

Alma Mater Studiorum Università di Bologna
Archivio istituzionale della ricerca

Combined use of natural antimicrobial based nanoemulsions and ultra high pressure homogenization to increase safety and shelf-life of apple juice

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

Published Version:

Combined use of natural antimicrobial based nanoemulsions and ultra high pressure homogenization to increase safety and shelf-life of apple juice / Patrignani F.; Siroli L.; Braschi G.; Lanciotti R.. - In: FOOD CONTROL. - ISSN 0956-7135. - ELETTRONICO. - 111:May 2020(2020), pp. 107051.1-107051.10. [10.1016/j.foodcont.2019.107051]

Availability:

This version is available at: <https://hdl.handle.net/11585/716069> since: 2024-04-12

Published:

DOI: <http://doi.org/10.1016/j.foodcont.2019.107051>

Terms of use:

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>).
When citing, please refer to the published version.

(Article begins on next page)

Journal Pre-proof

Combined use of natural antimicrobial based nanoemulsions and ultra high pressure homogenization to increase safety and shelf-life of apple juice

Francesca Patrignani, Lorenzo Siroli, Giacomo Braschi, Rosalba Lanciotti



PII: S0956-7135(19)30640-1

DOI: <https://doi.org/10.1016/j.foodcont.2019.107051>

Reference: JFCO 107051

To appear in: *Food Control*

Received Date: 29 July 2019

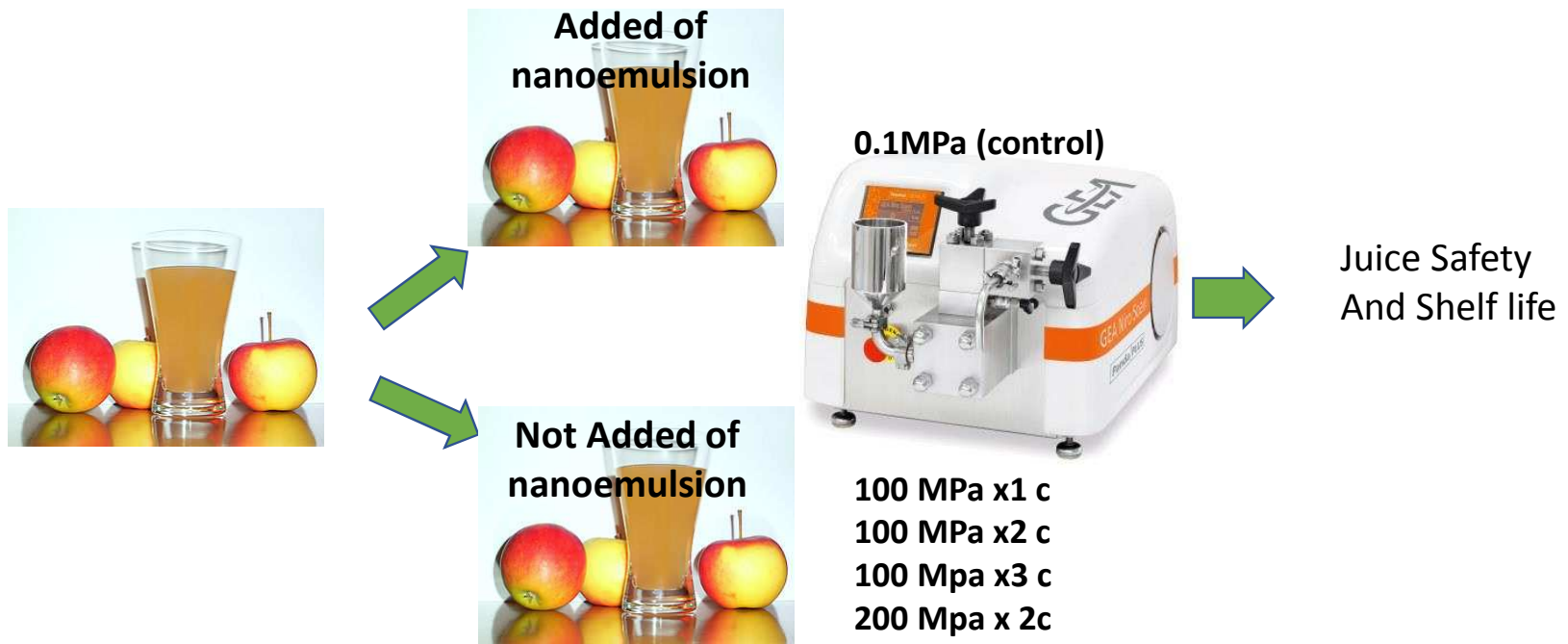
Revised Date: 8 November 2019

Accepted Date: 9 December 2019

Please cite this article as: Patrignani F., Siroli L., Braschi G. & Lanciotti R., Combined use of natural antimicrobial based nanoemulsions and ultra high pressure homogenization to increase safety and shelf-life of apple juice, *Food Control* (2020), doi: <https://doi.org/10.1016/j.foodcont.2019.107051>.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2019 Published by Elsevier Ltd.



**Combined use of natural antimicrobial based nanoemulsions and ultra high pressure
homogenization to increase safety and shelf-life of apple juice**

Francesca Patrignani^{a, b*}, Lorenzo Siroli^a, Giacomo Braschi^a and Rosalba Lanciotti^{a, b}

^a *Department of Agricultural and Food Sciences, University of Bologna, viale Fanin 44, 40127
Bologna*

^b *Interdepartmental Centre for Agri-Food Industrial Research, University of Bologna, Cesena, Italy*

* Corresponding Author: Francesca Patrignani

p.zza Goidanich 60, 47521 Cesena, Italy

phone: +39 0547338133

francesca.patrignani@unibo.it

Abstract

The present research was aimed to investigate the potentialities of ultra high pressure homogenization (UHPH) to produce stable natural antimicrobial based nanoemulsions. Initially, the nanoemulsions were characterized for their size, stability over time and antimicrobial properties against several pathogenic and spoilage microorganisms. After that nanoemulsions were tested to increase the safety and shelf-life of apple juice deliberately inoculated with pathogenic (*Listeria monocytogenes* Scott A, *Staph. aureus* SR231, *Escherichia coli* 555) and spoilage microorganisms (*Saccharomyces cerevisiae* SPA, *Lactobacillus plantarum* 82) and treated with different high pressure homogenization treatments. The analyses performed by dynamic laser light scattering showed that the hexanal and trans-2-hexenal based nanoemulsions were characterized by an average size of 86 and 100 nm, respectively while they were characterized by a stability, over time, of 14 months without separation. Moreover, the nanoemulsions resulted, after the UHPH treatment, colourless. The pathogenic species deliberately inoculated in apple juice decreased their cell loads with different kinetics in relation to the use of hexanal and trans-2-hexenal and high pressure homogenization treatment applied. Regarding spoilage microorganisms, *S. cerevisiae* cell loads decreased under detection limit (1 log CFU/mL) in juice containing nanoemulsions and treated at 200MPa for 2 cycles. The data of the present research contribute to support the application of natural antimicrobial based nanoemulsions into complex food system, enlarging the experimental evidence for their use in food sector. In particular, the obtained hexanal and tran-2-hexenal based nanoemulsions have demonstrated great application, due also to their organoleptic compatibility, to the fruit juice sector, promoting also an increase of quality of apple juice.

52

Keywords: nanoemulsion, natural antimicrobials, ultra high pressure homogenization, apple juice, safety.

55

56

57 **1. Introduction**

58 Natural antimicrobials and essential oils (EO) are of great appeal in the global market because they
59 are perceived by the consumers as natural and green alternatives to traditional industrial
60 preservatives. The analysis of the literature has pointed out that they are gifted of several properties
61 such antimicrobial, antioxidant and anticancer activities (Patrignani, Siroli, Serrazanetti, Gardini, &
62 Lanciotti, 2015). Despite their high antimicrobial activity against pathogenic and spoilage
63 microorganisms, their practical application in food is currently limited due to several factors. In
64 fact, their strong flavour impact, their great variability in composition, depending in its turn by the
65 plant geographic origin, agricultural techniques, seasonality, and methods of extraction, and, finally,
66 the scarce knowledge on their interactions with the food matrix macromolecules are the principal
67 factors limiting their use in foods (Gutierrez et al., 2008). In fact, antimicrobial agents are
68 chemically reactive species, which can cause considerable problems when embedded into a
69 complex food system, such as negative effects on the physical stability or integrity of the food
70 chemistry as well as the degradation of the biological activity of bioactive compounds (Donsì &
71 Ferrari, 2016, Kumar et al., 2019). These drawbacks result into the need to use concentrations able
72 to inhibit the microbial growth within the limits imposed by food regulations and the product shelf-
73 life span time and, at the same time, without modification on the food qualitative properties (Weiss,
74 Gaysinsky, Davidson, & McClements, 2009). However, the use of natural antimicrobials as
75 preservatives is reduced by their fast degradation and the lacking of appropriate carriers to
76 vehiculate and protect them in foods against oxidation. In fact, according to their lipophylic nature,
77 they need to be conveyed by a right carrier to be dissolved in the matrix such as ethanol. However,
78 the recent literature has proposed also the nanoencapsulation of bioactive compounds or EOs as
79 viable and efficient approach to increase their physical stability, to facilitate their vehiculation and
80 protecting them also from the interactions with the food ingredients. A huge literature on the
81 encapsulation of essential oils or natural antimicrobials, such as hexanal, carvacrol, terpenic
82 molecules, deals with the use of micrometric size capsules, used to protect the active compounds

83 against oxygen, light, moisture and low pH values. For example, β -cyclodextrin–pectin blends have
84 been proposed for the microencapsulation of hexanal by radiant energy vacuum drying to inhibit
85 *Penicillium expansum* in apple tissue (Sáenz-Garza, Delaquis, & Durance, 2013). On the other side,
86 several biopolymers have been widely used as wall materials of microparticles in the protection of
87 essential oils, both in aqueous phase and/or in spray-dried powders. Among them chitosan (Pedro,
88 Cabral-Albuquerque, Ferreira, & Sarmiento, 2009), Ca-alginate (Wang, Gong, Huang, Yu, & Xue,
89 2009), modified starch (mainly for agrochemical applications of pest control) (Glenn et al., 2010;
90 Varona, Martin, & Cocero, 2009), milk proteins (Baranauskiene, Venskutonis, Dewettinck, &
91 Verhe, 2006) and different polysaccharides (Adamiec & Kalemba, 2006; Krishnan, Bhosale, &
92 Singhal, 2005) are the most important and widely studied. While microcapsules may guarantee
93 excellent protection of essential oils against degradation or evaporation, they do not affect
94 antimicrobial activity. In contrast, nanometric size delivery systems, due to the subcellular size,
95 may increase the passive cellular absorption mechanisms, thus reducing mass transfer resistances
96 and increasing antimicrobial activity (Donsì et al. (2011, 2012a, 2012b). The increase of bioactivity
97 due to the reduction to sub-cellular size allow their usage at levels effective against the spoilage
98 microorganisms while maintaining the sensory properties of foods and avoiding the use of carrier
99 such as ethanol. Among the nanometric encapsulation systems currently being used for the delivery
100 of bioactive compounds, nanoemulsions are particularly suitable for food applications (Donsì &
101 Ferrari, 2016; Chaudhari et al., 2019; Kumar et al., 2019; Liu et al 2020), owing to the possibility of
102 formulation with natural ingredients and the easy industrial scalability of the production process.
103 The literature has pointed out the use of Ultra High Pressure up to 350 MPa to obtain
104 nanoemulsions of a mixture of terpenes using natural ingredients such as lecithin and sun flower oil
105 as carriers (Donsì et al., 2011)

106 Thus, in this contest, first aim of the research was to assess the capacity of Ultra High Pressure
107 Homogenization (UHPH) at 300 MPa to produce stable hexanal and trans 2-hexenal based
108 nanoemulsions. Moreover, the second aim of this research was to evaluate the antimicrobial activity

109 of the obtained nanoemulsions comparing their activity to those of the same compounds carried on
110 in ethanol. Finally, the obtained nanoemulsions were tested in order to evaluate their potential to
111 increase safety and shelf-life of apple juice, deliberately inoculated with pathogenic and spoilage
112 microorganisms, treated at different levels of pressure (100 MPa x1,2,3 cycles and 200 MPa for 2
113 cycles) stored at 10 °C

114

115 **2. Materials and methods**

116 *2.1. Preparation of nanoemulsions*

117 Nanoemulsions were prepared at Gea (Parma, Italy) according to the method reported by Donsì et
118 al. (2011) with some modification. Nanoemulsions of hexanal and trans-2-hexenal were prepared
119 separately. Briefly, each compound (50 g/kg) (Sigma Aldrich, Milan, Italy) was primarily mixed
120 with commercial soy lecithin (10 g/kg) by Ultra Turrax at 10000 rpm for 10 min and then added
121 with water (940 g/kg). The emulsions were treated by Ultra High Pressure Homogenization (Atena
122 model, Gea, Italy) at 300 MPa for 14 passes up to the reduction to nanoparticles.

123

124 *2.2. Nanoemulsion physical characterization*

125 The nanoemulsions were characterized by Gea for their size by Laser Particle size analyzer type
126 Coulter LS13320 (Beckman Coulter, Brea, CA). Each measurement was repeated three times. The
127 samples were prepared by mixing them gently and adding to the vessel. The optical model used is
128 Fraunhofer with Mie correction.

129 The stability of nanoemulsions were tested by Gea by the High-end Dispersion Analyzer
130 Lumisizer® (Lumisizer, Berlin, Germany). The stability testing can be accelerated by up to 2147X
131 compared to traditional test tube tests

132

133 *2.3. Determination of nanoemulsion Minimum Inhibitory (MIC) and Minimum Bactericidal*
134 *Concentration (MBC)*

135 The determination of MIC and MBC of the two obtained nanoemulsions was performed on five
136 different microbial strains such as *L. monocytogenes* Scott A, *E. coli* 555, *Staph. aureus* SR231, *L.*
137 *plantarum* 82 and *S. cerevisiae* SPA. The bacterial species were cultured two times in Brain Heart
138 Infusion (Oxoid, Basigstone, UK) at 37 °C for overnight while the yeast strain was propagated in
139 Sabouraud broth (Oxoid, Basigstone, UK) at 25 °C for 24h. The assay was performed in tubes
140 according to the method proposed by Siroli et al. (2014) with some modifications. For each
141 nanoemulsion, the tested concentrations ranged between 100 to 10000 ppm. For each
142 microorganism, three different inoculum levels were tested (2, 4 and 6 log CFU/mL). In addition,
143 the MIC and MBC of the nanoemulsions were compared with the MIC and MBC of the two
144 aldehydes carried on in ethanol (<1%). The MIC and MBC were evaluated after 24 and 48 h at the
145 microorganism optimal growth temperature.

146

147 2.4. Safety and shelf-life of apple juice in relation to the presence of nanoemulsions

148 2.4.1. Preparation of juice samples and Challenge test

149 The apple juice was obtained by apples, var. *Golden delicious*, purchased at local supermarket. The
150 juice was obtained by a lab extractor (Russel, Hobbs, 27700-56). The obtained juice was divided in
151 two batches. One batch (B1) was inoculated with selected microorganisms (see below) and treated
152 by high pressure homogenization (HPH) at different pressure levels and cycles, the other batch (B2)
153 was represented by inoculated apple juice, added of nanoemulsions (35 ppm for E-2-Hexenal and
154 70 ppm for Hexanal) and treated by HPH at the same pressure levels and cycles of B1.

155 The two batches were subjected to HPH treatments, performed at 0.1 MPa, 100 MPa for 1, 2 and 3
156 cycles and at 200 MPa for 2 cycles. The resulting samples are reported in Table 1.

157 In all the cases, the HPH treatments were performed using a continuous high pressure homogenizer
158 PANDA (Gea, Parma, Italy). The machine was supplied with a homogenizing R type valve with a
159 flow rate of 10 l/ h; the valve assembly includes a ball-type impact head made of ceramics, a
160 stainless steel large inner diameter impact ring and a tungsten-carbide passage head. The inlet

161 temperature of the untreated apple juices was 4 °C and it increased of about 1.5 °C/ 10 MPa. After
162 each pass at 100 MPa and 200 MPa, apple juices were cooled by using a thermal exchanger (Geai,
163 Parma, Italy). The maximum temperature reached by the samples did not exceed 40 °C.

164 All the obtained juices were incubated at 10 °C and analysed immediately after treatment and over
165 storage for microbial cell loads, pH.

166 The samples from B2 were also characterized for colour and volatile molecule profiles.

167

168 2.4.2. *Microorganisms used in the Challenge test and microbial sampling*

169 *L. monocytogenes* Scott A, *E. coli* 555, *Staph. aureus* SR231, *L. plantarum* 82 and *S. cerevisiae*
170 SPA, belonging to the Department of Agricultural and Food Sciences (DISTAL, University of
171 Bologna) collection, were used for the Challenge test. The bacterial species were cultured two times
172 in Brain Heart Infusion (Oxoid, Basigstone, UK) at 37 °C for overnight while the yeast strain was
173 propagated in YPD broth (Oxoid, Basigstone, UK) at 25 °C for 24h. The microbial species were
174 inoculated in mix the two apple juice batches at level of 3-4 log CFU/mL. Immediately after the
175 application of the decontamination treatments and during the product storage, the microbial cell
176 loads were determined by sampling on different selective media such as Listeria Selective Agar
177 (Oxford formulation) added of Listeria Supplement, Violet Red Bile Agar added of MUG, Baird
178 Parker, MRS and Sabouraud (Oxoid, Basigstone, UK) for *L. monocytogenes*, *E. coli*, *Staph. aureus*,
179 *Lb. plantarum* and *S. cerevisiae*, respectively. The plates were incubated at 37 °C for 24-48 h for
180 the bacterial species and 25 °C for 48 h for *S. cerevisiae*.

181

182 2.4.3. *Colour analysis and molecule volatile profiles*

183 Colour was measured using the CIELab scale and Illuminant D65. The instrument was calibrated
184 with a white tile (L^* 98.03, a^* -0.23, b^* 2.05) before the measurements. Results were expressed as
185 L^* , a^* and b^* . The molecule volatile profiles were detected by using the GC/MS/SPME technique
186 as described by Patrignani et al. (2013).

187

188 *2.5. Statistical analysis*

189 Microbial cell loads, colour and volatile profile data are means of three independent experiments.
190 For colour, three different measures were taken for each sample considered. The coefficients of
191 variability for microbiological data, expressed as the percentage ratios between the standard
192 deviations and the mean values, ranged between 2 and 5%. The significance of colour parameters
193 was evaluated using ANOVA followed by LSD test at $p < 0.05$. The volatile molecule profiles were
194 analyzed using a principal component analysis (PCA) performed by Statistica software (STAT soft
195 tools).

196

197 **3. Results**198 *3.1. Formulation, dimension and stabilization of nanoemulsions*

199 The hexanal and trans-2-hexenal based nanoemulsions were obtained using soy lecithin as carrier
200 and following the treatment by Ultra High Pressure at 300 MPa for 14 cycles. The dimensions and
201 stabilization parameters of the obtained nanoemulsions are reported in Table 1. The analyses
202 performed by dynamic laser light scattering showed that the hexanal and trans-2-hexenal based
203 nanoemulsions were characterized by an average size of 86 ± 23 and 100 ± 31 nm, respectively. The
204 results obtained by Lumisizer showed for these two samples stability profiles of almost 14 months
205 avoiding phase separation. Moreover, the nanoemulsions resulted, after the performed UHPH
206 treatment, colourless.

207

208 *3.2. Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs)*
209 *of the natural antimicrobial based nanoemulsions*

210 The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs)
211 of the two natural antimicrobial based nanoemulsions were studied against pathogenic and spoilage
212 microorganisms and in comparison with the pure hexanal and trans-2-hexenal carried in 1%

213 ethanol. The results are reported in Tables 2. The MICs were evaluated after 24 and 48 h of
214 incubation and in relation to different inoculum levels of the pathogenic and spoilage species
215 considered while, the MBCs were detected after 24 h spotting 10 μ l of the cellular suspension in
216 Petri dishes. The results obtained highlighted the major efficacy of trans-2- hexenal compared to
217 hexanal, independently on the carrier (lecithin or ethanol) and the considered microorganisms. The
218 MIC values, after 24 h and using an initial inoculum of level of 2 log CFU/mL, were always lower
219 than 700 ppm. *Saccharomyces cerevisiae*, used at level of 2 log CFU/mL, resulted more sensitive to
220 trans-2-hexenal, showing a MIC value of 100 ppm both in ethanol and in nanoemulsion. On the
221 other side, *L. plantarum*, at the same operative conditions, resulted the most resistant to trans-2-
222 hexenal, exhibiting MIC values of 700 and 500 ppm when carried out in lecithin and ethanol,
223 respectively. By contrast, hexanal showed a lower antimicrobial efficacy against all the considered
224 microorganisms. In general, hexanal, when delivered as nanoemulsion, exhibited higher MIC values
225 at 24 h, compared to hexanal in ethanol; however, the differences among the two, at 32 and 48 h,
226 decreased showing a slow release of this aldehyde when delivered as nanoemulsion. Thus, the
227 results obtained showed a fast release of nanoencapsulated trans-2-hexenal and a slow release of
228 hexanal when nanoencapsulated, suggesting a proper combined use of the two nanoemulsion.

229

230 3.3. Safety and shelf-life of apple juice: challenge test

231 In Figures 1-3, the evolution of *L. monocytogenes*, *E. coli* and *Staph. aureus* cell loads at 10 °C is,
232 respectively, reported in relation to the high pressure homogenization (HPH) treatment alone or in
233 presence of hexanal (70 ppm) and trans-2-hexenal (35 ppm) nanoemulsions. The concentration of
234 each nanoemulsion to employ in the experiments were selected on the basis of previous results
235 performed using the same food matrix. The pathogenic species considered decreased their cell loads
236 with different kinetics in relation to the hyperbaric treatment considered, the combination of the two
237 hurdles (HPH treatment and the presence of nanoemulsions) and as consequence of the low pH of
238 the juice. The inactivation kinetic of *L. monocytogenes* was increased after 2 days of storage in the

239 presence of nanoemulsions and without application of HPH. After 6 days of product storage, *L.*
240 *monocytogenes* cell load was under the detection limit. The combined use of HPH and
241 nanoemulsions increased the inactivation kinetics of this pathogenic species. The combination of
242 the level of pressure at 200 MPa for 3 cycles and the nanoemulsion determined an instantaneous
243 inactivation of 3 log order.

244 *Staph. aureus* inactivation kinetic was initially more affected by the pressure (200 MPa x 2 cycles)
245 than the presence of nanoemulsion. However, after 8 days of storage at 10 °C, *Staph. aureus* cell
246 loads were under the detection limit in samples treated at 200 MPa for 2 cycles and in the presence
247 of nanoemulsion while it was still detectable in the conditions where only the pressure was applied.

248 *E. coli* was very sensitive both to the pressure and to the use of nanoemulsions, alone or in
249 combination.

250 The inactivation kinetics of *S. cerevisiae* is reported in Fig. 4. The inactivation kinetics were
251 different in relation to the pressure applied. When *S. cerevisiae* was treated at 200 MPa x 2 cycles,
252 in the presence or not of nanoemulsions, its cell loads decreased of almost 3 log cycles. However,
253 during the first 14 days at 10°C, *S. cerevisiae* was able to recover in the juice initially treated only
254 by hyperbaric process and reach the spoilage threshold fixed at 6 log CFU/mL in 12-14 days. On
255 the other hand, *S. cerevisiae* cell loads decrease under detection limit (1 log CFU/mL) in juice
256 treated at 200 MPa for 2 cycles and added of nanoemulsions. *Lactobacillus plantarum* showed
257 barotolerance to the HPH treatments applied (data not showed). However, its cell loads decreased
258 over time. The presence of nanoemulsions further increased this behaviour.

259

260 3.4. Colour and volatile profiles of the apple juices added of nanoemulsions

261 The juice colour data in relation to the addition of nanoemulsions and HPH treatment applied are
262 reported in Table 3. The L* parameter increased initially in relation to the pressure applied but not
263 in significant way among samples. In general, this parameter increased over time and after 1d of
264 storage, all the samples added of nanoemulsions before pressure treatment were characterized by a

265 significant increase of this parameter with respect to the control not added of nanoemulsion and not
266 HPH treated. On the other hand, L^* parameter decreased over time in the control (juice treated at
267 0.1 MPa). At the end of the storage, it reached in the samples values ranging between 44.05 and
268 50.59 in relation to the level of pressure applied. The highest significant value was detected in juice
269 added of nanoemulsions and treated at 200 MPa for 2 cycles. The highest a^* values, that for
270 positive values tends toward red, were found at level of 8.48, 8.81, 8.06 in samples added of
271 nanoemulsions and treated at 100 MPa for 1, 2, 3 cycles, respectively. However, they were not
272 significant different from the remaining samples. Overtime, this parameter decreased in all the
273 samples, without any significant change at the end of the storage.

274 About b^* parameter, which for positive values tends toward yellow, it was found at highest values,
275 but not in significant way, in samples treated at 100 MPa for 1,2, and 3 cycles. At the end of the
276 juice storage, the highest significant values were detected in samples added of nanoemulsions and
277 in samples added of nanoemulsion and treated at 100 MPa for 1 and 2 cycles and 200 MPa for 2
278 cycles.

279 The apple juices added of nanoemulsions and treated by HPH were analysed, over time, for their
280 molecule volatile profiles by GC/MS-SPME. Also, a control sample (juice only treated at 0.1 MPa)
281 was included. The analysis permitted to identify about 40 molecules belonging to different classes
282 of compounds (Table 4). It is clear that the most present compounds belonged to aldehydes and
283 alcohols. The major peak areas for aldehydes in the samples were detected for the treatments
284 performed at 100 MPa x 3 and 200MPa x 2 after 8 days of sample storage. However, after 22 days
285 of storage at 10 °C, the total areas of hexanal and trans-2-hexenal decreased, while the principal
286 detoxification product of these two aldehydes, hexanol, increased in relation to the HPH treatments
287 applied. To better highlight the effects of the combined use of nanoemulsions and HPH treatments,
288 the GC/MS/SPME data were analyzed by Principal Component Analysis (PCA). The projection of
289 the samples is reported in Fig. 5 (5a) where it is clear that PC1 and PC2 are able to explain the 55%
290 of the total variance among the samples. The first cluster included, with the exception of the control

291 (C, Juice treated at 0.1 MPa and not added of nanoemulsions), all the samples added of
292 nanoemulsions immediately after the HPH treatment, irrespective of the level of application. The
293 second cluster, separated by the first along the PC2, was represented, with the exception of the
294 control sample which clustered in group 3, by the samples after 8 days of storage at 10 °C. In the
295 cluster 3 grouped all the samples after 22 days of storage and the control sample after 8 days. The
296 sample added of nanoemulsion and treated at 0.1 MPa was separated from all the other ones. In
297 Fig.5b, the molecules which permitted the clusterization of the samples are reported. Hexanol,
298 considered a detoxification product, affected particularly the grouping of cluster 3 where samples
299 after 22 days of storage were included.

300

301 **4. Discussion**

302 The increase of fruit juice safety and shelf-life by using innovative technologies, alternative to
303 thermal treatment, is a great industrial challenge. The successful results obtained on cell disruption
304 of dense microbial cultures stimulated researches on the application of ultra high pressure
305 homogenization (UHPH) for food safety and shelf-life improvement showing a maximal retention
306 of physico-chemical product properties, as well as nutritional and sensory features. Recently,
307 UHPH was indicated by several authors as a useful approach, together with colloid milling and
308 ultrasonication, to obtain natural antimicrobial based nanoemulsions to be used, instead of thermal
309 treatment or in combination with innovative technologies, to increase fruit juice safety and shelf-life
310 (Donsì et al., 2011; Donsì and Ferrari, 2016; Fathi, Martín, & McClements, 2014). On the other
311 hand, also Pucek et al. (2016) have exploited the HPH technology to produces multiple drug based
312 nanocarriers. Due to reached nanometric size, nanoemulsions have unique properties. First of all,
313 differently from emulsions, thermodynamically instable and tending to separate, they are very stable
314 (McClements and Rao, 2011). Our results demonstrated that the hexanal and tran-2-hexenal based
315 nanoemulsions were stable for more than 1 year when treated at 300 MPa for 14 passes. These
316 findings are in agreement also with the results of Donsì et al. (2011) who found for terpenic

317 molecule based nanoemulsions a comparable stability. Secondly, since the nanoemulsion droplet
318 diameters are much smaller than the wavelength of light, they form transparent or only slightly
319 turbid systems, which are suitable also for addition to clear beverages, sauces, soups, and syrups
320 (Salvia-Trujillo, Rojas-Grau, Soliva-Fortuny, & Martin-Belloso, 2014). The antimicrobial based
321 nanoemulsions obtained in this research work appeared colourless and showed high solubility in
322 water, passing up the problem of the delivery in food systems of these lipophilic compounds
323 through ethanol solution which represent for food industry an additional cost. Moreover,
324 nanoemulsions, due to their chemico-physical properties, did not affect the palatability of the tested
325 apple juice.

326 Thus, for these important features, and because compatible from a sensory point of view with
327 vegetable food matrixes, the obtained nanoformulations showed a great potential application in the
328 field of juice and beverage as reported also by several Authors (Salvia-Trujillo, Rojas-Grau, Soliva-
329 Fortuny, & Martin-Belloso, 2015; Silva, Cerqueira, & Vicente, 2012).

330 Although, in general, the reduction to nanoparticle size should increase the transport mechanisms
331 through the cell membrane of spoilage and pathogenic microorganisms (Donsì et al., 2011), the data
332 of the present research showed MIC and MBC values not different, in the first 24 h of analysis,
333 from the compounds vehiculated in ethanol. This could be due to the strong interaction between the
334 compounds and the material used for nanoencapsulation which delayed the released of the
335 compounds in the first 24 h of analysis. In fact, the results obtained showed that the kinetics of
336 release of the two antimicrobials in nanoemulsion were different overtime suggesting a potential
337 use in combination in real system. In fact, the microbiological results achieved in apple juice,
338 regarding the nanoemulsion alone or in combination with high pressure homogenization, showed
339 their great potential to inactivate the target pathogenic bacteria and *S. cerevisiae*, deliberately
340 inoculated in the juice. On the other hand, *S. cerevisiae* represents, for this kind of products,
341 characterized by low pH and high level of sugar, the principal spoiling agent (Patrignani et al.,
342 2013). The data resulting from the Challenge test showed that also *E. coli* dynamic inactivation was

343 fastened by the presence of nanoencapsulated antimicrobials, also in the absence of the application
344 of high pressure homogenization. Nanoemulsion droplets, thanks to their size and to the exposition
345 of the hydrophilic groups of the emulsifying molecules, can be efficiently transported through the
346 porin proteins of the outer-membrane, generally impermeable to free essential oil or natural
347 antimicrobials, enabling an effective delivery of EOs also to the cell membrane of Gram negative
348 bacteria (Nazzaro, Fratianni, De Martino, Coppola, & De Feo, 2013). From a technological point of
349 view, the combined use of high pressure homogenization and nanoemulsions increased the lightness
350 of the apple treated juice, due both to the effects of HPH and to the antioxidant activity of the
351 natural antimicrobial (Patrignani, & Lanciotti, 2016; Prakash et al., 2018). On the other hand, the
352 HPH treatment is reported to increase L^* parameter due to the higher light scattering properties of
353 smaller size particles (Calligaris et al., 2012, Patrignani et al 2019). A similar result was also
354 observed by Yi et al. (2017) on apple juice with 50% of kiwifruit addition upon the application of
355 dynamic pressure. In addition, the use of natural antimicrobial based nanoemulsions can reduce the
356 sensory impact that the free molecules generally have (Espina et al., 2014). Additionally, the use of
357 suitable delivery systems can favour the dispersion of the natural antimicrobials into the food
358 matrix, avoiding the use of huge amount of EOs which adversely affect the sensory properties of the
359 product. Moreover, as reported by Donsì et al., (2012a) the choice of the good delivery system can
360 prevent the EO evaporation rate, as well as the negative interaction with other food components
361 (Shah, Davidson, & Zhong, 2012). The data from GC/MS-SPME analysis showed that the addition
362 of the two natural antimicrobials, although as nanoemulsions, affected the apple juice molecule
363 volatile profiles since hexanal and trans-2-hexenal was the most abundant identified compounds.
364 However, their kinetic release in the product were different over time. In fact, the apple juice
365 samples clusterized together dependently on the storage time. In addition, the presence of hexanol,
366 as detossification product deriving from hexanal by microbial enzymatic activity and vegetable
367 tissue one, was more pronounced in samples subjected to a faster microbial spoilage.

368

369 5. Conclusion

370 The data of the present research contribute to support the application of natural antimicrobial based
371 nanoemulsions into complex food system, enlarging the experimental evidence for their use in food
372 sector. In particular, the obtained hexanal and tran-2-hexenal based nanoemulsions demonstrated
373 great application potential, due also to their compatibility with the tested food matrix, promoting
374 also an increase of quality of apple juice. The data reported in the present research also highlighted
375 the potential use of nanoemulsions in combination with another hurdle, such as high pressure
376 homogenization. However, for a further application in food industry, the action mechanisms of
377 encapsulated antimicrobials need to be deeply understood and studied also in order to replace other
378 carrier such as ethanol which can represent an additional cost for the food industry.

379

380 Figure caption

381 **Figure 1)** *L. monocytogenes* cell load (log CFU/mL) evolution detected in apple juice in relation to
382 the initial inoculum level, treatment applied and time of storage at 10 °C.

383 **Figure 2)** *Staphylococcus aureus* cell load (log CFU/mL) evolution detected in apple juice in
384 relation to the initial inoculum level, treatment applied and time of storage at 10 °C.

385 **Figure 3)** *Escherichia coli* cell load (log CFU/mL) evolution detected in apple juice in relation to
386 the initial inoculum level, treatment applied and time of storage at 10 °C.

387 **Figure 4)** *Saccharomyces cerevisiae* cell load (log CFU/mL) evolution detected in apple juice in
388 relation to the initial inoculum level, treatment applied and time of storage at 10 °C.

389 **Figure 5)** Projection on the factor plane (1x2) of variables (b) and apple juice samples (a) added
390 (samples 1,2,3,4,5) or not (C) of nanoemulsions, treated at different pressures and stored at 10°C for
391 several days (T0, T8, T22).

392

393 References

- 394 Adamiec, J., & Kalembe, D. (2006). Analysis of microencapsulation ability of essential oils during
395 spray drying. *drying technology*, 24(9), 1127-1132.
396 <https://doi.org/10.1080/07373930600778288>.
- 397 Baranauskiene, R., Venskutonis, P. R., Dewettinck, K., & Verhé, R. (2006). Properties of oregano
398 (*Origanum vulgare* L.), citronella (*Cymbopogon nardus* G.) and marjoram (*Majorana*
399 *hortensis* L.) flavors encapsulated into milk protein-based matrices. *Food Research*
400 *International*, 39(4), 413-425. <https://doi.org/10.1016/j.foodres.2005.09.005>.
- 401 Calligaris, S., Foschia, M., Bartolomeoli, I., Maifreni, M., and Manzocco, L. (2012). Study on the
402 applicability of high-pressure homogenization for the production of banana juices. *LWT Food*
403 *Science & Technology*, 45, 117-121. doi: 10.1016/j.lwt.2011.07.026
- 404 Chaudhari, A.K., Dwivedy, A. K., Singh, V.K., Das, S., ; Singh, A., & Dubey, N.K. (2019).
405 Essential oils and their bioactive compounds as green preservatives against fungal and
406 mycotoxin contamination of food commodities with special reference to their
407 nanoencapsulation. *Environmental Science and Pollution Research*, 26, 25414-25431.
408 <http://10.1007/s11356-019-05932-2>
- 409 Donsì, F., Annunziata, M., Sessa, M., & Ferrari, G. (2011). Nanoencapsulation of essential oils to
410 enhance their antimicrobial activity in foods. *LWT - Food Science and Technology*, 44(9),
411 1908–1914. <http://dx.doi.org/10.1016/j.lwt.2011.03.003>.
- 412 Donsì, F., Annunziata, M., Vincensi, M., & Ferrari, G. (2012a). Design of nanoemulsion-based
413 delivery systems of natural antimicrobials: effect of the emulsifier. *Journal of Biotechnology*,
414 159(4), 342–350. <http://dx.doi.org/10.1016/j.jbiotec.2011.07.001>.
- 415 Donsì, F., Sessa, M., & Ferrari, G. (2012b). Effect of emulsifier type and disruption chamber
416 geometry on the fabrication of food nanoemulsions by high pressure homogenization.
417 *Industrial & Engineering Chemistry Research*, 51(22), 7606–7618. [http://dx.doi.org/10.](http://dx.doi.org/10.1021/ie2017898)
418 [1021/ie2017898](http://dx.doi.org/10.1021/ie2017898).

- 419 Donsì, F., & Ferrari, G. (2016). Essential oil nanoemulsions as antimicrobial agents in food.
420 *Journal of Biotechnology*, 233, 106-120. <https://doi.org/10.1016/j.jbiotec.2016.07.005>.
- 421 Espina, L., Somolinos, M., Loràn, S., Conchello, P., Garcia, D., & Pagàn, R. (2011). Chemical
422 composition of commercial citrus fruit essential oils and evaluation of their antimicrobial
423 activity acting alone or in combined processes. *Food Control*, 22(6), 896-902.
424 <https://doi.org/10.1016/j.foodcont.2010.11.021>.
- 425 Fathi, M., Martín, A., & McClements, D. J. (2014). Nanoencapsulation of food ingredients using
426 carbohydrate based delivery systems. *Trends in Food Science & Technology*, 39(1), 18-39.
427 <https://doi.org/10.1016/j.tifs.2014.06.007>.
- 428 Glenn, G., Klameczynski, A. P., Woods, D. F., Chiou, B., Orts, W. J., & Imam, S. H. (2010).
429 Encapsulation of Plant Oils in Porous Starch Microspheres. *Journal of Agricultural and Food*
430 *Chemistry*, 58(7), 4180-4184. doi: 10.1021/jf9037826.
- 431 Gutierrez, J., Barry-Ryan, C., & Bourke, P. (2008). The antimicrobial efficacy of plant essential oil
432 combinations and interactions with food ingredients. *International Journal of Food*
433 *Microbiology*, 124(1), 91-97. <https://doi.org/10.1016/j.ijfoodmicro.2008.02.028>.
- 434 Krishnan, S., Bhosale, R., & Singhal, R. S. (2005). Microencapsulation of cardamom oleoresin:
435 evaluation of blends of gum arabic, maltodextrin and a modified starch as wall materials.
436 *Carbohydrate Polymers*, 61(1), 95-102. <https://doi.org/10.1016/j.carbpol.2005.02.020>.
- 437 Kumar, A; Kujur, A Singh, PP; Prakash, B. (2020). Nanoencapsulated plant-based bioactive
438 formulation against food-borne molds and aflatoxin B-1 contamination: Preparation,
439 characterization and stability evaluation in the food system. *Food Chemistry*, 287, 139-150.
440 dOI: 10.1016/j.foodchem.2019.02.045.
- 441 Liu, Q., Zhang, M., Bhandari, B., Xu, J., Yang, C. (2020). Effects of nanoemulsion-based active
442 coatings with composite mixture of star anise essential oil, polylysine, and nisin on the quality
443 and shelf life of ready-to-eat Yao meat products. *Food Control*, 107 1-7.
444 10.1016/j.foodcont.2019.106771

445
446
447

448 McClements, D. J., & Rao, J. (2011). Food-grade nanoemulsions: formulation, fabrication,
449 properties, performance, biological fate, and potential toxicity. *Critical Reviews in Food*
450 *Science and Nutrition*, 51(4), 285–330. <http://dx.doi.org/10.1080/10408398.2011.559558>.

451 Nazzaro, F., Fratianni, F., De Martino, L., Coppola, R., & De Feo, V. (2013). Effect of Essential
452 Oils on Pathogenic Bacteria. *Pharmaceuticals*, 6(12), 1451-1474. doi: 10.3390/ph6121451.

453 Patrignani, F., Tabanelli, G., Siroli, L., Gardini, F., & Lanciotti, R. (2013). Combined effects of
454 high pressure homogenization treatment and citral on microbiological quality of apricot juice.
455 *International Journal of Food Microbiology*, 160(3), 273-281.
456 <https://doi.org/10.1016/j.ijfoodmicro.2012.10.021>.

457 Patrignani, F., Siroli, L., Serrazanetti, D. I., Gardini, F., & Lanciotti, R. (2015). Innovative
458 strategies based on the use of essential oils and their components to improve safety, shelf-life
459 and quality of minimally processed fruits and vegetables. *Trends in Food Science &*
460 *Technology*, 46(2), 311-319. <https://doi.org/10.1016/j.tifs.2015.03.009>.

461 Patrignani, F., & Lanciotti, R. (2016). Applications of High and Ultra High Pressure
462 Homogenization for Food Safety. *Frontiers in Microbiology*, 7, 1132.
463 <https://doi.org/10.3389/fmicb.2016.01132>.

464 Patrignani, F., Mannozi, C., Tappi, S., Tylewicz, U., Pasini, F., Castellone, V., Riciputi, Y.,
465 Rocculi, P., Romani, S., Caboni, M.F., Gardini, F., Lanciotti, R., & Dalla Rosa, M. (2019).
466 (Ultra) High Pressure Homogenization Potential on the Shelf-Life and Functionality of
467 Kiwifruit Juice. *Frontiers in Microbiology*, Volume 10 | Article 246. doi:
468 10.3389/fmicb.2019.00246

469

- 470 Pedro, A. S., Cabral-Albuquerque, E., Ferreira, D., & Sarmiento, B. (2009). Chitosan: an option for
471 development of essential oil delivery systems for oral cavity care?. *Carbohydrate Polymers*,
472 76(4), 501-508. <https://doi.org/10.1016/j.carbpol.2008.12.016>.
- 473 Prakash, B., Kujur, A., Yadav, A., Kumar, A., Singh, P. P., & Dubey, N. K. (2018).
474 Nanoencapsulation: An efficient technology to boost the antimicrobial potential of plant
475 essential oils in food system. *Food Control*, 89, 1-11.
476 <https://doi.org/10.1016/j.foodcont.2018.01.018>.
- 477 Pucek, A., Niezgoda, N., Kulbacka, J., Wawrzeńczyk, C., & Wilk, K. A. (2017).
478 Phosphatidylcholine with conjugated linoleic acid in fabrication of novel lipid nanocarriers,
479 *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 532, 377-388.
480 <https://doi.org/10.1016/j.colsurfa.2017.04.061>.
- 481 Sáenz-Garza, N., Delaquis, P., & Durance, P. (2013). Microencapsulation of hexanal by radiant
482 energy vacuum microwave-molecular inclusion for controlled release and inhibition of
483 *Penicillium expansum* in a model system and on apple tissue. *Food Research International*,
484 52(2), 496-502. <https://doi.org/10.1016/j.foodres.2013.01.040>.
- 485 Salvia-Trujillo, L., Rojas-Grau, M. A., Soliva-Fortuny, R., & Martín-Belloso, O. (2014).
486 Formulation of antimicrobial edible nanoemulsions with pseudo-ternary phase experimental
487 design. *Food and Bioprocess Technology*, 7(10), 3022–3032. [http://dx.](http://dx.doi.org/10.1007/s11947-014-1314-x)
488 [doi.org/10.1007/s11947-014-1314-x](http://dx.doi.org/10.1007/s11947-014-1314-x).
- 489 Salvia-Trujillo, L., Rojas-Graü, A., Soliva-Fortuny, R., & Martín-Belloso, O. (2015).
490 Physicochemical characterization and antimicrobial activity of food-grade emulsions and
491 nanoemulsions incorporating essential oils. *Food Hydrocolloids*, 43, 547–556.
492 <https://doi.org/10.1016/j.foodhyd.2014.07.012>.
- 493 Shah, B., Davidson, P. M., & Zhong, Q. (2012). Nanocapsular Dispersion of Thymol for Enhanced
494 Dispersibility and Increased Antimicrobial Effectiveness against *Escherichia coli* O157:H7

- 495 and *Listeria monocytogenes* in Model Food Systems. *Applied and Environmental*
496 *Microbiology*, 78(23), 8448-8453. doi: 10.1128/AEM.02225-12.
- 497 Silva, H. D., Cerqueira, M. A., & Vicente, A. A. (2012). Nanoemulsions for Food Applications:
498 Development and Characterization. *Food and Bioprocess Technology*, 5(3), 854-867.
499 <http://dx.doi.org/10.1007/s11947-013-1094-8>.
- 500 Siroli, L., Patrignani, F., Montanari, C., Tabanelli, G., Bargossi, E., Gardini, F., & Lanciotti, R.
501 (2014). Characterization of oregano (*Origanum vulgare*) essential oil and definition of its
502 antimicrobial activity against *Listeria monocytogenes* and *Escherichia coli* in vitro system and
503 on foodstuff surfaces. *African Journal of Microbiology Research*, 8(29), 2746-2753. doi:
504 10.5897/AJMR2014.6677.
- 505 Varona, S., Martin, A., & Cocero, M. J. (2009). Formulation of a natural biocide based on lavandin
506 essential oil by emulsification using modified starches. *Chemical Engineering and Processing*,
507 48(6), 1121-1128. <https://doi.org/10.1016/j.cep.2009.03.002>.
- 508 Yi, J., Kebede, B., Kristiani, K., Grauwet, T., Van Loey, A., and Hendrickx, M. (2017). Minimizing
509 quality changes of cloudy apple juice: the use of kiwifruit puree and high pressure
510 homogenization. *Food Chemistry* 249, 202-212. doi: 10.1016/j.foodchem.2017.12.088
- 511 Wang, Q., Gong, J., Huang, X., Yu, H., & Xue, F. (2009). In vitro evaluation of the activity of
512 microencapsulated carvacrol against *Escherichia coli* with K88 pili. *Journal of Applied*
513 *Microbiology*, 107(6), 1781-1788. doi: 10.1111/j.1365-2672.2009.04374.x.
- 514 Weiss, J., Gaysinsky, S., Davidson, M., & McClements, J. (2009). Nanostructured encapsulation
515 systems: food antimicrobials. In G. V. Barbosa-Cánovas, A. Mortimer, D. Lineback, W.
516 Spiess, & K. Buckle (Eds.), *IUFoST world congress book: Global issues in food science and*
517 *technology* (pp. 425-479). Amsterdam: Elsevier Inc. [https://doi.org/10.1016/B978-0-12-](https://doi.org/10.1016/B978-0-12-374124-0.00024-7)
518 [374124-0.00024-7](https://doi.org/10.1016/B978-0-12-374124-0.00024-7).

519

Table 1. Samples obtained within the present research

Batch 1

Inoculated juice treated at 0.1 MPa (control)

Inoculated juice treated at 100MPa x 1cycle

Inoculated juice treated at 100MPa x 2cycles

Inoculated juice treated at 100MPa x 3cycles

Inoculated juice treated at 200MPa x 2cycles

Batch 2

Inoculated juice added of nanoemulsion (NE) and treated at 0.1 MPa

Inoculated juice added of nanoemulsion (NE) and treated at 100MPa x 1cycle

Inoculated juice added of nanoemulsion (NE) and treated at 100MPa x 2cycles

Inoculated juice added of nanoemulsion (NE) and treated at 100MPa x 3cycles

Inoculated juice added of nanoemulsion (NE) and treated at 200MPa x 2cycles.

Table 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the natural antimicrobials carried in nanoemulsion or ethanol

	Log cfu/ml	<i>Staphylococcus aureus</i>			<i>L.monocytogenes</i>			<i>E.coli</i>			<i>S.cerevisiae</i>			<i>Lb.plantarum</i>		
		MIC 24h (ppm)	MIC 48h (ppm)	MBC 48h (ppm)	MIC 24h (ppm)	MIC 48h (ppm)	MBC 48h (ppm)	MIC 24h (ppm)	MIC 48h (ppm)	MBC 48h (ppm)	MIC 24h (ppm)	MIC 48h (ppm)	MBC 48h (ppm)	MIC 24h (ppm)	MIC 48h (ppm)	MBC 48h (ppm)
Hexanal nanobased emulsion	6	4000	5000	10000	3000	3000	5000	2000	3000	4000	800	3000	4000	5000	8000	>10000
	4	3000	5000	8000	3000	3000	5000	2000	2000	3000	800	2000	3000	5000	8000	>10000
	2	2000	4000	5000	1000	3000	5000	700	800	1000	800	1000	2000	4000	8000	>10000
Hexanal in ethanol	6	4000	4000	>10000	2000	2000	2000	2000	2000	3000	1000	2000	3000	5000	5000	>10000
	4	2000	3000	8000	1000	1000	2000	800	2000	2000	800	2000	2000	4000	5000	>10000
	2	2000	2000	5000	800	1000	2000	300	500	1000	500	800	2000	2000	5000	>10000
(E)-2-hexenal nanobased emulsion	6	700	3000	3000	500	800	1000	700	700	1000	500	700	700	700	1000	2000
	4	700	1000	2000	300	800	1000	500	700	700	300	500	500	700	1000	2000
	2	500	800	1000	300	500	800	500	500	500	100	100	300	700	1000	1000
(E)-2-hexenal in ethanol	6	1000	1000	2000	500	500	800	500	700	700	300	500	500	500	700	700
	4	500	700	800	300	500	800	500	700	700	300	500	500	500	700	700
	2	300	700	700	300	500	500	500	500	500	100	300	300	500	700	700

Table 3. Apple juice colour parameters detected in different samples in relation to the presence of natural antimicrobial nano-based emulsions (NE), pressure applied and time of storage

L*	Time (d)									
	0	1	4	6	8	12	15	19	22	
0.1 MPa (control)	35.09±3.18 ^a	34.90±2.58 ^a	33.35±1.21 ^a	33.70±3.27 ^a	33.10±3.11 ^a	33.00±2.87 ^a	29.70±3.79 ^a	30.10±3.26 ^a	28.75±3.22 ^a	
NE +0.1MPa	35.15±2.56 ^a	36.57±3.05 ^b	41.79±3.24 ^b	38.23±2.89 ^{ab}	41.88±2.28 ^b	42.05±2.56 ^b	42.56±2.25 ^b	43.10±2.98 ^b	48.05±1.55 ^c	
NE +100 MPa x1c	39.91±2.79 ^a	41.08±1.89 ^b	40.60±3.08 ^b	38.48±3.02 ^{ab}	42.42±3.02 ^b	41.04±3.22 ^b	41.89±3.06 ^b	43.68±3.09 ^b	45.75±3.27 ^{bc}	
NE +100 MPa x2c	39.66±3.03 ^a	40.99±1.22 ^b	36.77±1.79 ^{ab}	42.02±1.89 ^{bc}	42.02±2.74 ^b	42.74±2.98 ^b	42.66±2.88 ^b	50.82±2.21 ^c	46.15±2.54 ^{bc}	
NE +100 MPa x 3c	39.54±2.55 ^a	41.38±2.05 ^b	37.51±3.11 ^b	46.69±2.02 ^c	41.39±1.96 ^b	40.96±3.18 ^b	45.85±2.77 ^b	42.26±2.57 ^b	41.93±3.01 ^b	
NE +200 MPa x 2c	37.17±1.86 ^a	40.27±2.36 ^b	39.86±2.94 ^b	41.05±2.02 ^b	39.86±2.09 ^b	41.45±2.22 ^b	42.84±3.02 ^b	50.32±2.64 ^c	50.59±2.88 ^c	
a*	0	1	4	6	8	12	15	19	22	
0.1 MPa (control)	6.90±0.96 ^{ab}	6.30±0.78 ^a	4.10±1.46 ^a	4.50±1.50 ^a	4.35±0.84 ^a	3.89±1.25 ^a	3.75±2.02 ^a	3.70±0.58 ^a	3.10±0.95 ^a	
NE +0.1MPa	6.78±1.07 ^{ab}	7.94±0.95 ^{ab}	5.44±1.55 ^a	6.65±1.18 ^a	4.82±1.05 ^a	4.80±0.95 ^a	4.85±1.34 ^a	2.63±1.89 ^a	4.26±0.79 ^a	
NE +100 MPa x1c	8.48±1.18 ^{bc}	8.39±1.10 ^b	6.63±1.37 ^a	5.13±0.95 ^a	5.58±1.18 ^a	3.55±1.85 ^a	5.58±1.08 ^a	5.06±1.10 ^a	5.02±1.05 ^a	
NE +100 MPa x2c	8.81±1.33 ^{bc}	7.83±0.88 ^{ab}	4.25±1.03 ^a	6.40±1.22 ^a	5.97±1.29 ^a	5.25±1.16 ^a	5.41±0.95 ^a	4.22±0.85 ^a	5.48±1.25 ^a	
NE +100 MPa x 3c	8.06±1.09 ^{bc}	8.26±1.06 ^b	5.15±1.18 ^a	4.69±1.33 ^a	5.36±0.85 ^a	5.79±1.33 ^a	5.79±1.45 ^a	5.60±0.96 ^a	4.53±0.86 ^a	
NE +200 MPa x 2c	7.58±0.74 ^b	8.04±1.18 ^{ab}	5.21±0.99 ^a	6.23±1.56 ^a	5.19±1.10 ^a	5.62±1.05 ^a	5.66±1.23 ^a	5.84±0.88 ^a	4.24±1.13 ^a	

b*	0	1	4	6	8	12	15	19	22
0.1 MPa (control)	17.35±3.25 ^a	16.10±1.85 ^a	13.20±2.45 ^a	13.00±2.20 ^a	12.55±1.65 ^a	12.00±2.01 ^a	12.20±1.42 ^a	10.50±3.03 ^a	10.78±2.55 ^a
NE +0.1MPa	17.25±2.89 ^a	16.20±2.03 ^a	22.66±1.54 ^b	19.35±1.75 ^{bc}	20.93±1.55 ^b	31.47±3.22 ^c	17.01±2.22 ^b	29.65±3.89 ^d	21.65±1.78 ^b
NE +100 MPa x1c	21.62±2.55 ^a	24.44±2.64 ^b	21.57±1.88 ^b	16.78±2.18 ^{ab}	22.39±2.01 ^b	17.32±1.18 ^b	15.99±1.38 ^{ab}	16.33±2.00 ^b	19.26±2.08 ^b
NE +100 MPa x2c	20.73±1.93 ^a	21.01±1.52 ^b	14.33±2.02 ^a	21.87±1.61 ^{cd}	20.72±1.64 ^b	19.76±1.56 ^b	17.87±1.77 ^b	23.65±1.93 ^c	20.24±1.35 ^b
NE +100 MPa x 3c	21.12±1.85 ^a	21.86±1.38 ^b	16.47±1.85 ^a	25.20±2.05 ^d	19.41±1.09 ^b	19.07±2.01 ^b	22.07±2.09 ^c	14.53±2.18 ^{ab}	13.59±2.09 ^a
NE +200 MPa x 2c	17.89±2.01 ^a	24.47±1.86 ^b	21.04±1.45 ^b	20.86±2.04 ^{bc}	18.40±0.98 ^b	19.48±1.34 ^b	17.21±1.32 ^b	23.58±1.64 ^c	21.88±1.96 ^b

Data are reported as average values and standard deviations. Values with different letter within the column are significantly different ($p < 0.05$).

Table 4. Apple juice GC/MS/SPME profiles (expressed as total area/10000) in relation to the presence of natural antimicrobial nano-based emulsions, pressure applied and time of storage

Molecules	0d						8d						22d				
	1	2	3	4	5	C	1*	2*	3*	4*	5*	C*	1*	2*	3*	4*	5*
	Ethanol	27	31	28	29	22	48	39	124	81	38	81	897	3307	2253	1870	2192
1-propanol	-	-	-	-	-	-	-	-	-	-	-	40	73	92	50	56	85
1-propanol-2 methyl	-	-	-	-	-	-	-	-	-	-	-	-	38	14	9	10	38
1-Butanol, 3-methyl-	-	-	-	-	-	-	-	-	-	-	-	53	331	169	50	74	154
1-Hexanol	111	61	83	83	79	92	3467	4683	2344	1116	1512	2827	4866	6166	6059	5976	4863
3-Hexen-1-ol	-	-	-	-	-	-	462	38	45	29	42	14	-	-	-	9	25
2-Methylene cyclopentanol	31	22	22	20	23	32	16	17	12	28	19	-	-	-	-	29	28
6-Tetradecanol	25	35	-	15	19	-	18	30	10	20	25	-	-	-	-	38	31
2-Butyl-2,7-octadien-1-ol	935	898	805	683	1022	995	888	925	818	1103	697	351	661	516	601	553	384
1-Octanol, 2-butyl-	-	-	-	-	-	-	-	53	23	25	33	-	14	99	86	282	96
Cyclohexanol, 1-(1-hexenyl)-, (E)-	237	125	213	258	208	234	196	169	184	92	80	-	68	194	30	169	106
Phenylethyl Alcohol	-	-	-	-	-	-	-	-	-	-	-	7	60	84	14	30	56
Totale Alcohols	1366	1173	1150	1088	1373	1401	5087	6039	3517	2450	2488	4189	9420	9586	8768	9417	8643
ethyl acetato	-	-	-	-	-	-	-	-	-	-	-	31	176	153	36	75	121
Hexanoic acid, ethyl ester	-	-	-	-	-	-	-	-	-	-	-	7	528	34	-	13	27

Acetic acid, hexyl ester	104	47	32	8	21	62	11	19	35	23	7	7	381	203	172	475	582
Octanoic acid, ethyl ester	-	-	-	-	-	-	-	-	-	-	-	-	93	72	-	-	-
2-Propenoic acid, octyl ester	-	-	-	-	-	-	-	-	-	-	-	-	124	60	-	-	-
2-Thiopheneacetic acid, 2-methyloct-5-yn-4-yl ester	278	200	242	49	152	299	114	144	93	214	136	145	227	148	237	137	125
Decanoic acid, ethyl ester	-	-	-	-	-	-	-	-	-	-	-	-	331	30	25	-	-
Oxalic acid, cyclobutyl decyl ester	-	-	-	-	-	-	-	-	21	-	8	-	251	-	-	-	-
Oxalic acid, cyclohexylmethyl tetradecyl ester	208	163	164	167	156	204	85	167	97	116	142	20	96	107	155	168	153
Hexadecenoic acid, ethyl ester	116	33	24	31	36	89	53	45	56	46	36	-	-	38	8	11	-
Total Esters	706	443	462	255	364	654	262	375	302	400	330	210	2205	845	633	878	1008
2-Butanone	10	6	2	10	8	12	8	1	4	2	2	-	-	-	4	2	-
2-hexanone- 4 metil	15	38	6	45	11	16	8	15	12	13	11	38	21	8	19	4	50
3-Hexen-2-one	55	75	82	96	103	118	23	21	56	59	41	-	-	-	-	-	-
Bicyclo[3.2.0]heptan-2-one	-	25	-	23	15	10	-	4	4	7	11	-	39	9	37	16	51
Propanal, dipropylhydrazone	46	47	24	28	19	62	14	16	14	16	17	-	22	-	-	-	-
Total Ketones	126	191	115	203	156	218	53	57	90	96	81	38	81	17	61	22	101
2-Butenal, (Z)	7	13	25	15	12	25	7	7	7	8	11	-	8	6	9	8	9
Hexanal	7207	6885	6132	5542	5913	35	3014	3171	4350	4370	4382	25	24	15	31	56	51
2-Hexenal, (E)-	5314	6357	4785	5484	5621	12	3402	3042	4150	4266	4291	6	12	18	12	52	37
2-Heptenal	-	-	9	30	63	9	6	-	14	23	25	5	26	17	9	26	13
2-4-Hexadienal,(E,E)	227	226	60	107	68	128	17	26	78	61	72	-	-	46	64	-	-
2-Octenal,(E)	44	57	19	26	62	19	8	17	27	25	15	-	-	-	-	-	-

2,4 Heptadienal, (E,E)	41	13	14	16	16	14	13	17	21	20	18	-	-	-	-	-	
Benzaldehyde	23	62	22	55	33	22	21	22	48	40	34	33	31	43	21	22	33
Totale Aldehydes	12862	13613	11067	11275	11788	264	6487	6302	8694	8813	8847	69	101	145	148	164	142
Hexanoic acid	852	531	610	601	666	720	578	409	583	622	460	15	28	24	5	8	-
Octanoic Acid	-	-	-	-	-	-	-	-	-	-	-	-	100	24	62	89	52
Totale Acids	852	531	610	601	666	720	578	409	583	622	460	15	129	48	66	97	52
Total Molecules	15912	15951	13403	13422	14347	3257	12467	13181	13186	12382	12207	4522	11936	10641	9676	10579	9947

1: juice treated 0.1 MPa+nano emulsion (NE)

2: juice + nano emulsion (NE) and treated at 100 MPax1c

3: juice + nano emulsion (NE) treated at 100 MPa x 2c

4: juice + nano emulsion (NE) treated at 100 MPa x 3c

5: juice + nano emulsion (NE) treated at 200 MPa x 2c

C: juice treated at 0.1MPa (control)

-: Under the detection limit

Figure 1.

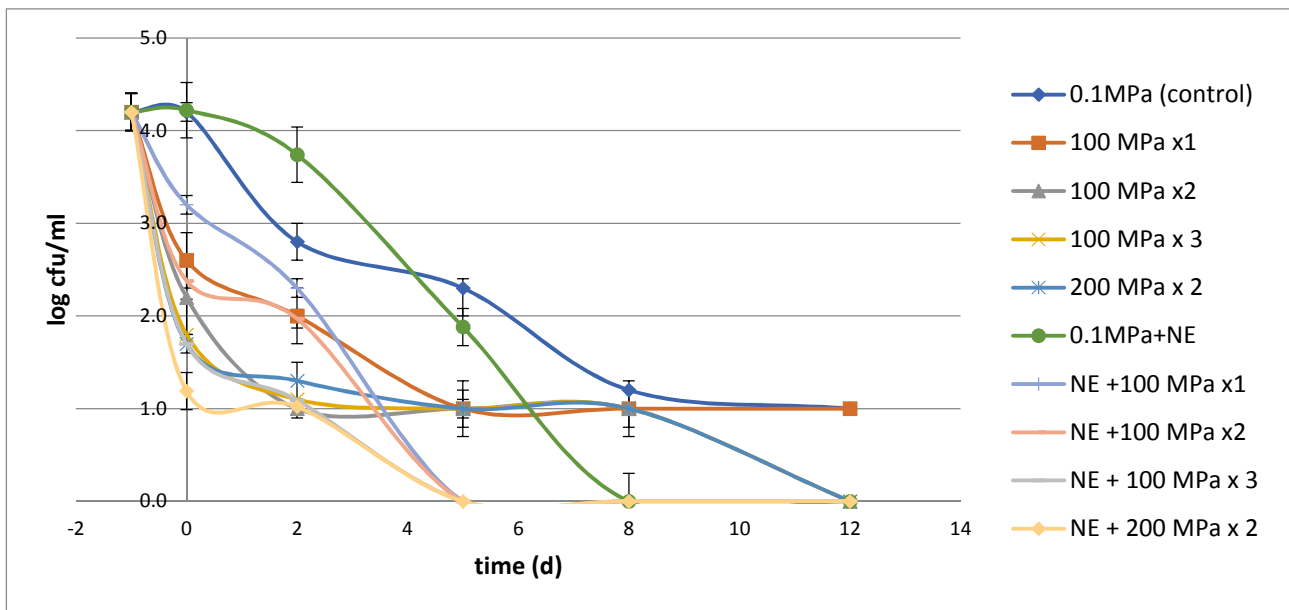


Figure 3

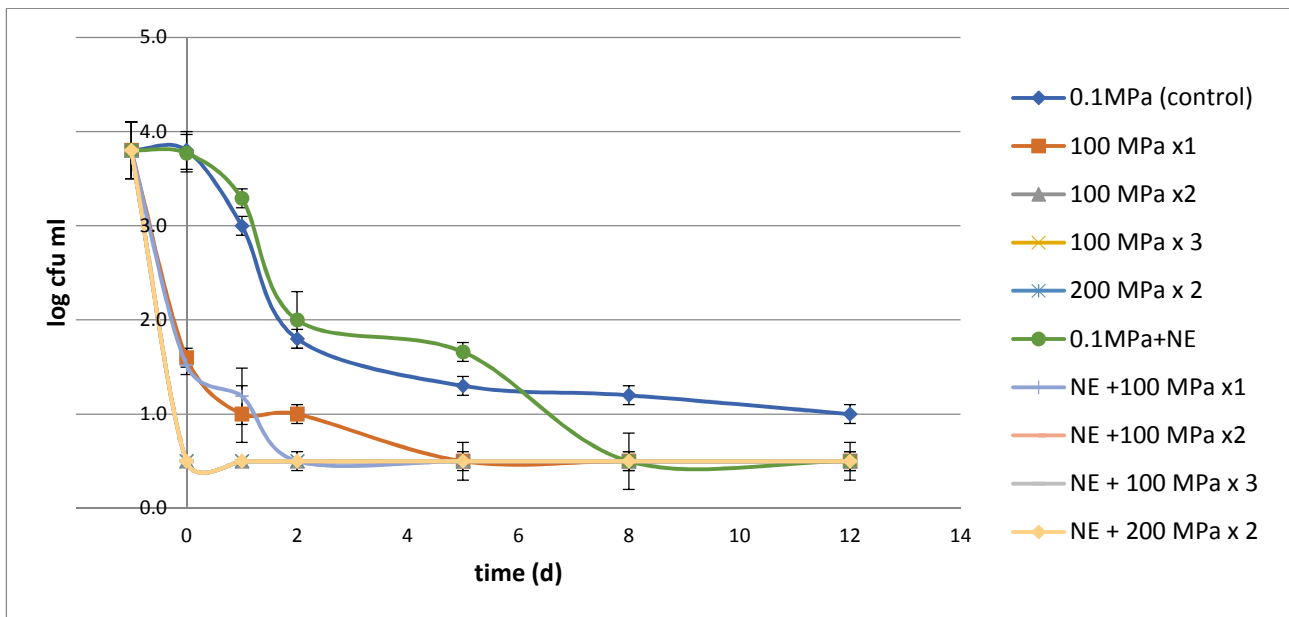


Figure 4.

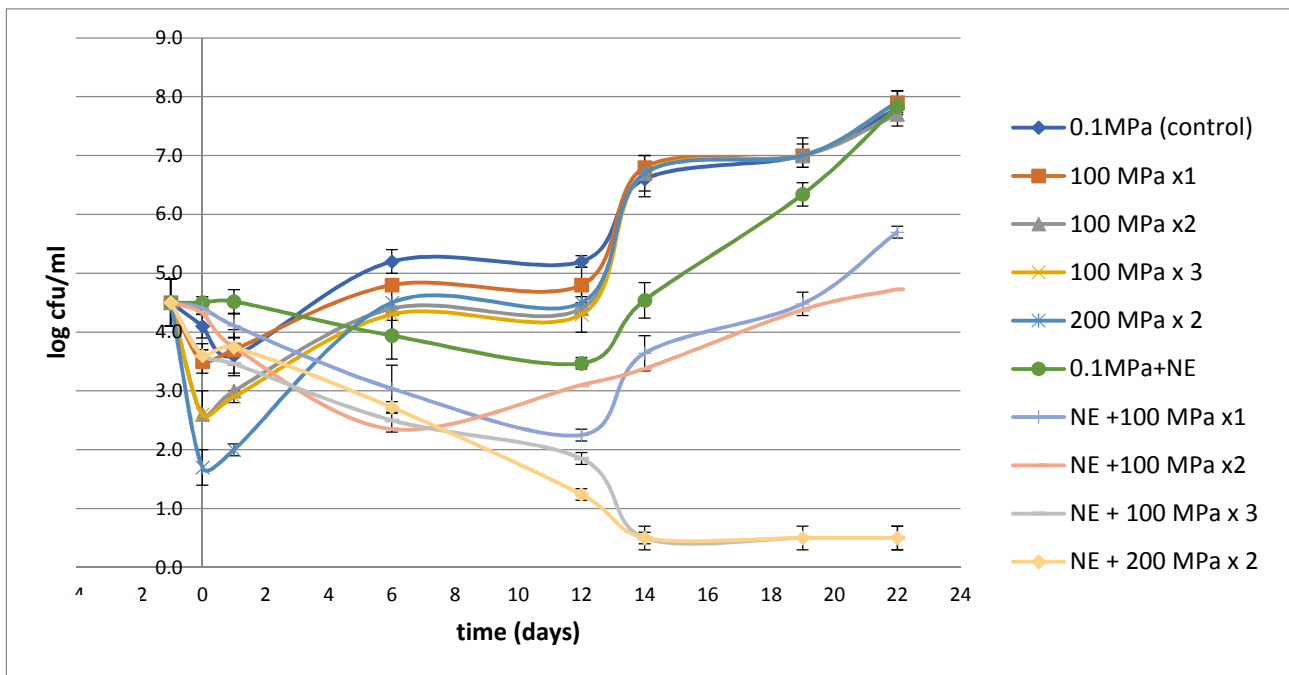
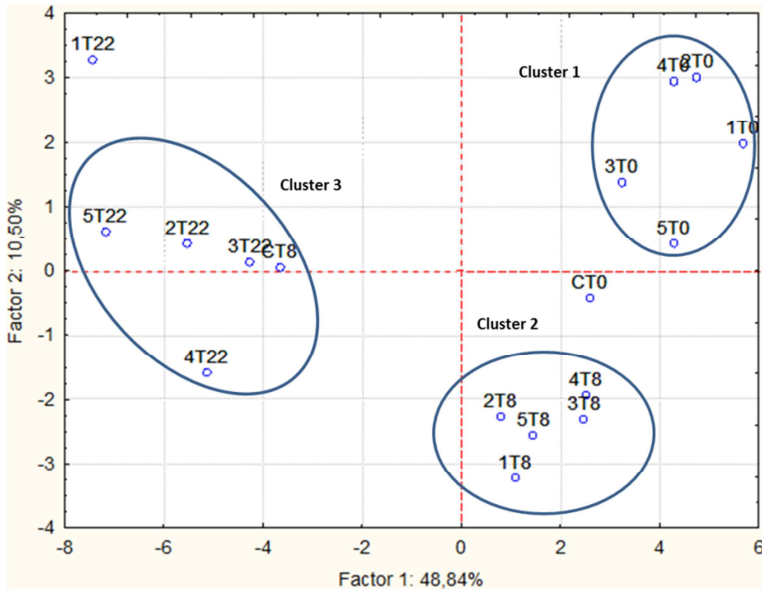


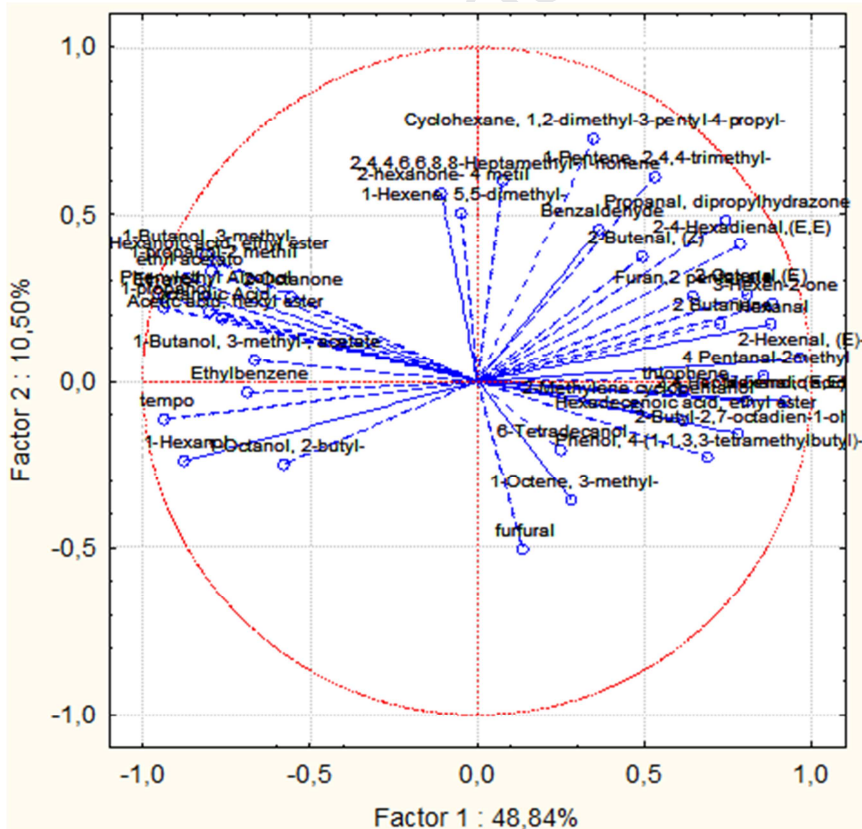
Figure 5.

a



C: 0.1 MPa (control); 1: sample treated at 0.1 MPa added of NE obtained after 14c at 300 MPa; 2: sample added of NE (obtained after 14c at 300 MPa) and treated at 100 MPa x 1; 3: sample added of NE (obtained after 14c at 300 MPa) and treated at 100 MPa x 2; 4: sample added of NE (obtained after 14 c at300 MPa) and treated at 100 MPa x 2; 5: sample added of NE (obtained after 14 c at300 MPa) and treated at 200 MPa x 2

b



Highlights

Obtained Nanoemulsions were stable for more than one year

Obtained Nanoemulsions were colourless, water soluble, and gifted of antimicrobial properties

Obtained Nanoemulsions combined with high pressure homogenization increased juice shelf-life

Journal Pre-proof

Dear Editor in Chief

Hereby, I declare that no conflict of interest exists for the publication of the present research.

Faithfully

Francesca Patrignani

Journal Pre-proof