thus, it appears that the antioxidants present in the bioaccessible fractions of the tomato juices 344 where able to partially prevent the cytotoxic effect induced by H_2O_2 on the Caco-2 cells. 345 346 Laparra, Alegría, Barberá & Farré (2008) reported that the antioxidant compounds present in fruit beverages consisting of grape, orange and apricot concentrates, after in vitro digestion, 347 reduced the cytotoxic effect of H₂O₂ induced oxidative stress on Caco-2 cell viability, as 348 determined by the MTT (3-[4,5-dimethylthiazol-2-yl]-2,3-diphenyl tetrazolium bromide) 349 assay. Bellion, Digles, Will, Dietrich, Baum, Eisenbrand & Janzowski (2010) found that 350 351 extracts from apple juice, apple pomace extraction juice and apple peel were able to significantly reduce DNA damage induced in Caco-2 cells with apple peel extract being the 352 most effective. Although, in this study both juices exerted a cytoprotective effect against 353 354 H₂O₂ induced oxidation, the MVH juice showed a significantly higher protective effect compared to the conventional one (p < 0.05) which may indicate a higher antioxidant 355 capacity of the microwave processed juice. The protective effects of tomato products might 356 357 be derived from the antioxidant components that can prevent cell damage by means of synergistic interactions (Friedman, 2002; George et al., 2004). Although the higher 358 cytoprotective effect observed for the MVH juice might be explained by the higher amounts 359 of antioxidant in the bioaccessible fractions, it could also be due to other reasons. Recently, 360 the relationship between food microstructure and the food's nutritional value has been 361 362 highlighted. The study of Lemmens, Van Buggenhout, Oey, Van Loey, & Hendrickx, (2009) showed that the microstructure characteristics of carrot tissue affect hardness which was 363 found to be negatively correlated to β -carotene in vitro bioaccessibility. Colle et al. (2010) 364 365 found that for high pressure homogenisation increasing the pressure levels resulted in the formation of a stronger fibre network in tomato pulp which leads to the decrease of lycopene 366 in vitro bioaccessibility by making it less approachable to digestive enzymes and bile salts. 367

368 Therefore, the increased protective effect observed in this study for the MVH tomato juice might also be derived by the increased bioaccessibility of certain nutrients present in the 369 juice. The differences in cloudiness levels between the two tomato juices might be an 370 371 indicator of their different microstructure however more in depth analysis is needed to conclusively state this. In this regard, parameters such as the temperature kinetics of the heat 372 treatment play an important role in lycopene bioaccessibility as rapid heating of tomato puree 373 374 (with the use of a microwave oven) can lead to higher bioaccessibility compared to a slow temperature increase (Page, Van Stratum, Degrou & Renard, 2012). A comparison to a 375 376 conventional continuous flow system will give further evidence on the effect of processing on antioxidant bioaccessibility. 377

378

379 **3.5. Organoleptic analysis**

380 Since the food industry is showing interest in the adoption of novel processing technologies in order to meet the needs of consumers investigating the impact that these technologies have 381 382 on the acceptability of processed products is essential. The organoleptic analysis results of the pasteurised juices are presented samples in Fig. 4. In general, both the CP and MVH tomato 383 juices had similar scores. The results of the analysis showed that no differences between the 384 two juices could be distinguished by the organoleptic panel for the odour, acidity, flavour and 385 sweetness attributes. A statistically significant difference was observed for the appearance 386 attribute with the CP juice scoring slightly higher. However, the overall acceptability (p > p)387 0.05) did not differ significantly between the two types of juice. The lower scores for 388 appearance of MVH juice could be explained by the higher cloudiness values observed (Fig. 389 2). Similar results were found by Valero et al. (2000) who stated that there were no 390 391 perceivable differences in organoleptic characteristics between microwave and conventionally processed milk in a heat exchanger both after processing and during storage. 392

393 It has also been reported that microwave processing can result in improved organoleptic characteristics. The study of Benlloch-Tinoco et al. (2014) demonstrated that based on all the 394 organoleptic characteristics tested, panellists showed a clear preference to the microwave 395 396 processed kiwifruit puree compared to conventional heat treated one in a batch retort. In the present study, given that there was no difference in the overall acceptability in almost all 397 attributes evaluated, it is concluded that the continuous microwave processing is a promising 398 399 and viable alternative to conventional pasteurisation. More work comparing the MVH system to an industrial scale conventional continuous flow pasteuriser will provide more information 400 401 on the potential advantages of this novel technology.

402

403 **4. CONCLUSIONS**

Tomato juice pasteurisation with the novel industrial scale continuous microwave system had 404 very similar physicochemical and microbial characteristics compared with the conventional 405 406 pasteurisation, during refrigerated storage. The antioxidant capacity measured with the ABTS 407 assay, but not with ORAC, immediately after treatment was higher for the MVH juice compared to the CP one. However, antioxidant capacity of the juices during storage was very 408 409 similar. Moreover, bioaccessible fractions of the MVH juice were able to provide a significantly higher protective effect against H₂O₂ induced oxidation in Caco-2 cells. The 410 organoleptic trial showed no significant differences between the two juices for almost all 411 attributes evaluated. Microwave processing with the use of this novel continuous microwave 412 volumetric heating system appears to a viable alternative for tomato juice pasteurisation since 413 414 it can produce a physicochemically and microbiologically stable product with higher antioxidant capacity, in significantly reduced processing time. The application of new 415 generation microwave technologies in food processing has not reached its full potential so 416 417 far, however, it shows promise in delivering a range of products and ensuring microbiological

safety without compromising quality. Given that industrial scale equipment was used, theresults from this study should facilitate the adoption of this technology by the industry.

420

- 421 Conflict of interest statement
- 422 The authors declare no conflict of interest

423

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FIGURE LEGENDS

Figure 1. Schematic representation of the continuous flow microwave system.

Figure 2. Cloudiness values of conventionally and microwave pasteurised tomato juice 443 during storage at 4°C. Results are expressed as means \pm SD (n = 4).

Figure 3. Typical light microscopic pictures (x10) of conventionally and microwave pasteurised tomato juice after processing - day 0 (CP = conventional, MVH = Microwave volumetric heating).

Figure 4. Caco-2 cell cultures pre-incubated for 24 h with bioaccessible fractions of 450 conventional and microwave pasteurised tomato juice and exposed to 5 mM H₂O₂. Results 451 are expressed as means \pm SD (n = 6) of the control (100%). Different lower case letters 452 denote statistically significant differences (p < 0.05).

455 Figure 5. Organoleptic comparison of conventionally and microwave pasteurised tomato
456 juice (after processing - day 0)

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