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Microfluidization of Ginger Rhizome (*Zingiber officinale* Roscoe) Juice: Impact of Pressure and Cycles on Physicochemical Attributes, Antioxidant, Microbial, and Enzymatic Activity

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1 **Microfluidization of Ginger Rhizome (*Zingiber Officinale Roscoe*) Juice: Impact of Pressure and Cycles on**
2 **Physico-chemical Attributes, Antioxidant, Microbial and Enzymatic Activity**

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Abstract

23 The aim of this study was to investigate the effects of high-pressure microfluidization on the physico-chemical
24 attributes, antioxidant, enzymatic and microbiological activity of ginger rhizome (*Zingiber officinale Roscoe*) juice
25 at different microfluidization pressures (68.94, 103.42, and 137.89 MPa) and cycles (1, 2, and 3). An important
26 physical attribute, the mean particle size of ginger rhizome juice, was significantly ($p < 0.05$) decreased from 15.85
27 (control) to 12.86 μm at 137.89 MPa and 1 cycle, which was further supported by microscopic inspection revealing
28 cell disintegration. Due to the release of phenolic compounds and pigments brought on by cell disruption, total
29 phenolic content, antioxidant activity assays DPPH, and ABTS values increased from 56.79 to 65.28 mg GAE/100
30 mL, 69.99 to 77.51% inhibition and 74.61 to 84.74% inhibition, respectively, at 103.42 MPa and 3 cycles. The
31 polyphenol oxidase (PPO) enzyme was shown to be baroresistant because microfluidization had no noticeable
32 impact on its relative activity. A higher microfluidization pressure of 137.89 MPa resulted in the complete reduction
33 of aerobic bacteria, yeast, and molds. Due to its solvent-free nature and success in microbial reduction,
34 microfluidization may aid in the delivery of high quality clean-labelled ginger juice. The findings demonstrated that
35 microfluidization treatment has the potential to produce clean-label, safe ginger juice with improved overall quality.
36 This study provides useful information to industries for the commercialization of ginger juice and will assist in
37 promoting the usage of undervalued rhizome species.

38 **Keywords:** Ginger; Novel processing; Antioxidant activity; Microbial safety; Juices.

39

40 1 Introduction

41 Ginger rhizome (*Zingiber officinale Roscoe*) is a popular spice known for its wide spectrum of therapeutic
42 properties. It contains carbohydrates (60–70%), crude fiber (3–8%), protein (9%), ash (8%), fatty oil (3–6%) and
43 volatile oil (2–3%). The characteristic flavor of ginger is due to zingerone, shogaols, gingerols, and volatile
44 (essential) oils, which constitute up to 3% of ginger on fresh weight basis (Ali et al. 2008). The composition of
45 volatile and non-volatile components in ginger varies depending on the species and climatic conditions. The volatile
46 fragrant essential oil of ginger contains mainly sesquiterpenoids, with α -zingiberene (30–70%) being the main
47 component, along with smaller amounts of other sesquiterpenoids: β -sesquiphellandrene (15–20%), β -bisabolene

48 (10–15%), and α -farnesene, and monoterpenoids (β -phellandrene, camphene, cineol, geraniol, citral, etc.). Ginger
49 also contains diterpenes and ginger glycolipids (Ali et al. 2008; Srinivasan 2017). Gingerol, shogaol, paradol and
50 zingerone (F. Chen et al., 2019; Shukla et al., 2019) are the key phytochemicals found in the ginger rhizome which
51 are accountable for different therapeutic properties, i.e. antioxidant, anti-inflammatory, neuroprotective, protective
52 effects against respiratory disorders, hepatoprotective, antiviral, anti-fungal, anti-parasitic, immunomodulatory,
53 gastrointestinal activity, anti-arthritic, hypoglycemic activity, anti-ulcer and relief in constipation and flatulence
54 (Ban et al., 2020). The abundance of phytochemicals contributes to the increased consumption of ginger in daily life.
55 It is consumed in different forms of products, including beverages, confectionery, bakery and health supplement
56 products. For example, ginger juice is used as a flavoring component in ice cream (Gabbi et al. 2018; Pinto et al.
57 2009). The addition of ginger rhizome juice to ice cream reduced the total solids, fat and protein content, and
58 overrun, while increased the antioxidant activity, total phenols and melting resistance (Gabbi et al. 2018).
59 Furthermore, it has been used in a ready-to-serve (RTS) herbal beverage (K Gaikwad 2012), yoghurt (Zhong et al.
60 2017) and Sandesh (Bandyopadhyay et al. 2008).

61 From a safety standpoint, thermal processing is a commonly used technique to inactivate natural enzymes and
62 detrimental microbes. However, when spices like ginger are exposed to high temperatures, they lose their vital
63 volatile components (D. Chen et al. 2016). Also, thermal processing has detrimental effects on the color, taste, and
64 nutrients of processed food products (Chakraborty et al. 2016). Non-thermal processing techniques, like high
65 pressure processing, high-energy pulsed electric fields and high pressure carbon dioxide, have been used to
66 inactivate the PPO enzyme and preserve the color of carrot juice (Koley et al. 2020a) and cloudy ginger juice (D.
67 Chen et al. 2016). Similarly, the application of high pressure microfluidization as a non-thermal technique has
68 intensified recently because of the flexibility of microfluidizers in processing conditions, as both pressure and cycles
69 can be optimized to specific requirements (Dhiman and Prabhakar 2021). Microfluidization is a novel technique that
70 includes slamming liquid-liquid or solid-liquid structures together at high velocity and strong shear (Kumar et al.
71 2022). This is achieved by splitting a pressurized liquid stream and directing it toward one another so that they
72 collide head-on in an interaction chamber (Suhag et al. 2022). Because of the collision and the high pressure in the
73 interaction chamber, shear and impact forces arise, resulting in particle size reduction, cellular disruption, and
74 structural alterations of food components (e.g. proteins and carbohydrates) (Bitik et al. 2019; Mert et al. 2014a;
75 Suhag et al. 2021).

76 Microfluidization has recently piqued the interest of researchers and industries due to a number of advantages: (i) it
77 is a green technology because it does not use any chemicals or solvents during processing; (ii) it produces smaller
78 and more uniform particle sizes; (iii) less processing time, therefore, reduced processing costs; (iv) very few to no
79 chances of contamination during processing; (v) easy scalability from small-batch processes to continuous
80 processing; (vi) there are no moving parts in the equipment, which increases its lifespan; and (vii) produces highly
81 consistent and reproducible results under the same operating parameters and conditions. Microfluidization has
82 mostly been used for the development of emulsions, such as pea globulin-based emulsion (Oliete et al. 2019), olive
83 oil-in-water pickering emulsion (Espinosa-Solís et al. 2021), gel-like emulsion with whey protein isolate (Y. Liu et
84 al. 2019), nanoemulsion containing natural antibacterial mixture of citral, trans-2-hexen-1-ol, and linalool (1:1:1
85 w/w) (Taghavi et al. 2018), double (water-in-oil-in-water) emulsion to carry hydrophilic (chlorophyllin) and/or
86 hydrophobic (lemongrass essential oil) active compounds (Artiga-Artigas et al. 2019) and rutin loaded oil-in-water
87 emulsion (Dammak and do Amaral Sobral 2017). Furthermore, it has also been used for the modification of legume
88 (chickpea and lentil) starch (Bitik et al. 2019) and as a milling process for wheat bran fibers to be used as an
89 functional ingredient in bakery products (Mert et al. 2014b).

90 Certain juices have been microfluidized at different pressure-cycle combinations, which has shown significant
91 changes in the physico-chemical, functional, microbial and enzymatic properties of microfluidized juices, however
92 the results vary from juice to juice. Koley et al., (2020b) observed an increase in the carotenoid content of
93 microfluidized carrot juice, but no clear trends for antioxidant activity and total phenolic content. Karacam et al.,
94 (2015) on microfluidizing strawberry juice reported that low pressure (60 MPa) had no significant effect, whereas,
95 high pressure (100 MPa) lead to an increase of 22% in antioxidant activity. Tarafdar et al. (2019) also showed an
96 increase in the total phenolic content, total flavonoid content and free radical scavenging activity of sugarcane juice
97 microfluidized at 120 MPa as opposed to a reduction at pressure of 100 MPa and 140 MPa. Furthermore, a recent
98 study by Ke et al. (2022) treated whole mango juice using an industrial scale microfluidizer and demonstrated that it
99 lead an improvement in the antioxidant activity. In the case of micronizing mushrooms and *P. pyrifolia* (Chinese
100 pear) juice, Liu et al., (2009) found that PPO was not inactivated, in contrast to the treatment of pressure and number
101 of cycles that increased the relative activity of PPO. On the contrary, Tarafdar, Kaur, et al. (2021) reported a
102 reduction of 39.4-64.7% in the PPO activity upon microfluidization of sugarcane juice, but complete inactivation
103 was not achieved.

104 According to Grand View Research, (2022) report, fruit juices held the largest market share in the global juice
105 industry in 2021, representing 61.2% of the total global revenue. Furthermore, the majority of studies described
106 above focused on the microfluidization of fruit juice and a few vegetable juice. D. Chen et al. (2016) reported that
107 the high hydrostatic pressure (500 MPa, 10 min) was better treatment compared to ultrahigh temperature (110 °C for
108 8.6 s) for keeping quality of cloudy ginger juice. This study only reported the results of one pressure level (500
109 MPa), during which there was an increase in temperature of 18 °C (from 20 to 38 °C), which may be detrimental
110 due to the longer holding time of 10 min in high hydrostatic pressure. As a result, high pressure microfluidization
111 that has a residence time of 1-5 milliseconds inside the interaction chamber (velocities up to 500 m/s) (Microfluidics
112 chamber user guide 2015) may be a viable alternative for processing ginger juice. In addition, there is very limited
113 research on the effects of microfluidization, which is being explored in this study to investigate ginger juice. In this
114 study, the authors aims to determine the resulting changes by microfluidization on physico-chemical properties and
115 antioxidant, enzymatic, and microbial activity of ginger juice at different pressures and cycle combinations. The
116 findings demonstrated that microfluidization treatment has the potential to produce clean-label, safe ginger juice
117 with improved overall quality. This study provides useful information to industries for the commercialization of
118 ginger juice and will assist promote the usage of undervalued rhizome species.

119 **2 Materials and methods**

120 **2.1 Materials**

121 Freshly harvested ginger rhizomes (*Zingiber officinale Roscoe*) were bought (in November 2022) from a local
122 market in Kundli, Haryana, India. Folin-Ciocalteu reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-
123 ethylbenzothiazoline-6-sulfonic acid (ABTS), gallic acid, sodium carbonate, potassium persulfate, methanol,
124 ethanol, phosphate and catechol were purchased from Sigma Aldrich, India. Whereas, plate count, peptone water
125 and potato dextrose agar were bought from HIMEDIA, India. All other reagents were of analytical grade.

126 **2.2 Ginger juice extraction**

127 Ginger rhizomes were manually sorted, graded, and cleaned before being peeled off with a stainless steel peeler.
128 Ginger was then subjected to a juicer (Bajaj JEX 16, India) to extract the juice, which was later filtered using a four-
129 fold muslin cloth as ginger juice is rich in fiber content. Traces of non-soluble fiber in the juice may clog the
130 microchannels of the microfluidizer. After filtration, the amount of juice extracted was measure and noted in liters

131 (L). Additionally, to determine the density of juice, a 50 mL empty measuring cylinder was placed on a weighing
132 balance (Sartorius AG Germany), and its weight was tared. The measuring cylinder was filled with 50 mL of juice,
133 and its weight (in g) was noted.

134 **2.3 Microfluidization treatment**

135 Ginger juice (200 mL) was microfluidized through an electric benchtop lab scale microfluidizer (M-110P,
136 Microfluidics Corp., MA, USA) equipped with a diamond fixed Y-type geometry (F12Y) interaction chamber (75
137 μm pore size), followed by an auxiliary processing module (APM) for fluid flow stabilization. Three microfluidizer
138 pressure levels: 68.94, 103.42 and 137.89 MPa, with three different numbers of cycles: 1, 2 and 3, were applied to
139 the ginger juice. Following each treatment, the ginger juice's temperature was immediately measured using a digital
140 thermometer (Mextech ST-9283B, resolution 0.1 $^{\circ}\text{C}$), before being collected in sterile bottles. For further qualitative
141 analysis, samples were kept at 4 $^{\circ}\text{C}$.

142 **2.4 Total soluble solids, pH and temperature**

143 The total soluble solids and pH values of the ginger juice samples were calculated using a pre-calibrated digital
144 refractometer (RX-70001, ATAGO, Japan) and pH meter (HANNA instruments, Italy). To determine the impact of
145 microfluidization parameters on the temperature of ginger juice, readings were taken before and after each cycle of
146 microfluidization using a handheld thermometer.

147 **2.5 Color**

148 The color characteristics (L^* , a^* and b^*) of the ginger juice samples were studied using a handheld digital Chroma
149 meter (CR- 400, Konica Minolta, Japan) calibrated using black and white reference plates as described by Koley et
150 al., (2014). Total color difference (ΔE^*) of microfluidized ginger juice compared to control was calculated using eq.
151 (1). Subscripts c and m represent the color values for control and microfluidized samples, respectively.

$$152 \quad \Delta E^* = \sqrt{(L_c^* - L_m^*)^2 + (a_c^* - a_m^*)^2 + (b_c^* - b_m^*)^2} \quad (1)$$

153 **2.6 Particle size analysis**

154 The particle size distribution of ginger juice samples was analyzed using liquid-mode in PSA 1090 (Anton Paar,
155 Austria). Debubbling and ultrasound were allowed as dispersion parameters, water was used as the solvent with a
156 refractive index of 1.329-1.330. After cleaning the machine with deionized water, the samples were placed in the

157 small volume dispersion unit and uniformly dispersed with a stirrer. Measurement started when the obscuration of
158 the samples reached about 1.5%. Measurements were taken in triplicate for each sample. Particle size measurements
159 of all the samples were taken and analyzed by the Kalliope™ software. The results were presented in terms of D₁₀,
160 D₅₀, and D₉₀ percentile values, which represent the particle sizes below which 10%, 50%, and 90% of all particles
161 are respectively located.

162 **2.7 Microscopic analysis**

163 A fluorescent microscope (Eclipse Ci-L, Nikon, Japan) was used to capture microscopic images to observe the effect
164 of microfluidization on the extent of particle breakdown in ginger juice. Analysis was carried out by pouring 20 µL
165 of the ginger juice samples on a clean glass slide and gently rotating the coverslip to a 45° angle to have samples
166 with the same orientation. Images were captured using 100x magnification and white light.

167 **2.8 Total phenolic content**

168 The total phenolic content of the ginger juice samples was evaluated using the Folin-Ciocalteu reagent as described
169 by Singleton et al., (1999) with some changes. In brief, diluted ginger juice samples (1:10) were mixed with 250 µL
170 Folin-Ciocalteu reagent. Next, the mixture was added with 500 µL (7.5% sodium carbonate), and 4 ml of distilled
171 water and incubated in the dark at 25 °C for 2 h. After incubation, absorbance readings were taken at 725 nm using a
172 spectrophotometer (SL159, Elico connecting science & Lab, India). Gallic acid was used (0–100 mg/mL) as a
173 standard. Average values of three independent replications were reported in milligrams of gallic acid equivalent per
174 100 milliliters mg GAE/100 mL..

175 **2.9 Antioxidant assays**

176 **2.9.1 DPPH assay**

177 The method proposed by Mishra et al., (2011) was used to determine the antioxidant activity using the DPPH
178 radical, with minor modifications. In brief, 3.9 mg of DPPH was dissolved in 100 mL methanol to prepare the stock
179 solution. Then, 3.9 mL of DPPH stock solution was added to dilute ginger juice (1:20) (100 µL) followed by
180 incubation in the dark at 25 °C for 1 h. Also, 3.9 mL of DPPH stock solution was mixed with 100 µL methanol to
181 prepare the control sample. Absorbance readings were taken at 517 nm using a spectrophotometer (SL159, Elico
182 connecting science & Lab, India) and the % inhibition of DPPH was determined using equation 2.

183
$$\% \text{ inhibition of DPPH} = \frac{\text{Abs Control} - \text{Abs Sample}}{\text{Abs Control}} \times 100 \quad (2)$$

184 **2.9.2 ABTS assay**

185 A mixture of ABTS stock solution (7 mM) and potassium persulfate (2.45 mM) in 1:1 v/v was kept at 25 °C for 18 h
186 to prepare the radical cation. Then, the mixture was diluted using ethanol until it had an optical density of 0.700 at
187 734 nm as described by Shalaby, (2013) with minor modifications. For analysis, 1mL of stable ABTS solution was
188 mixed with 100 µL of dilute ginger juice (1:20). Absorbance readings were taken at 734 nm using a
189 spectrophotometer (SL159, Elico connecting science & Lab, India) after a 30 min incubation period. An ABTS
190 solution without antioxidants was applied as the control and the % inhibition of ABTS was determined using
191 equation 3.

192
$$\% \text{ inhibition of ABTS} = \frac{\text{Abs Control} - \text{Abs Sample}}{\text{Abs Control}} \times 100 \quad (3)$$

193 **2.10 Polyphenol oxidase activity**

194 To determine the relative activity of the PPO enzyme, 500 µL of ginger juice was mixed with 2 mL of phosphate
195 buffer (50mM, pH 6.5) followed by the addition of 1000 µL of catechol (0.2 M) as described by Tarafdar, Kaur, et
196 al., (2021). Absorbance values were recorded at 420 nm for 0 min and at 4 min using a spectrophotometer (SL159,
197 Elico connecting science & Lab, India). The percentage reduction in PPO activity was determined using equation 4..

198
$$\% \text{ PPO activity reduction} = \left(\frac{\text{PPO}_c - \text{PPO}_m}{\text{PPO}_c} \right) \times 100 \quad (4)$$

199 Where, PPO_c and PPO_m are the activity of PPO in control and microfluidized ginger juice, respectively.

200 **2.11 Microbial analysis**

201 The total plate count was made by pouring plate count agar over 1000 µL of serially diluted (in peptone water)
202 ginger juice sample. Similarly, yeast and molds count was carried out by pouring potato dextrose agar on 1000 µL of
203 diluted juice sample. For total plate count and yeast and mold count, the plates were incubated for 24 h at 37 °C and
204 72 h at 25 °C, respectively. The results were presented in log₁₀ colony forming units (CFU) per mL (Batra et al.
205 2016).

206 **2.12 Statistical analysis**

207 All the analyses were made using three independent microfluidized and control ginger juice samples. Results were

208 reported as averages \pm standard deviations. Principal component analysis (PCA), correlation and two-way ANOVA
209 followed by Tukey's post hoc analysis ($p < 0.05$) were applied to determine the effects of microfluidization
210 parameters (pressure and number of cycles), using OriginPro 2021, version 9.8.0.200, OriginLab Corporation, USA.

211 **3 Results and discussion**

212 **3.1 Yield of ginger juice**

213 After sorting, grading, cleaning and peeling the procured sample, 2.76 kg of ginger was obtained, which was further
214 used for juice extraction. From this 2.76 kg of ginger, 1.20 L of juice was extracted. The yield of juice extraction
215 was estimated to be 42.57% based on the juice with a density of 0.9792 g/ml, obtained from the pulp (data not
216 shown). Because ginger contains a high percentage of non-soluble starch, nearly 50%, the juice yield was low.

217 **3.2 TSS, pH and temperature of ginger juice**

218 The effects of high-pressure microfluidization treatment on TSS and pH of ginger juice are summarized in Table 1.
219 Microfluidization parameters, both pressure and number of cycles showed a significant effect ($p < 0.05$) on the TSS
220 of ginger juice. TSS decreased from 3.35% (control) to 2.70% - 3.29% after microfluidization treatment. After first
221 microfluidization cycle, the TSS was reduced by 6.27%, 19.40%, and 16.12% at 68.94, 103.42, and 137.89 MPa,
222 respectively. Ginger juice that was subjected to a 103.42 MPa and 1-cycle treatment demonstrated the maximum
223 TSS decrease. It should be highlighted that the first microfluidization cycle decreased the TSS, however, the second
224 and third treatment cycles increased the TSS, with no significant difference within these cycles. This suggests that
225 the second microfluidization cycle was effective for mass transfer from the juice particles and further solubilization
226 in the surrounding medium. In terms of microfluidization pressure, the pressure of 103.42 MPa had a decreasing
227 effect, whereas raising the pressure to 137.89 MPa had an increasing effect, demonstrating that a moderate
228 microfluidization pressure has a noticeable effect on the TSS of ginger juice. TSS in the juice may be reduced due to
229 the release of insoluble solid particles, denaturation of enzymes (amylase, proteases and polygalacturonases), or the
230 loss of the components (sugars and organic acids) that make up the soluble solids in the juice during processing (M.
231 Chen et al. 2017). For instance, when juice is homogenized at high pressure, a little proportion of soluble protein's
232 hydrophobic structure is revealed. Subsequently, more hydrophobic areas are produced, which reduces the protein's
233 solubility and lowers the TSS (Guo et al. 2016). Similar results were reported after microfluidization of sugarcane
234 (Tarafdar and Kaur 2021), sea buckthorn (Abliz et al. 2021), and pomegranate juice (M. Chen et al. 2017).
235 Microfluidization pressure had a significant ($p < 0.05$) effect on the pH of ginger juice, whereas microfluidization

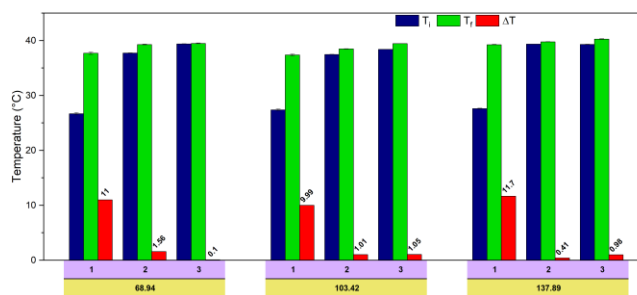
236 cycles had a non-significant ($p>0.05$) effect. At lower pressure (68.94 MPa), the pH value was practically identical
 237 to that of the control (6.55) (Table 1). Microfluidization pressure of 103.42 MPa caused a small increase in pH, and
 238 increasing the pressure to 137.89 MPa led to no significant difference ($p>0.05$) within these pressures. The pH value
 239 indicates the concentration of free H^+ ions. During microfluidization treatment, the breakdown of cellular
 240 components such as pectin (J. Chen et al. 2012) or proteins might reduce the concentration of free H^+ , resulting in a
 241 slight increase in pH (Abliz et al. 2021).

242 The microfluidization treatment raised the temperature of ginger juice. The temperature of ginger juice changed the
 243 most after the first cycle and little after the second and third cycles at all microfluidization pressures (Figure 1).
 244 Overall, the maximum temperature change of 11.7 °C was recorded after the first cycle at 137.89 MPa, this increase
 245 was lower than the rise of 18 °C observed during the high pressure processing of cloudy ginger juice at 500 MPa for
 246 10 min (D. Chen et al. 2016). Furthermore, all of the microfluidization pressures had similar final temperatures after
 247 the third cycle, and all of the final temperatures were lower than 40 °C. The temperature of the microfluidized ginger
 248 juice was raised as a result of high velocity, extreme shear, and cavitations in the interaction chamber of the
 249 microfluidizer (Dhiman and Prabhakar 2021). When the juice passes through the interaction chamber, the provided
 250 compression energy is turned into kinetic energy of the juice, which is then converted into friction energy, resulting
 251 in a temperature rise that increases as the number of cycles increase (Diels and Michiels 2006).

252 **Table 1:** TSS and pH of control and microfluidized ginger juice samples.

Microfluidization pressure (MPa)	Cycles	TSS (%)	pH
Control	0	3.35±0.03 ^{Aa}	6.55±0.03 ^{Bb}
	1	3.14±0.08 ^{Bc}	6.54±0.02 ^{Ba}
	2	3.29±0.05 ^{Bb}	6.56±0.03 ^{Ba}
68.94	3	3.22±0.06 ^{Bb}	6.56±0.03 ^{Ba}
	1	2.70±0.08 ^{Dc}	6.69±0.01 ^{Aa}
	2	2.75±0.06 ^{Db}	6.70±0.02 ^{Aa}
103.42	3	2.78±0.03 ^{Db}	6.77±0.02 ^{Aa}
	1	2.81±0.03 ^{Cc}	6.76±0.01 ^{Aa}
	2	2.97±0.04 ^{Cb}	6.70±0.01 ^{Aa}
137.89	3	2.94±0.04 ^{Cb}	6.72±0.02 ^{Aa}

253 Data were expressed as averages ± standard deviations from measurements of triplicate experiments.
 254 Average ± standard deviation that do not share the superscript letters capital case (pressure) and small case (cycle)
 255 are significantly different ($p < 0.05$).



257

258 **Figure 1:** Change in the temperature of ginger juice after microfluidization treatment. T_i = Inlet temperature of
 259 ginger juice before microfluidization treatment (°C); T_f = Outlet temperature of ginger juice after microfluidization
 260 treatment (°C); ΔT = Rise in temperature of ginger juice due to microfluidization treatment (°C); 1, 2 and 3 on x-
 261 axis represent the number of cycles and 68.94, 103.42 and 137.89 represent the microfluidization pressure in MPa.

262 3.3 Color

263 Due to its inherent ability to predict changes in food products, color is regarded as one of the most crucial food
 264 product characteristics. Juice pigments, which are influenced by enzymatic activity, phenolic content, particle size
 265 reduction, and temperature changes, are what cause color variations in juices. The lightness of microfluidized ginger
 266 juice was significantly influenced by microfluidization pressure ($p < 0.05$), but not by the number of cycles ($p < 0.05$)
 267 (Table 2). At the highest pressure of 137.89 MPa, a maximum mean value of 52.85 for lightness was noted.
 268 Microfluidization increased the greenness value from -2.63 (control) to -4.06 (103.42 MPa and 1 cycle), but the
 269 effect of varying the microfluidization pressure and number of cycles was non-significant ($p > 0.05$). Furthermore,
 270 microfluidized ginger juice had lower yellowness values than the control. Microfluidization pressure of 68.94 MPa
 271 reduced yellowness values from 20.97 (control) to 16.53 (3 cycles), while increasing the pressure to 103.42 MPa
 272 resulted in a non-significant ($p > 0.05$) reduction, but applying pressure of 137.89 MPa resulted in a significant
 273 ($p < 0.05$) reduction with a mean value of 16.88. Similarly, the number of cycles reduced the yellowness with a
 274 statistically significant ($p < 0.05$) effect (Table 2). The change in color (ΔE^*) of microfluidized samples was also
 275 affected in similar pattern. Microfluidization pressures of 68.94 and 103.42 MPa had a non-significant effect
 276 ($p > 0.05$) on ΔE^* , while a pressure of 137.89 MPa caused a significant ($p < 0.05$) increase. Additionally, the number of
 277 cycles had a significant ($p < 0.05$) effect, ΔE^* increasing with the number of cycles. Thereby, a maximum ΔE^* of 5.18
 278 was observed at 137.89 MPa and 3 cycles. Karacam et al., (2015) observed similar patterns after the
 279 microfluidization of strawberry juice. Also, hydrodynamic cavitation processing showed reduction in yellowness of
 280 aonla juice with an increase in pressure (Annapoorna et al. 2023). Curcumin, demethoxycurcumin, and 6-

281 dehydrogingerdione were identified as the major compounds responsible for ginger's yellow color (Yamaguchi et al.
 282 2010). Color is influenced not only by the pigment content of the food, but also by its physical structure and light-
 283 scattering capabilities. Microfluidization reduces particle size (Table 3), which increases surface area and, as a
 284 result, light-scattering properties (Sharma et al. 2021).

285 **Table 2:** Color parameters of control and microfluidized ginger juice.

Microfluidization pressure (MPa)	Cycles	L*	a*	b*	ΔE
Control	0	52.08±0.88 ^{Bb}	-2.63±0.55 ^{Aa}	20.97±0.57 ^{Aa}	-
	1	52.84±0.39 ^{ABa}	-3.60±0.31 ^{Bb}	18.54±0.37 ^{Bb}	2.76±0.39 ^{Bc}
68.94	2	52.37±0.29 ^{ABab}	-3.75±0.15 ^{Bb}	17.25±0.48 ^{Bc}	3.90±0.46 ^{Bb}
	3	52.68±0.47 ^{ABab}	-4.01±0.61 ^{Bb}	16.53±0.16 ^{Bd}	4.73±0.18 ^{Ba}
103.42	1	52.54±0.10 ^{Ba}	-4.06±0.12 ^{Bb}	17.92±0.17 ^{BCb}	3.41±0.15 ^{Bc}
	2	52.24±0.11 ^{Bab}	-3.77±0.10 ^{Bb}	17.25±0.26 ^{BCc}	3.90±0.25 ^{Bb}
	3	52.47±0.27 ^{Bab}	-3.93±0.10 ^{Bb}	16.73±0.10 ^{BCd}	4.46±0.03 ^{Ba}
137.89	1	52.85±0.14 ^{Aa}	-3.62±0.11 ^{Bb}	17.67±0.10 ^{Cb}	3.54±0.10 ^{Ac}
	2	53.13±0.19 ^{Aab}	-3.94±0.10 ^{Bb}	17.10±0.13 ^{Cc}	4.27±0.18 ^{Ab}
	3	52.76±0.10 ^{Aab}	-3.63±0.10 ^{Bb}	15.94±0.10 ^{Cd}	5.18±0.10 ^{Aa}

286 Data were expressed as averages ± standard deviations from measurements of triplicate experiments.
 287 Average ± standard deviation that do not share the superscript letters capital case (pressure) and small case (cycle)
 288 are significantly different (p < 0.05).

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Table 3: Particle size of control and microfluidized ginger juice.

Microfluidization pressure (MPa)	Cycles	D ₁₀ (μm)	D ₅₀ (μm)	D ₉₀ (μm)	Mean particle size (μm)
Control	0	4.02±0.01 ^{Aa}	16.04±0.02 ^{Aa}	24.03±0.63 ^{Aa}	15.85±0.07 ^{Aa}
	1	3.60±0.05 ^{Ab}	14.34±0.11 ^{BCb}	23.06±0.52 ^{ABab}	14.56±0.10 ^{Bb}
68.94	2	3.92±0.05 ^{Aa}	14.51±0.10 ^{BCb}	21.66±1.06 ^{Abb}	14.57±0.09 ^{Bb}
	3	4.25±0.33 ^{Aab}	14.63±0.13 ^{BCb}	22.14±1.41 ^{ABab}	14.83±0.05 ^{Bb}
103.42	1	4.04±0.01 ^{Ab}	15.50±0.16 ^{Bb}	23.35±1.07 ^{ABab}	14.72±0.14 ^{Bb}
	2	4.05±0.02 ^{Aa}	14.57±0.19 ^{Bb}	22.62±0.54 ^{Abb}	14.68±0.20 ^{Bb}

	3	3.61±0.09 ^{Aab}	14.10±0.10 ^{Bb}	22.57±0.60 ^{ABab}	14.06±0.10 ^{Bb}
137.89	1	2.63±0.04 ^{Bb}	13.25±0.10 ^{Cb}	21.89±1.08 ^{Bab}	12.86±0.11 ^{Cb}
	2	3.55±0.07 ^{Ba}	14.35±0.22 ^{Cb}	22.46±0.94 ^{Bb}	13.16±0.10 ^{Cb}
	3	3.54±0.11 ^{Bab}	14.39±0.15 ^{Cb}	22.32±1.05 ^{Bab}	13.19±0.07 ^{Cb}

295 Data were expressed as averages ± standard deviations from measurements of triplicate experiments.
 296 Average ± standard deviation that do not share the superscript letters capital case (pressure) and small case (cycle)
 297 are significantly different ($p < 0.05$).

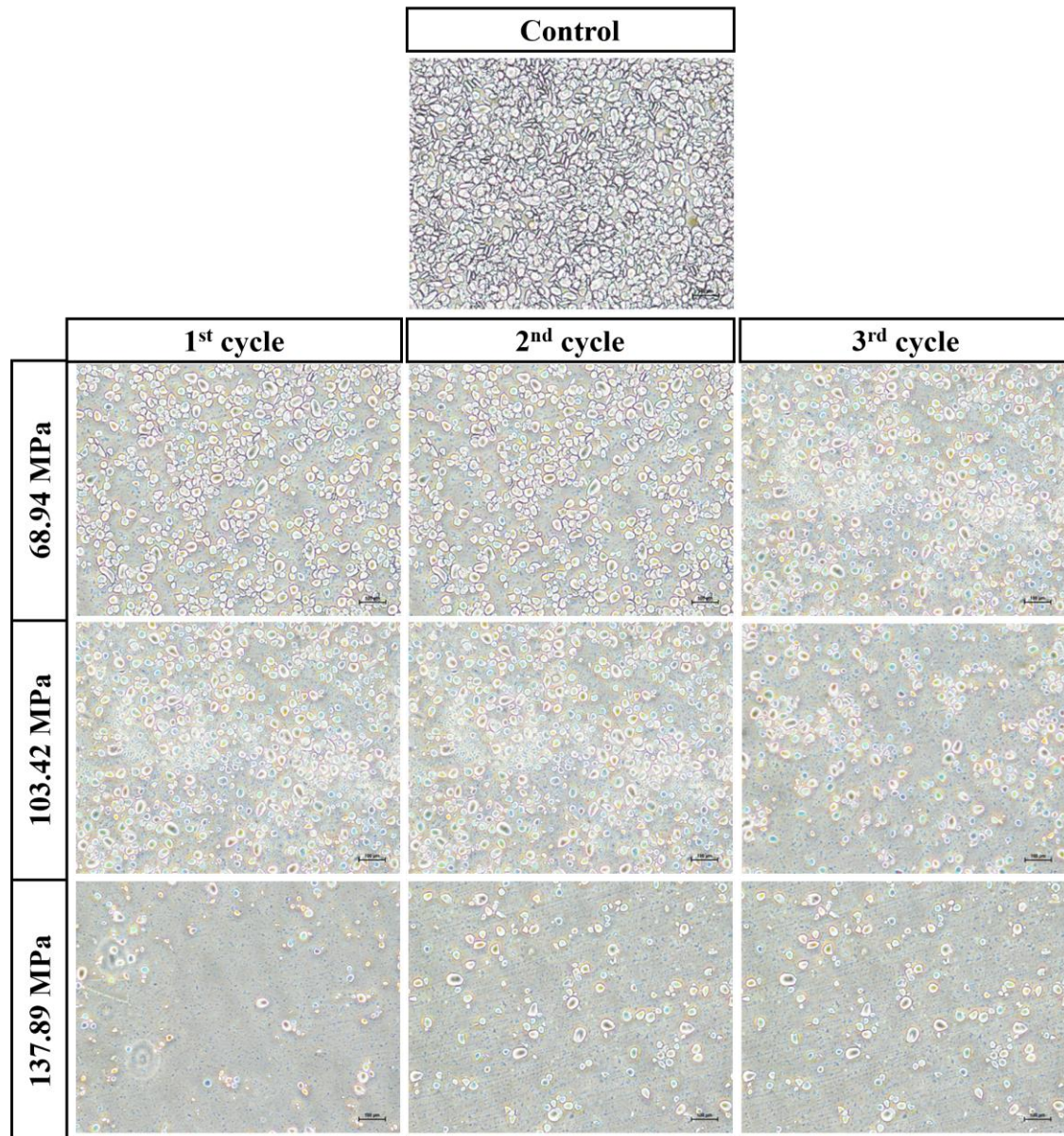
298 3.4 Particle size

299 Particle size plays a significant role in the rheological and sedimentation properties of juice, as well as in its
 300 appearance and changes at the macromolecular level. When compared to the control sample, the microfluidization
 301 treatment decreased the D_{10} , D_{50} , D_{90} , and mean particle size of ginger juice. Microfluidization pressure
 302 significantly ($p < 0.05$) decreased the D_{10} particle size from 4.02 μm (control) to 2.63 μm (137.89 MPa and 1 cycle),
 303 whereas the number of cycles caused D_{10} particle size to increase significantly ($p < 0.05$) up to the second
 304 microfluidization cycle treatment. Further increase in the number of cycles had a statistically non-significant
 305 ($p > 0.05$) effect, but the values were still lower than the control sample (Table 3). Surprisingly, the D_{10} particle size
 306 was found to be 4.25 μm at a microfluidization pressure of 62.94 MPa and 3 cycles, which is higher than that of the
 307 control sample. With an increase in microfluidization pressure, the D_{50} particle size showed a statistically significant
 308 ($p < 0.05$) reduction of about 2 μm and a statistically non-significant ($p > 0.05$) increase with an increase in the number
 309 of cycles, but it did not exceed the value of the control sample (16.034 μm). In addition, the microfluidization
 310 treatment lead to a reduction in the D_{90} particle size of ginger juice compared to the control sample. However,
 311 altering the microfluidization parameters, including both pressure and number of cycles, did not show any
 312 statistically significant ($p > 0.05$) effect on the D_{90} particle size. Comparing the treated samples to the control sample,
 313 microfluidization decreased the mean particle size. When the microfluidization pressure was increased, the mean
 314 particle size decreased significantly ($p < 0.05$), with mean values of 14.65, 14.48, and 13.07 μm at 68.94, 103.42, and
 315 137.89 MPa, respectively. When the number of cycles were increased, the mean particle size increased, but the
 316 effect was not statistically significant ($p > 0.05$). Overall, a common pattern observed was a decrease in the particle
 317 size with an increase in pressure, and an increase in the particle size with an increase in the number of cycles at the
 318 same pressure. Similar results were observed with the microfluidization of sea buckthorn juice by Abliz et al.,
 319 (2021) at different pressure-cycles combinations. Aggregation of protein particles at high temperatures justified the
 320 increase in particle size with an increase in the number of cycles, which may be similar to ginger juice as the

321 temperature of microfluidized ginger juice increases with an increase in the number of cycles (Figure 1). Moreover,
322 microfluidization works on the combination of powerful shear, high frequency vibration, high velocity impact,
323 instantaneous pressure drops and cavity effect (Suhag et al. 2021). The multiple forces produced in microfluidization
324 could lead to a smaller particle size of ginger juice with an increase in the microfluidization pressure. Similar,
325 observations of reduction in particle with increase in pressure were made by Annapoorna et al. (2023) for
326 hydrodynamic cavitation processed aonla juice due to shockwaves, strong turbulence, and violent collapsing of
327 microbubbles to form microjets. Furthermore, changes in particle size may be due to changes in starch, as increasing
328 pressure changes the structure, crystallinity, gelatinization temperature, enthalpy of gelatinization, and solubility of
329 starch (X. M. Wang et al. 2018). Microscopic analysis (discussed in section 3.5) revealed that the small starch
330 particles tend to aggregate into larger ones. This aggregation process may result from partial gelatinization (Song et
331 al. 2013), and it could be the underlying cause for the observed changes in particle size of microfluidized ginger
332 juice. .

333 3.5 Microscopic analysis

334 The control sample showed higher aggregation than all other samples, which broke down more as the pressure
335 increased. A decrease in particle size with an increase in pressure can be observed in Figure 2. Among the three
336 pressure levels, the maximum decrease in particle size can be observed at 137.89 MPa, compared to 68.94 and
337 103.42 MPa. These findings imply that following microfluidization treatment, more cellular contents are liberated.
338 At the same time, more tiny particles made of bits of cell wall and cell contents started to appear. The findings of
339 Jurić et al., (2019) also demonstrated that after homogenization, cell clusters and cell walls were damaged. Particle
340 disruption may cause intact cell walls to rupture, disrupted cells to break, and disrupted cells to release intracellular
341 components, changing the composition, size, and polydispersity of suspended particles (Rojas et al. 2016). However,
342 an increase in the number of cycles showed an increase in the mean diameter at the same pressure, which might be
343 due to the formation of aggregates. Overall, the microscope images showed a gradual disruption of ginger juice cells
344 with increasing microfluidization pressure, which aligns with the reduction in particle size displayed in Table 3.



345
346 **Figure 2:** Control and microfluidized ginger juice under 100x microscope view – the providence of particles.
347

348 3.6 Total phenolic content

349 The total phenolic content of the ginger juice samples shown in Table 4 was analyzed using the Folin-Ciocalteu
350 method. Total phenolic content was significantly ($p < 0.05$) affected by microfluidization cycles, whereas pressure
351 had a non-significant ($p > 0.05$) effect. The total phenolic content of the control sample was 56.79 mg GAE/100 mL,
352 which was close to the value of 57.40 mg GAE/100 mL for cloudy ginger juice reported by Chen et al., (2016). In
353 comparison to the control, the total phenolic content decreased considerably after the first microfluidization cycle at

354 68.94 MPa, with a mean value of 51.35 mg GAE/100 mL. This decrease after first microfluidization cycle can be
 355 attributed to the loss of heat-sensitive ginger's sesquiterpene compounds, which form phenolic compounds (Jugreet
 356 et al. 2020), due to localized heat generation ($\Delta T = 11^\circ\text{C}$) inside the interaction chamber. However, the total
 357 phenolic content increased to a mean value of 63.36 (68.94 MPa, second cycle) and 65.28 mg GAE/100 mL (103.42
 358 MPa, third cycle), both of which were higher than the control value. This increase in the total phenolic content can
 359 only be explained by considering that the disruption of cellular membrane and release of intracellular material,
 360 phenolic compounds and pigments dominated the thermal breakdown brought about by the small increase in the
 361 temperature ($\Delta T = 1.56^\circ\text{C}$ and 1.05°C). Similarly, the increased total phenolic content of ginger after convective
 362 drying at 60°C was attributed to the release of bound phenolic compounds brought about by the breakdown of cell
 363 wall (Mustafa and Chin 2023). Comparable results were described by Tarafdar, Kumar, et al., (2021), showing a
 364 significant effect of the number of cycles and no prominent impact of microfluidization pressure on the total
 365 phenolic content of sugarcane juice.

366 **Table 4:** Total phenolic content and antioxidant activity of control and microfluidized ginger juice.

Microfluidization pressure (MPa)	Cycles	Total phenolic content (mg GAE/100 mL)	DPPH (% inhibition)	ABTS (% inhibition)
Control	0	56.79±0.26 ^{Aab}	69.99±0.10 ^{Aab}	74.61±0.16 ^{Aab}
	1	51.35±0.30 ^{Ab}	56.63±0.10 ^{Ab}	73.20±0.16 ^{Ab}
	2	63.36±0.20 ^{Aab}	72.49±0.17 ^{Aa}	82.13±0.20 ^{Aab}
68.94	3	53.44±0.19 ^{Aa}	67.50±0.21 ^{Aa}	73.67±0.12 ^{Aa}
	1	47.23±0.29 ^{Ab}	59.24±0.11 ^{Ab}	70.85±0.10 ^{Ab}
	2	52.13±0.10 ^{Aab}	63.34±0.26 ^{Aa}	74.61±0.14 ^{Aab}
103.42	3	65.28±0.14 ^{Aa}	77.51±0.22 ^{Aa}	84.74±0.18 ^{Aa}
	1	56.50±0.28 ^{Ab}	69.75±0.10 ^{Ab}	79.99±0.24 ^{Ab}
	2	56.21±0.12 ^{Aab}	69.64±0.15 ^{Aa}	80.09±0.16 ^{Aab}
137.89	3	58.05±0.13 ^{Aa}	69.50±0.40 ^{Aa}	81.40±0.10 ^{Aa}

367 Data were expressed as averages ± standard deviations from measurements of triplicate experiments.
 368 Average ± standard deviation that do not share the superscript letters capital case (pressure) and small case (cycle)
 369 are significantly different ($p < 0.05$).

370 3.7 Antioxidant activity

371 To assess the antioxidant activity of ginger juice, DPPH and ABTS radicals were utilized as stable free radicals
 372 (Öztürk et al. 2007). Because of their potential to donate hydrogen atoms or electrons and trap free radicals, plant

373 extracts containing polyphenolic components exhibit antioxidant scavenging activity. DPPH scavenging activity
374 showed no significant ($p>0.05$) trend with an increase in pressure, and also no noticeable increase was observed with
375 respect to the control, except at 103.42 MPa and 3 cycles, which was 7.513 % more than that of control (Table 4).
376 DPPH scavenging activity and total phenolic content showed a strong correlation ($R^2 = 0.91$), which helps to explain
377 that maybe total phenolic compounds are the cause of the irregular trend. Similarly, for the ABTS assay, the
378 influence of the microfluidization treatment was irregular. In our study, the irregular effect of microfluidization on
379 antioxidant activity appears to be an inconsistent result. One interesting phenomenon was that the maximum values
380 for total phenolic content, DPPH scavenging, and ABTS antioxidant activity were all found at 103.42 MPa and the
381 third cycle. This variation in the findings of different assays may be explained by the fact that these assays are
382 influenced by various parameters such as the technique used, free radical generators used, and the complexity of the
383 molecular structure being tested (X. Wang et al. 2019). Similar findings and inferences on the DPPH and ABTS
384 assay for microfluidized peach juice were published by Wang et al., (2019).

385 **3.8 Enzymatic activity**

386 Polyphenol oxidase (PPO) activity was investigated to determine the change in enzymatic activity following
387 microfluidization treatment (Table 5). The microfluidization parameters, both pressure and number of cycles, caused
388 no significant ($p>0.05$) reduction in the PPO activity of ginger juice. Although a decrease of about 2% compared to
389 the control was observed, this change is not significant enough to warrant using this technology to reduce the
390 enzymatic activity of ginger juice. The activity of PPO in various fruit and vegetable juices has been shown to be
391 affected differently by microfluidization treatment. In sugarcane juice, PPO activity decreased significantly ($p<0.05$)
392 after microfluidization, according to Tarafdar, Kaur, et al., (2021). They also reported that sugarcane stems were
393 blanched before the extraction of juice, which may facilitate microfluidization to reduce the enzymatic activity.
394 While, Liu et al., (2009) found a considerable rise in the relative PPO activity in Chinese pear. They reported an
395 increase of 85.01% after microfluidization at 160 MPa and 3 cycles. Additionally, a reduction in enzymatic activity
396 is associated with changes in tertiary and quaternary structures brought on by the dissolution of a non-covalent
397 hydrogen bond, which typically requires pressures greater than 150 MPa (Porto et al. 2018). However, the maximum
398 pressure used in our study was 137.89 MPa, which could explain the insignificant reduction in ginger juice
399 enzymatic activity. Furthermore, Leite Júnior et al., (2016) predicted that it would be challenging to anticipate the
400 impact of high pressures on the inactivation of enzymes due to the involvement of multifactorial parameters, such as

401 operational parameters, sample pretreatments, product features, and enzyme characteristics.

402 **Table 5:** Polyphenol oxidase (PPO) enzyme activity and microbial count for control and microfluidized ginger juice.

Microfluidization (MPa)	pressure	Cycles	% PPO activity reduction	Total plate count (log ₁₀ CFU/mL)	Yeast and mold count (log ₁₀ CFU/mL)
Control		0	-	6.63±0.02 ^{Aa}	6.55±0.02 ^{Aa}
68.94		1	1.83±0.01 ^{Aa}	6.57±0.02 ^{Bb}	6.53±0.02 ^{Ab}
		2	1.14±0.02 ^{Aa}	6.51±0.01 ^{Bbc}	6.48±0.02 ^{Ab}
		3	1.68±0.03 ^{Aa}	6.47±0.03 ^{Bc}	6.36±0.01 ^{Ac}
103.42		1	1.93±0.03 ^{Aa}	6.01±0.02 ^{Cb}	5.21±0.04 ^{Bb}
		2	1.56±0.05 ^{Aa}	6.00±0.05 ^{Cbc}	5.04±0.04 ^{Bb}
		3	1.48±0.01 ^{Aa}	5.97±0.03 ^{Cc}	4.79±0.11 ^{Bc}
137.89		1	1.17±0.02 ^{Aa}	ND	ND
		2	2.34±0.02 ^{Aa}	ND	ND
		3	1.95±0.02 ^{Aa}	ND	ND

403 ND: Not detected; Data were expressed as averages ± standard deviations from measurements of triplicate
 404 experiments. Average ± standard deviation that do not share the superscript letters capital case (pressure) and small
 405 case (cycle) are significantly different (p < 0.05).

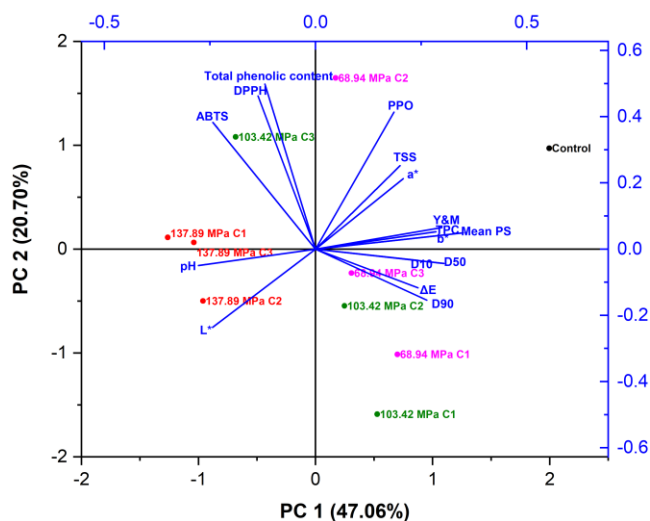
406 3.9 Microbiological analysis

407 The microbial count of control and microfluidized ginger juice is displayed in Table 5. The total plate, yeast, and
 408 mold counts in ginger juice were significantly (p<0.05) influenced by microfluidization parameters. As the
 409 microfluidization pressure and number of cycles increased, the total plate, yeast, and mold count decreased. The
 410 microbes in ginger juice could not be completely destroyed by lower pressures of 68.94 and 103.42 MPa and 3
 411 cycles. In contrast, the pressure of 137.89 MPa and 1 cycle was able to completely reduce the microbial count,
 412 producing a ginger juice that was microbiologically safe in terms of both aerobic and yeast and mold count,
 413 indicating that this pressure was safe and effective. Microfluidization exerts its main mechanism of destroying
 414 vegetative bacteria through mechanical forces, which encompass spatial pressure, turbulence, impingement,
 415 cavitations, and/or velocity gradients within the liquid (Diels and Michiels 2006). According to Aertsen et al.,
 416 (2009), the increased pressure causes changes in membrane fluidity, protein denaturation, and RNA transcription, all
 417 of which contribute to the inactivation of microorganisms. Diels & Michiels, (2006) asserted that the random motion
 418 of numerous eddies of different sizes is what causes velocity changes upon microfluidization. Eddies smaller than
 419 the cell dimensions produce an oscillating action of cell and cell fluid due to forces varying in intensity and scale,
 420 ultimately disrupting the cell. While, eddies larger than the cell dimensions cause the cell to be displaced.

421 Furthermore, it is known that high pressure cycling is more effective than continuous high pressure processing for
422 killing vegetative microbes (Maresca et al. 2011).

423 3.10 Principal component analysis (PCA)

424 Biplot of principle component analysis shown in Figure 3 explained nearly 68% of total variance. Data was widely
425 scattered for the different pressure and cycles combinations, suggesting that these combinations significantly
426 influenced the physico-chemical and functional attributes of ginger juice. Furthermore, as the number of cycles
427 increased, the score points for the lower (68.94 MPa) and moderate (103.42 MPa) microfluidization pressures
428 became increasingly scattered, signifying that the number of cycles had a greater impact at these pressure levels.
429 Conversely, the score points for the higher (137.89 MPa) microfluidization pressure remained more consistent as the
430 number of cycles increased, implying that the number of cycles had less of an effect at this pressure level. Loadings
431 of antioxidant assays (DPPH and ABTS) and total phenolic content were in the same direction, indicating a positive
432 correlation, as expected, given that the phenols contained in ginger juice may contribute to its antioxidant activity
433 (Tanweer et al. 2020). Furthermore, the antioxidant assays and total phenolic content loadings clustered near ginger
434 juice microfluidized at 103.42 MPa and 3 cycles, showing that this pressure cycle combination is suitable for
435 delivering ginger juice rich in phenolic compounds and enhanced antioxidant activity. Although the loadings of PPO
436 exhibited no cluster close to any pressure-cycle combination, indicating that the processing conditions used in this
437 study failed to inactivate the PPO enzyme present in the ginger juice.



438

439 **Figure 3:** Principal component analysis (PCA) score and loading plot of PC1 and PC2 of ginger rhizome juice.
440 Where, TSS – total soluble solids, L*- lightness, a*- redness, b*- yellowness, ΔE - total color difference, D10, D50,
441 D90 - the particle sizes below which 10%, 50%, and 90% of all particles are respectively located, Mean PS – mean
442 particle size, DPPH - % inhibition of DPPH, ABTS - % inhibition of ABTS, PPO - % Polyphenol oxidase activity
443 reduction, TPC – total plate count, Y&M – yeast and mold count, C1, C2 and C3 – number of cycles.

444 **4 Conclusion**

445 Microfluidization significantly affected the physico-chemical properties of ginger rhizome juice with minimal effect
446 on the polyphenol oxidase enzyme. Ginger rhizome juice underwent mild heating during microfluidization, with a
447 final temperature of less than 40°C. In addition, the microfluidization process resulted in a reduction in particle size
448 by cell breakage, which releases phenolic components and pigments, in turn affecting the antioxidant and color
449 characteristics. A higher microfluidization pressure of 137.89 MPa was effective in completely reducing the aerobic
450 microbes as well as the yeasts and molds, resulting in a microbes free ginger juice with increased antioxidant
451 activity. Overall, the microfluidization technique has the potential to deliver clean-label, safe, and high-quality
452 ginger juice. Also, microfluidized ginger rhizome juice may be used for the production of stable flavored beverages
453 such as ginger ale, ginger beer, ginger-infused teas, smoothies, as well as functional foods like energy bars, health
454 shots, and nutritional supplements. Further research will focus on the changes in volatile components, sensory
455 aspects, storage quality analysis and the development of different juice blends of ginger juice using
456 microfluidization treatment and the commercialization of microfluidized juices.

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464 **Authors' contributions**

465 **Rajat Suhag:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original
466 draft, Writing-revision and editing. **Shivam Singh:** Formal analysis, Investigation, Data curation. **Yogesh Kumar:**
467 Formal analysis, Investigation, Data curation, Writing – original draft. **Pramod K Prabhakar:** Conceptualization,
468 Writing-revision and editing, Project administration, Supervision. **Murlidhar Meghwal:** Conceptualization,
469 Writing-revision and editing, Project administration, Supervision.

470 All authors read and approved the final manuscript.

471 **Competing interests**

472 The authors have no relevant financial or non-financial interests to disclose.

473 **Availability of data and material/ Data availability**

474 Data available on request to the corresponding author.

475 **References**

476 Abliz, A., Liu, J., Mao, L., Yuan, F., & Gao, Y. (2021). Effect of dynamic high pressure microfluidization treatment
477 on physical stability, microstructure and carotenoids release of sea buckthorn juice. *LWT*.

478 <https://doi.org/10.1016/j.lwt.2020.110277>

479 Aertsen, A., Meersman, F., Hendrickx, M. E. G., Vogel, R. F., & Michiels, C. W. (2009). Biotechnology under high
480 pressure: applications and implications. *Trends in Biotechnology*. <https://doi.org/10.1016/j.tibtech.2009.04.001>

481 Ali, B. H., Blunden, G., Tanira, M. O., & Nemmar, A. (2008). Some phytochemical, pharmacological and
482 toxicological properties of ginger (*Zingiber officinale* Roscoe): a review of recent research. *Food and*
483 *chemical Toxicology*, *46*(2), 409–420.

484 Annapoorna, R. P., More, P. R., & Arya, S. S. (2023). Effect of pressure and time on bioactive content, PPO
485 inactivation, physicochemical and sensory properties of aonla (*Emblica officinalis*) juice during hydrodynamic
486 cavitation processing. *Food Science and Biotechnology*, *32*(1), 71–82. [https://doi.org/10.1007/s10068-022-](https://doi.org/10.1007/s10068-022-01164-2)
487 [01164-2](https://doi.org/10.1007/s10068-022-01164-2)

488 Artiga-Artigas, M., Molet-Rodríguez, A., Salvia-Trujillo, L., & Martín-Belloso, O. (2019). Formation of Double
489 (W1/O/W2) Emulsions as Carriers of Hydrophilic and Lipophilic Active Compounds. *Food and Bioprocess*

490 *Technology*, 12(3), 422–435. <https://doi.org/10.1007/s11947-018-2221-3>

491 Ban, Z., Zhang, J., Li, L., Luo, Z., Wang, Y., Yuan, Q., et al. (2020). Ginger essential oil-based microencapsulation
492 as an efficient delivery system for the improvement of Jujube (*Ziziphus jujuba* Mill.) fruit quality. *Food*
493 *Chemistry*. <https://doi.org/10.1016/j.foodchem.2019.125628>

494 Bandyopadhyay, M., Chakraborty, R., & Raychaudhuri, U. (2008). Antioxidant activity of natural plant sources in
495 dairy dessert (Sandesh) under thermal treatment. *LWT*. <https://doi.org/10.1016/j.lwt.2007.06.001>

496 Batra, N. G., Sharma, A., & Agarwal, N. (2016). Microbiological analysis of packed fruit juices locally available in
497 Jaipur, India. *International Journal of Pharma and Bio Sciences*.
498 <https://doi.org/10.22376/ijpbs.2016.7.4.b395-401>

499 Bitik, A., Sumnu, G., & Oztop, M. (2019). Physicochemical and Structural Characterization of Microfluidized and
500 Sonicated Legume Starches. *Food and Bioprocess Technology*, 12(7), 1144–1156.
501 <https://doi.org/10.1007/s11947-019-02264-4>

502 Chakraborty, S., Rao, P. S., & Mishra, H. N. (2016). Changes in Quality Attributes During Storage of High-Pressure
503 and Thermally Processed Pineapple Puree. *Food and Bioprocess Technology*, 9(5), 768–791.
504 <https://doi.org/10.1007/s11947-015-1663-0>

505 Chen, D., Pan, S., Chen, J., Pang, X., Guo, X., Gao, L., et al. (2016). Comparing the Effects of High Hydrostatic
506 Pressure and Ultrahigh Temperature on Quality and Shelf Life of Cloudy Ginger Juice. *Food and Bioprocess*
507 *Technology*. <https://doi.org/10.1007/s11947-016-1759-1>

508 Chen, F., Tang, Y., Sun, Y., Veeraraghavan, V. P., Mohan, S. K., & Cui, C. (2019). 6-shogaol, a active constituents
509 of ginger prevents UVB radiation mediated inflammation and oxidative stress through modulating Nrf2
510 signaling in human epidermal keratinocytes (HaCaT cells). *Journal of Photochemistry and Photobiology B:*
511 *Biology*. <https://doi.org/10.1016/j.jphotobiol.2019.111518>

512 Chen, J., Liang, R. H., Liu, W., Liu, C. M., Li, T., Tu, Z. C., & Wan, J. (2012). Degradation of high-methoxyl pectin
513 by dynamic high pressure microfluidization and its mechanism. *Food Hydrocolloids*.
514 <https://doi.org/10.1016/j.foodhyd.2011.12.018>

515 Chen, M., Xu, Y., & Zong, W. (2017). Effect of ultra-high pressure micro-jet on the quality of pomegranate juice.
516 *Food Research and Development*, 38(3), 81–85. <https://doi.org/10.3969/j.issn.1005-6521.2017.03.018>.

517 Cui, Y., Shi, Y., Bao, Y., Wang, S., Hua, Q., & Liu, Y. (2018). Zingerone attenuates diabetic nephropathy through

518 inhibition of nicotinamide adenine dinucleotide phosphate oxidase 4. *Biomedicine and Pharmacotherapy*.
519 <https://doi.org/10.1016/j.biopha.2018.01.051>

520 Dammak, I., & do Amaral Sobral, P. J. (2017). Formulation and Stability Characterization of Rutin-Loaded Oil-in-
521 Water Emulsions. *Food and Bioprocess Technology*, 10(5), 926–939. <https://doi.org/10.1007/s11947-017-1876-5>

522

523 Dhiman, A., & Prabhakar, P. K. (2021). Micronization in food processing: A comprehensive review of mechanistic
524 approach, physicochemical, functional properties and self-stability of micronized food materials. *Journal of*
525 *Food Engineering*, 292, 110248. <https://doi.org/10.1016/j.jfoodeng.2020.110248>

526 Diels, A. M. J., & Michiels, C. W. (2006). High-pressure homogenization as a non-thermal technique for the
527 inactivation of microorganisms. *Critical Reviews in Microbiology*.
528 <https://doi.org/10.1080/10408410601023516>

529 Espinosa-Solís, V., García-Tejeda, Y. V., Portilla-Rivera, O. M., & Barrera-Figueroa, V. (2021). Tailoring Olive Oil
530 Microcapsules via Microfluidization of Pickering o/w Emulsions. *Food and Bioprocess Technology*.
531 <https://doi.org/10.1007/s11947-021-02673-4>

532 Gabbi, D. K., Bajwa, U., & Goraya, R. K. (2018). Physicochemical, melting and sensory properties of ice cream
533 incorporating processed ginger (*Zingiber officinale*). *International Journal of Dairy Technology*, 71(1), 190–
534 197. <https://doi.org/10.1111/1471-0307.12430>

535 Grand View Research. (2022). *Fruit And Vegetable Juice Market Size, Share & Trends Analysis Report By Product*
536 *(Fruit, Vegetable Juices), By Distribution Channel (Supermarkets/Hypermarkets, Online), By Region, And*
537 *Segment Forecasts, 2022 - 2030*. [https://www.grandviewresearch.com/industry-analysis/fruit-vegetable-juice-](https://www.grandviewresearch.com/industry-analysis/fruit-vegetable-juice-market#:~:text=b.,USD 138.54 billion in 2022)
538 [market#:~:text=b.,USD 138.54 billion in 2022](https://www.grandviewresearch.com/industry-analysis/fruit-vegetable-juice-market#:~:text=b.,USD 138.54 billion in 2022).

539 Guo, X. J., Zong, W., Zhao, G. Y., Zhang, L. H., Wang, X. Y., & Wu, S. H. (2016). Effect of high pressure
540 microfluidization (HPM) on the physical stability of yam juice. *Science and Technology of Food Industry*,
541 37(17), 125–128.

542 Jugreet, B. S., Suroowan, S., Rengasamy, R. R. K., & Mahomoodally, M. F. (2020). Chemistry, bioactivities, mode
543 of action and industrial applications of essential oils. *Trends in Food Science and Technology*.
544 <https://doi.org/10.1016/j.tifs.2020.04.025>

545 Jurić, S., Ferrari, G., Velikov, K. P., & Donsi, F. (2019). High-pressure homogenization treatment to recover

546 bioactive compounds from tomato peels. *Journal of Food Engineering*.
547 <https://doi.org/10.1016/j.jfoodeng.2019.06.011>

548 K Gaikwad, K. (2012). Studies on the Development and Shelf Life of Low Calorie Herbal Aonla- Ginger RTS
549 Beverage by Using Artificial Sweeteners. *Journal of Food Processing & Technology*, 04(01).
550 <https://doi.org/10.4172/2157-7110.1000200>

551 Karacam, C. H., Sahin, S., & Oztop, M. H. (2015). Effect of high pressure homogenization (microfluidization) on
552 the quality of Ottoman Strawberry (F. Ananassa) juice. *LWT*. <https://doi.org/10.1016/j.lwt.2015.06.064>

553 Ke, Y., Chen, J., Dai, T., Xiao, M., Chen, M., Liang, R., et al. (2022). Industry-Scale Microfluidizer: a Novel
554 Technology to Improve Physiochemical Qualities and Volatile Flavor of Whole Mango Juice. *Food and*
555 *Bioprocess Technology*. <https://doi.org/10.1007/s11947-022-02979-x>

556 Koley, T. K., Nishad, J., Kaur, C., Su, Y., Sethi, S., Saha, S., et al. (2020a). Effect of high-pressure microfluidization
557 on nutritional quality of carrot (*Daucus carota* L.) juice. *Journal of Food Science and Technology*.
558 <https://doi.org/10.1007/s13197-020-04251-6>

559 Koley, T. K., Nishad, J., Kaur, C., Su, Y., Sethi, S., Saha, S., et al. (2020b). Effect of high-pressure
560 microfluidization on nutritional quality of carrot (*Daucus carota* L.) juice. *Journal of Food Science and*
561 *Technology*, 57(6), 2159–2168. <https://doi.org/10.1007/s13197-020-04251-6>

562 Koley, T. K., Singh, S., Khemariya, P., Sarkar, A., Kaur, C., Chaurasia, S. N. S., & Naik, P. S. (2014). Evaluation of
563 bioactive properties of Indian carrot (*Daucus carota* L.): A chemometric approach. *Food Research*
564 *International*, 60, 76–85. <https://doi.org/10.1016/j.foodres.2013.12.006>

565 Kumar, A., Dhiman, A., Suhag, R., Sehrawat, R., Upadhyay, A., & McClements, D. J. (2022). Comprehensive
566 review on potential applications of microfluidization in food processing. *Food Science and Biotechnology*,
567 31(1), 17–36. <https://doi.org/10.1007/s10068-021-01010-x>

568 Leite Júnior, B. R. de C., Tribst, A. A. L., & Cristianini, M. (2016). Comparative effects of high isostatic pressure
569 and thermal processing on the inactivation of *Rhizomucor miehei* protease. *LWT - Food Science and*
570 *Technology*. <https://doi.org/10.1016/j.lwt.2015.09.042>

571 Liu, W. E. I., Jianhua, L. I. U., Mingyong, X. I. E., Chengmei, L. I. U., Weilin, L. I. U., & Wan, J. I. E. (2009).
572 Characterization and high-pressure microfluidization-induced activation of polyphenoloxidase from chinese
573 pear (*Pyrus pyrifolia* Nakai). *Journal of Agricultural and Food Chemistry*. <https://doi.org/10.1021/jf9006642>

574 Liu, Y., Zhang, W., Wang, K., Bao, Y., Regenstein, J. Mac, & Zhou, P. (2019). Fabrication of Gel-Like Emulsions
575 with Whey Protein Isolate Using Microfluidization: Rheological Properties and 3D Printing Performance.
576 *Food and Bioprocess Technology*, 12(12), 1967–1979. <https://doi.org/10.1007/s11947-019-02344-5>

577 Maresca, P., Donsì, F., & Ferrari, G. (2011). Application of a multi-pass high-pressure homogenization treatment for
578 the pasteurization of fruit juices. *Journal of Food Engineering*. <https://doi.org/10.1016/j.jfoodeng.2010.12.030>

579 Mert, B., Tekin, A., Demirkesen, I., & Kocak, G. (2014a). Production of Microfluidized Wheat Bran Fibers and
580 Evaluation as an Ingredient in Reduced Flour Bakery Product. *Food and Bioprocess Technology*, 7(10), 2889–
581 2901. <https://doi.org/10.1007/s11947-014-1258-1>

582 Mert, B., Tekin, A., Demirkesen, I., & Kocak, G. (2014b). Production of Microfluidized Wheat Bran Fibers and
583 Evaluation as an Ingredient in Reduced Flour Bakery Product. *Food and Bioprocess Technology*, 7(10), 2889–
584 2901. <https://doi.org/10.1007/s11947-014-1258-1>

585 *Microfluidics chamber user guide*. (2015). [https://www.alfatest.it/keyportal/uploads/2017-microfluidics-chamber-](https://www.alfatest.it/keyportal/uploads/2017-microfluidics-chamber-user-guide.pdf)
586 [user-guide.pdf](https://www.alfatest.it/keyportal/uploads/2017-microfluidics-chamber-user-guide.pdf)

587 Mishra, B. B., Gautam, S., & Sharma, A. (2011). Shelf Life Extension of Sugarcane Juice Using Preservatives and
588 Gamma Radiation Processing. *Journal of Food Science*. <https://doi.org/10.1111/j.1750-3841.2011.02348.x>

589 Mustafa, I., & Chin, N. L. (2023). Antioxidant Properties of Dried Ginger (*Zingiber officinale* Roscoe) var.
590 Bentong. *Foods*, 12(1), 178. <https://doi.org/10.3390/foods12010178>

591 Oliete, B., Potin, F., Cases, E., & Saurel, R. (2019). Microfluidization as Homogenization Technique in Pea
592 Globulin-Based Emulsions. *Food and Bioprocess Technology*. <https://doi.org/10.1007/s11947-019-02265-3>

593 Öztürk, M., Aydoğmuş-Öztürk, F., Duru, M. E., & Topçu, G. (2007). Antioxidant activity of stem and root extracts
594 of Rhubarb (*Rheum ribes*): An edible medicinal plant. *Food Chemistry*, 103(2), 623–630.
595 <https://doi.org/10.1016/j.foodchem.2006.09.005>

596 Pinto, S. V., Patel, A. M., Jana, A. H., & Solanky, M. J. (2009). Evaluation of different forms of ginger as flavouring
597 in herbal ice cream. *Int. J. Food Sci. Technol. Nutr*, 3(1–2), 73–83.

598 Porto, B. C., Tribst, A. A. L., & Cristianini, M. (2018). Dynamic High Pressure Effects on Biopolymers:
599 Polysaccharides and Proteins. In *Biopolymers for Food Design* (pp. 313–350). Elsevier.
600 <https://doi.org/10.1016/B978-0-12-811449-0.00010-4>

601 Rojas, M. L., Leite, T. S., Cristianini, M., Alvim, I. D., & Augusto, P. E. D. (2016). Peach juice processed by the

602 ultrasound technology: Changes in its microstructure improve its physical properties and stability. *Food*
603 *Research International*. <https://doi.org/10.1016/j.foodres.2016.01.011>

604 Shalaby, A. (2013). Antioxidant compounds, assays of determination and mode of action. *African Journal of*
605 *Pharmacy and Pharmacology*, 7(10), 528–539. <https://doi.org/10.5897/AJPP2013.3474>

606 Sharma, H., Singh, A. K., Deshwal, G. K., Rao, P. S., & Kumar, M. D. (2021). Functional *Tinospora cordifolia*
607 (giloy) based pasteurized goat milk beverage: Impact of milk protein-polyphenol interaction on bioactive
608 compounds, anti-oxidant activity and microstructure. *Food Bioscience*.
609 <https://doi.org/10.1016/j.fbio.2021.101101>

610 Shukla, A., Goud, V. V., & Das, C. (2019). Antioxidant potential and nutritional compositions of selected ginger
611 varieties found in Northeast India. *Industrial Crops and Products*.
612 <https://doi.org/10.1016/j.indcrop.2018.10.086>

613 Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). [14] Analysis of total phenols and other
614 oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In *Methods in Enzymology* (pp.
615 152–178). [https://doi.org/10.1016/S0076-6879\(99\)99017-1](https://doi.org/10.1016/S0076-6879(99)99017-1)

616 Song, X., Zhou, C., Fu, F., Chen, Z., & Wu, Q. (2013). Effect of high-pressure homogenization on particle size and
617 film properties of soy protein isolate. *Industrial Crops and Products*.
618 <https://doi.org/10.1016/j.indcrop.2012.08.005>

619 Srinivasan, K. (2017). Ginger rhizomes (*Zingiber officinale*): A spice with multiple health beneficial potentials.
620 *PharmaNutrition*, 5(1), 18–28. <https://doi.org/10.1016/j.phanu.2017.01.001>

621 Suhag, R., Dhiman, A., Prabhakar, P. K., Sharma, A., Singh, A., & Upadhyay, A. (2022). Microfluidization of liquid
622 egg yolk: Modelling of rheological characteristics and interpretation of flow behavior under a pipe flow.
623 *Innovative Food Science & Emerging Technologies*, 81, 103119. <https://doi.org/10.1016/j.ifset.2022.103119>

624 Suhag, R., Dhiman, A., Thakur, D., Kumar, A., & Upadhyay, A. (2021). Physico-chemical and functional properties
625 of microfluidized egg yolk. *Journal of Food Engineering*, 294, 110416.
626 <https://doi.org/10.1016/j.jfoodeng.2020.110416>

627 Taghavi, E., Mirhosseini, H., Rukayadi, Y., Radu, S., & Biabanikhankahdani, R. (2018). Effect of Microfluidization
628 Condition on Physicochemical Properties and Inhibitory Activity of Nanoemulsion Loaded with Natural
629 Antibacterial Mixture. *Food and Bioprocess Technology*. <https://doi.org/10.1007/s11947-017-2037-6>

630 Tanweer, S., Mehmood, T., Zainab, S., Ahmad, Z., & Shehzad, A. (2020). Comparison and HPLC quantification of
631 antioxidant profiling of ginger rhizome, leaves and flower extracts. *Clinical Phytoscience*, 6(1), 12.
632 <https://doi.org/10.1186/s40816-020-00158-z>

633 Tarafdar, A., & Kaur, B. P. (2021). Microfluidization-Driven Changes in Some Physicochemical Characteristics,
634 Metal/Mineral Composition, and Sensory Attributes of Sugarcane Juice. *Journal of Food Quality*.
635 <https://doi.org/10.1155/2021/3326302>

636 Tarafdar, A., Kaur, B. P., & Pareek, S. (2021). Effect of Microfluidization on Deteriorative Enzymes, Sugars,
637 Chlorophyll, and Color of Sugarcane Juice. *Food and Bioprocess Technology*, 14(7), 1375–1385.
638 <https://doi.org/10.1007/s11947-021-02651-w>

639 Tarafdar, A., Kumar, Y., Kaur, B. P., & Badgajar, P. C. (2021). High-pressure microfluidization of sugarcane juice:
640 Effect on total phenols, total flavonoids, antioxidant activity, and microbiological quality. *Journal of Food*
641 *Processing and Preservation*, (March), 1–10. <https://doi.org/10.1111/jfpp.15428>

642 Tarafdar, A., Nair, S. G., & Pal Kaur, B. (2019). Identification of Microfluidization Processing Conditions for
643 Quality Retention of Sugarcane Juice Using Genetic Algorithm. *Food and Bioprocess Technology*, 12(11),
644 1874–1886. <https://doi.org/10.1007/s11947-019-02345-4>

645 Wang, X. M., Zhu, X. M., Zhang, N. H., Tu, Z. C., Wang, H., Liu, G. X., & Ye, Y. H. (2018). Morphological and
646 structural characteristics of rice amylose by dynamic high-pressure microfluidization modification. *Journal of*
647 *Food Processing and Preservation*. <https://doi.org/10.1111/jfpp.13764>

648 Wang, X., Wang, S., Wang, W., Ge, Z., Zhang, L., Li, C., et al. (2019). Comparison of the effects of dynamic high-
649 pressure microfluidization and conventional homogenization on the quality of peach juice. *Journal of the*
650 *Science of Food and Agriculture*. <https://doi.org/10.1002/jsfa.9874>

651 Yamaguchi, K., Kato, T., Noma, S., Igura, N., & Shimoda, M. (2010). The effects of high hydrostatic pressure
652 treatment on the flavor and color of grated ginger. *Bioscience, Biotechnology and Biochemistry*.
653 <https://doi.org/10.1271/bbb.90712>

654 Zhong, Z., Ming, W., & Feng, L. I. (2017). Effect of ginger juice on fermentative properties of yoghurt. URL
655 http://en.cnki.com.cn/Article_en/cjfdtotal-rylg200506007.htm. Accessed, 6.

656