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

3

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25 **Abstract**

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

39

40 **Keywords**

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

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47 **1. INTRODUCTION**

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

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

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

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

96

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 $85 \pm 0.4^\circ\text{C}$ (Fig. 1). The feed pump
120 supplied the system with a flow of 100 L of tomato juice per hour. The overall residence time

121 of the juice inside the processing tube was 81.8 ± 1.1 sec. The temperature was automatically
122 recorded before and immediately after treatment, as soon as the product left the processing
123 tube. The pasteurised samples were collected in sterile containers and cooled down in iced
124 water.

125

126 **2.3. Physicochemical analysis**

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

140

141 **2.4. Light microscopy**

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

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

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

160

161 **2.6. Microbiological analysis**

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

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

173

174 **2.7. In vitro digestion model**

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

188

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

190 Human intestinal Caco-2 cells (American Type Culture Collection (ATCC) were cultured in
191 medium comprising Minimum Essential Medium (MEM; Life Technologies, U.K.). Cultures
192 were maintained according to Cilla et al. (2008). For the assays, Caco-2 cells were seeded
193 onto 24-well plates, at a density of 1×10⁵ cells with 1 ml of MEM and the culture medium
194 was changed every three days. Twenty one days after confluency, the culture medium was
195 removed from the wells and the cell monolayers were washed with phosphate buffered saline

196 heated to 37°C. The cells were pre-incubated (37°C/5% CO₂/95% RH) for 24 h with the
197 bioaccessible fractions of the tomato juice samples, with a ratio of fraction to culture media
198 of 1:1 (v/v) in order to preserve cell viability. Afterwards, the MEM was removed and the
199 cells were washed with PBS. The induction of oxidative stress was carried out by exposure to
200 a 5 mM H₂O₂ solution in MEM for 1 h (37°C/5% CO₂/95% RH).

201

202 **2.9. Cell viability assay**

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

213

214 **2.10. Organoleptic analysis**

215 A hedonic test was conducted with 28 assessors in individual booths, aged between 21 and
216 60, who scored the acceptability of various tomato juice attributes using the following scoring
217 system: 1 - dislike extremely to 9 - like extremely. Each assessor was asked the score the
218 following attributes for each sample: sweetness, odour, flavour, acidity, appearance and
219 overall acceptability. Prior to organoleptic panelling, all samples were tested for

220 microbiological safety. Samples were served in transparent plastic glass, coded with three
221 digit random numbers. Organoleptic analysis took place in the sensory suite at College of
222 Agriculture Food and Rural Enterprise.

223

224 **2.11. Data analysis**

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

230

231 **3. RESULTS AND DISCUSSION**

232 **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
234 was 96.10 ± 0.20 % and 96.47 ± 0.10 %, respectively. The soluble solids content was 2.25
235 °Brix for both juices and this remained stable during storage. There was no significant
236 difference in titratable acidity (0.35-0.44 g citric acid/100g) or pH values (4.20-4.26) between
237 the two processing technologies and storage time had no significant effect on these
238 parameters. Limited or no effects on pH and soluble solid values has also been reported in
239 similar studies with orange juice processed with high intensity pulsed electric fields and
240 conventional thermal treatments (Yeom, Streaker, Zhang & Min, 2000; Elez-Martinez,
241 Soliva-Fortuny, and Martin-Belloso, 2006). Both redness (a^* ; 1.96 ± 1.07 and 1.48 ± 0.30
242 for the CP and MVH pasteurised tomato juice, respectively) and chroma values (C; $7.44 \pm$

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

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

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

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

297

298 **3.3. Effect of processing on the microbiological characteristics during storage**

299 The effect of both types of processing on total viable counts (TVC), lactic acid bacteria
300 (LAB), Enterobacteriaceae and yeasts and moulds counts of tomato juice during storage at
301 4°C for 56 days, was investigated. Immediately after processing (day 0 of storage) both types
302 of tomato juice had counts below the limit of detection for all the microorganisms tested.
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 ± 0.33
305 and 2.00 ± 0.33 log CFU/ml for CP and MVH juices, respectively) which remained stable
306 until day 56 (2.16 ± 0.25 and 2.05 ± 0.12 log CFU/ml for CP and MVH juices, respectively),
307 with no significant differences between storage days ($p > 0.05$) or between processing
308 technologies ($p > 0.05$). The results from the present study are in accordance with the results
309 of Hsu, Tan and Chi (2008) that showed LAB, Enterobacteriaceae and yeasts and moulds
310 counts remained below the detection limit in thermally pasteurised tomato juice for at least 28
311 days of refrigerated storage. The low microbial counts during storage are consistent with the
312 stable pH values observed for both types of tomato juice since a reduction in pH may be
313 attributable to organic acid production as a result of microbial growth. Even though the
314 heating mechanism of the two technologies is different the results reveal a very similar effect
315 on the microbial stability during storage. Microwave volumetric heating appears to be equally
316 as effective for microbial inactivation and the prolongation of the shelf life of tomato juice, as
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
320 digested, could be released and metabolised or remain within the food. Therefore, it is
321 important to quantify the fraction of the ingested antioxidants which are available for use by
322 the body (Wootton-Beard, Moran & Ryan 2011). This is referred to as bioaccessibility and
323 represents the quantity of nutrients which are released from the food matrix and are
324 accessible for transport into the mucosa (Hedrén et al. 2002). Recently, several studies have
325 used *in vitro* digestion models to determine the bioaccessibility of several nutrients such as
326 lycopene (Colle et al. 2010), and β -carotene (Knockaert et al. 2011) after processing with
327 novel or conventional technologies. These *in vitro* models are usually coupled with
328 chromatographic or spectrophotometric methods. In this study we evaluated the antioxidant
329 effect of the two juices by combining an *in vitro* digestion model with an intestinal epithelia
330 model (i.e. Caco-2 cells) in order to offer a more realistic view on what is occurring during
331 digestion. To the best of our knowledge this is the first study that determined the effect of
332 commercial scale processing technologies using an *in vitro* digestion/Caco-2 cells model.
333 Figure 4 illustrates the effect that CP and MVH pasteurised tomato juice had against H₂O₂-
334 induced oxidative stress in Caco-2 cells, after *in vitro* digestion. Incubation of Caco-2 cells
335 with a 5 mM solution of H₂O₂ resulted in a significant reduction in Caco-2 viability ($79.77 \pm$
336 1.67 % compared to the control) which is consistent with the study of Cilla et al. (2008) who
337 found a similar effect of H₂O₂ on Caco-2 cells. After H₂O₂ diffuses to mitochondria, it has
338 been found to cause a loss of mitochondrial integrity and function and ultimately cell death
339 (Mronga, Stahnke, Goldbaum, & Richter-Landsberg, 2004). In this study, for the Caco-2 cell
340 cultures that were pre-incubated with bioaccessible fractions of CP and MVH tomato juices
341 the AlamarBlue assay showed increased cell viability for both types of processed juices.
342 Tomato is considered a rich source of several antioxidants, such as ascorbic acid, vitamin E,

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

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

378

379 **3.5. Organoleptic analysis**

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

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

402

403 **4. CONCLUSIONS**

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

418 safety without compromising quality. Given that industrial scale equipment was used, the
419 results 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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438 **FIGURE LEGENDS**

439

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

441

442 **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).

444

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

448

449 **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).

453

454

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

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458

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