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Abstract: Verjuice is an acidic unfermented unripe grape juice that has recently been proposed as an alternative to vinegar in different food preparations. In this study, pickled cucumber preserves were industrially prepared using two dilutions of verjuice as acidifying agent and compared with the traditional vinegar-pickled samples. Samples were chemically characterized and subjected to sensory analyses using a trained panel and consumers. The different acidifying agents resulted in pickled cucumbers giving different olfactory and gustatory evaluations. No differences in appearance and texture were found. Despite showing chemical and sensory differences, pickles preserved with verjuice and vinegar had similar overall liking scores for visual, olfactory and gustatory aspects. A key feature of pickles prepared with verjuice is their lack of acetic acid potentially conferring them an additional value, suggesting that verjuice represents a viable alternative to vinegar for the production of pickles with distinctive sensorial properties.

DIPARTIMENTO DI AGRONOMIA ANIMALI ALIMENTI
RISORSE NATURALI E AMBIENTE

DAFNAE

DEPARTMENT OF AGRONOMY FOOD NATURAL RESOURCES
ANIMALS AND ENVIRONMENT



UNIVERSITÀ
DEGLI STUDI
DI PADOVA

Padova, 31st July 2018.

Dear Editor in Chief,

Please find a copy of the manuscript entitled "*Sensory characterization of cucumbers pickled with verjuice as novel acidifying agent*" that we wish to submit for publication in Food Chemistry.

All authors have contributed significantly to the work and are in agreement regarding the content of the manuscript and its publication. The manuscript is an original contribution, and neither it nor one substantially similar has been published or submitted for publication elsewhere.

Verjuice, an unfermented juice obtained from pressing unripe grape berries, is considered an innovative food product and has been used within the food industry of several countries principally for adding acidity and flavor, but without the sharpness present in vinegar. It has been predominantly used as an alternative to vinegar and lemon juice in dressings on salads and meat, and as an ingredient in various drinks/appetizers. One of the key features of verjuice is that it is made from a by-product of the wine industry, and therefore represents an opportunity to convert a waste into a high value grape product.

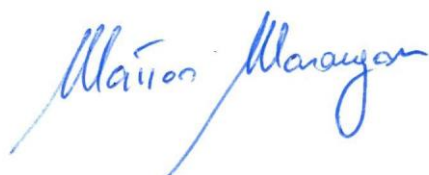
This manuscript assessed the use of verjuice as an acidifying ingredient in pickled cucumber preserves, which were industrially produced. We explored its chemical and sensorial properties in comparison to the commercial ingredient normally used in food preservation: vinegar. Several complementary sensory analyses (DA, TDS, Liking and Penalty analysis on JAR) were employed to profile different products and to get information about the drivers of liking and priorities of future reformulation. Our findings clearly show that verjuice can be used as an alternative to vinegar for the production of pickled cucumbers, and that it has the potential to be used for preservation of other vegetables via direct acidification.

We believe that this manuscript should satisfy the quality standard of Food Chemistry, and we consider that the new information presented in this article will be well received by researchers interested in pursuing the study on the potential applications of verjuice in different sectors of the food industry, as well as by researcher interested in the chemical and sensorial characterisation of commercial and novel food products.

Thank you for your consideration.

Sincerely,

Dr Matteo Marangon

A handwritten signature in blue ink that reads "Matteo Marangon". The signature is written in a cursive style with a long, sweeping underline that extends under both names.

Highlights

- Verjuice was used as acidifying ingredient for pickling in industrial conditions
- Pickles in verjuice were appreciated as the standard commercial product in vinegar
- Verjuice concentration affected pickles' taste and aroma, not appearance and texture
- Pickles in verjuice showed less issues for reformulation than those in vinegar
- Verjuice is a viable alternative to vinegar for pickled cucumbers

1 **Sensory characterization of cucumbers pickled with verjuice as**
2 **novel acidifying agent**

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13

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16 **Abstract**

17 Verjuice is an acidic unfermented unripe grape juice that has recently been
18 proposed as an alternative to vinegar in different food preparations. In this
19 study, pickled cucumber preserves were industrially prepared using two
20 dilutions of verjuice as acidifying agent and compared with the traditional
21 vinegar-pickled samples. Samples were chemically characterized and subjected
22 to sensory analyses using a trained panel and consumers. The different
23 acidifying agents resulted in pickled cucumbers giving different olfactory and
24 gustatory evaluations. No differences in appearance and texture were found.
25 Despite showing chemical and sensory differences, pickles preserved with
26 verjuice and vinegar had similar overall liking scores for visual, olfactory and
27 gustatory aspects. A key feature of pickles prepared with verjuice is their lack of
28 acetic acid potentially conferring them an additional value, suggesting that
29 verjuice represents a viable alternative to vinegar for the production of pickles
30 with distinctive sensorial properties.

31

32 **Keywords:** Acidifying; Consumers; Cucumbers; Pickling; Sensory Analysis;
33 Verjuice; Vinegar.

34 **1. Introduction**

35 A widespread approach for preserving vegetables is pickling, a technique based
36 on the addition of salt and/or an acidic liquid as primary means of preservation.
37 Direct acidification, with or without pasteurization, is applied to vegetable foods
38 to improve their shelf life and quality, as well as to enhance their palatability. In
39 particular, pickling is generally performed by the addition of an organic acid,
40 which can be coupled to pasteurization, addition of other preservatives
41 (normally sulfur dioxide or potassium sorbate), refrigerated storage or a
42 combination of these treatments, resulting in unfermented preserves with
43 distinctive sensorial properties (Siddid & Uebersax, 2018). Indeed, in addition to
44 the preserving effects against microbial spoilage obtained by reducing the pH,
45 these acids can also affect also the organoleptic properties of the preserved
46 food. Traditionally, this type of processing is obtained by using acid liquids
47 based on vinegar, which is largely employed as preservative for several food
48 preparations, providing them with a peculiar acetic flavor, a feature that can be
49 appreciated or not by consumers.

50 However, other natural acidic liquids seem to have the potential to be used for
51 direct acidification of vegetables. Among these, verjuice, the liquid with high
52 acidity obtained from pressing unripe grape berries (Nikfardjam, 2008; Öncül &
53 Karabiyikli, 2015), could be potentially used as an alternative to vinegar.
54 Actually, verjuice has been shown to be a valid alternative to lemon juice in
55 different food preparation such as salad seasoning, flavoring agent, digestive
56 drinks and some cocktails (Nikfardjam, 2008; Setorki, Asgary, Eidi, Rohani, &
57 Khazaei, 2010; Dupas de Matos et al., 2017; Dupas de Matos et al., 2018),
58 although the sensorial consequences of its use as a preserving agent for direct

59 acidification of vegetables has never been investigated. This is of particular
60 interest because verjuice lacks acetic acid, its acidity being due to non-volatile
61 tartaric and malic acids (Dupas de Matos et al., 2017). It is also noteworthy that
62 unripe grape products (e.g. verjuice, sour grape sauce, etc.) have gained
63 popularity among consumers due to their organoleptic, chemical and antioxidant
64 properties (Öncül & Karabiyikli, 2015). Because of this increasing interest in
65 using verjuice in food applications, the present study was undertaken to
66 evaluate the sensorial effects of its use as the substitute of vinegar in vegetable
67 preserves by using pickled cucumbers as a model. Specifically, cucumber
68 (*Cucumis sativus*) fruits, which are among the most popular vinegar-pickled
69 vegetables, were submitted to direct acidification according to the industrial
70 processing used in Italy for these products. This was performed with two
71 concentration of verjuice and the obtained pickles were compared with the
72 traditionally vinegar-pickled cucumbers by chemical characterization and
73 sensory analysis performed by both a trained panel and consumers.

74

75 **2. Methods**

76 *2.1 Production of verjuice*

77 Unripe grape clusters from Sauvignon blanc and Chardonnay varieties were
78 harvested in 2016 season at stage 33 of the modified E-L phenological
79 classification scheme (Coombe, 1995). Clusters were destemmed by hand,
80 washed with tap water, and then pressed by using a small-scale stainless-steel
81 basket press until extracting 51% of juice (w/w). The basket press was loaded
82 with berries in presence of 40 mg/Kg of potassium metabisulphite. The obtained
83 juices were stored at 0°C for 10 days to promote precipitation of potassium

84 bitartrate crystals, then centrifuged (500 rpm, 10 minutes), vacuum filtered
85 through 1.6 μm glass fiber filters (VWR, Milan, Italy), bottled in 500 mL glass
86 green bottles, closed with crown cap, and stored at 5°C until being used for the
87 production of pickled cucumbers.

88

89 *2.2 Production of pickled cucumber*

90 The pickled cucumber samples were prepared industrially by a food preserves
91 company according to their standard processing system. The preserving liquids
92 were prepared with citric and ascorbic acids, salt, water, and vinegar or verjuice
93 as acidifying agents. The final pH of the preserving liquids were all adjusted to
94 2.8 before utilization. For the experimental samples vinegar was substituted by
95 verjuice, prepared as described in the previous section (Sauvignon blanc and
96 Chardonnay verjuice mixed 50:50, v/v). Two different concentrations of verjuice
97 (1:2 and 1:10 (v/v) with water) were used for the preparation of the experimental
98 samples. Cucumbers (original pH 6) were placed in hot water (90 °C) for 3
99 minutes, drained off and transferred to glass jars of 580 mL volume (300 g of
100 cucumber/jar), filled with the three preserving liquids, closed with screw cap,
101 pasteurized and stored for 8 months before being analyzed.

102

103 *2.3 Analytical characterization*

104 *2.3.1 Compositional data*

105 pH was measured using a portable pH meter (HACH, Colorado, United States)
106 equipped with a 5050T pH electrode with temperature sensor. Sodium and
107 potassium concentrations were measured by ICP-OES Arcos EOP (Spectro A.

108 I. GmbH, Kleve, Germany), according to the AOAC Official method 2013.06
109 (2016).

110

111 *2.3.2 HPLC analysis*

112 Organic acids (tartaric, malic, citric, and acetic) as well as glucose and fructose
113 contents were analyzed by high-performance liquid chromatography (HPLC)
114 according to the method proposed by Coelho et al. (2018) with minor
115 modifications. Liquid samples were prepared by diluting them with the mobile
116 phase (5 mM H₂SO₄ in ultrapure water), before being syringe-filtered at 0.20 µm
117 prior to injection. Solid samples (pickled cucumbers) were extracted in the
118 mobile phase by homogenizing them with an ultraturrax homogeneiser (IKA,
119 Staufen, Germany). Upon centrifugation, the supernatant was collected,
120 adequately diluted with the mobile phase, and syringe-filtered at 0.20 µm before
121 injection.

122

123 *2.3.3 Color measurement*

124 Color was measured with a portable spectrophotometer (Minolta CM-600d,
125 Osaka, Japan) set on illuminant D65 (standard daylight) and with a 10°
126 observer. Cucumbers were placed on an appropriate tray and the reflectance of
127 the chromaticity coordinates was measured in triplicate (2 readings x 3
128 cucumbers = 6 readings for each jar) directly on their surface to a depth of 1
129 cm. Data were expressed according to the CIELab colorimetric system: “L”
130 (brightness), “a” (red color coordinate) and “b” (yellow color coordinate) from the
131 SCE (specular component excluded) measurement condition.

132

133 *2.3.4 Texture measurement*

134 The texture of the cucumbers, measured as maximum shear force (N/cm²)
135 required to cut the sample, was assessed using a LS5 dynamometer (Lloyd
136 Instruments Ltd., AMETEK Measurement & Calibration Technologies, Florida,
137 USA) equipped with a Warner-Bratzler probe, 500-N load cell, and crosshead
138 speed of 2 mm/s, as reported by Segato et al. (2007). The measurements were
139 conducted in triplicate by placing the pickled cucumbers at room temperature on
140 a non-lubricated flat platform.

141

142 *2.4 Sensory evaluation*

143 *2.4.1 Sample preparation and presentation*

144 The jars were opened and samples were prepared just before the analysis. One
145 cucumber was sliced, placed in a cup with 10 mL of its own preserving liquid to
146 keep the cucumber soaked. Pickles prepared with vinegar (VIN), verjuice
147 diluted 1:2 (VER1:2) and 1:10 (VER1:10) were served at room temperature in
148 white small plastic cups coded with 3-digit numbers. Presentation order of terms
149 was counterbalanced between and within participants (Ares et al., 2014a).
150 Mineral water was provided for the participants to rinse their mouths between
151 samples.

152

153 *2.4.2 Panel composition*

154 Twelve trained judges (7 females and 5 males) with prior experience in sensory
155 descriptive evaluation of fruit and vegetables, and familiarity with the sensory
156 software used, were recruited from the IBIMET-CNR staff. The panel received a

157 specific training on how to recognize and evaluate each descriptor based using
158 intensity scales (ISO 8586:2012).

159 The same panel was used to perform both Descriptive Analysis (DA) and
160 Temporal Dominance of Sensations (TDS) tests. Both tests were conducted in
161 individual booths and carried out in duplicate with the samples' order
162 randomized to prevent any statistical effects of order or carryover (Ares et al.,
163 2014a). Data were recorded on computer notebooks equipped with a specific
164 software for sensory data acquisition (FIZZ, Biosystemès, France).

165

166 *2.4.2.1 Descriptive Analysis (DA)*

167 Before starting the session, the judges received by the panel leader detailed
168 instructions on the attributes to be evaluated and on how to answer the
169 questions. They were informed that the attributes to be assessed would have
170 been related to appearance (green color and brightness), smell (vinegar and
171 cucumber) taste (acidity, sweetness, salt, bitterness), retronasal sensation
172 (cucumber, vinegar, and cooked vegetable aromas), and tactile (crunchiness,
173 firmness, astringency, pungency). Judges were given three slices of each
174 pickled cucumber sample (VIN, VER1:2, VER1:10) simultaneously and were
175 asked to rate, according to their experience, all the attributes using a 9-point
176 intensity scale. The evaluation of the samples was carried out under the
177 conditions described in UNI 10957:2003.

178

179 *2.4.2.2 Temporal Dominance of Sensations (TDS)*

180 Prior to the evaluation sessions, a focus group conducted a pilot tasting aimed
181 at determining the time required for all sensory sensations to end starting from

182 the beginning of the mastication. This information was used to decide the
183 duration of the test, which was set to 60 seconds. For each point of time, the
184 proportion of evaluations (subject x replication) for which the given attribute was
185 assessed as dominant is computed. These proportions were smoothed over
186 time using a spline regression and displayed as curves of the evolution of the
187 dominance rate for each attribute (Schlich & Pineau, 2017).

188 In the formal sensory session, the judges were asked to select all the in-mouth
189 sensations they felt while tasting the three samples (VIN, VER1:2, VER1:10).
190 One slice of pickled cucumber for each of the samples was given to them
191 simultaneously. The attributes evaluated were crunchiness, firmness, acidity,
192 sweetness, salt, bitterness, astringency, pungency, cucumber aroma, vinegar
193 aroma, and cooked vegetable aroma. The evaluation of the samples was
194 carried out under the conditions described in Pineau et al. (2009) and (2012).

195

196 *2.4.3 Consumer test*

197 A total of 113 subjects participated in the consumer test, 42% were female and
198 58% were male, aged between 18 and 65 years, and living in both the Bologna
199 (Emilia-Romagna, Italy) and Conegliano (Veneto, Italy) areas. Before starting
200 the session, preliminary questions were asked regarding the frequency of
201 consumption of vegetable preserves, the overall liking of different vegetables
202 preserves if consumed, on their knowledge about the presence in the market of
203 the “zero vinegar” vegetable preserves, and on their propensity to consume this
204 category of products.

205 Participants were simultaneously given three samples of whole pickled
206 cucumber and asked to rate, according to their preference, the visual, olfactory

207 and gustatory overall liking for each sample using a horizontal 9-point hedonic
208 scale (1 = dislike extremely; 5 = neither like nor dislike; 9 = like extremely). Only
209 for the gustatory aspect, judges were also asked to assess the appropriateness
210 of several attributes by using a 5-point just-about-right (JAR) scale (1 = too
211 weak; 3 = just about right; 5 = too strong). JAR was applied to attributes such as
212 firmness, acidity, sweetness, salt, bitterness, astringency, pungency, and
213 cucumber aroma. All subjects received written information about the study
214 before giving their consent as well as all sensorial experiments were performed
215 in compliance with the institutional framework. Consumers spontaneously
216 agreed in participating in a session without cash incentive.
217 The output from the JAR was submitted to penalty analysis to determine the
218 drop in overall liking associated with each attribute evaluated (Ares, Dauber,
219 Fernández, Giménez, & Varela, 2014b). The interpretation of the penalty
220 analysis has been conducted by setting at 20% the minimum percentages of
221 consumers and at 1 point of drop in overall liking required to consider an
222 attribute significant, thus defining a top right-hand quadrant or critical corner
223 (Marcano, Varela, Cunha, & Fiszman, 2015).

224

225 *2.5 Statistical analysis*

226 Statistical analysis was performed using SAS[®] software (SAS 9.4, SAS Institute
227 Inc., Cary, NC, USA) with statistical significance determined using an alpha
228 value of 0.05 unless otherwise stated.

229 Physical and chemical characteristics, sensory profiles and consumer data were
230 analyzed using ANOVA and Tukey's Honestly Significant Differences (HSD) for
231 post hoc mean separation.

232 TDS curves computation considers each attribute separately. For each point of
233 time, the proportion of runs for which the given attribute was assessed as
234 dominant is computed. These proportions, smoothed using the TRANSREG
235 procedure of SAS®, are plotted against time and called TDS curves.
236 Transformation used in the TRANSREG procedure is non-iterative smoothing
237 spline. For each product, TDS curves of all the attributes are depicted on the
238 same graph (Pineau et al., 2009).

239

240 **3. Results**

241 *3.1 Product analytical characterization*

242 Pickled cucumbers and their preserving liquids were characterized in order to
243 obtain information about their chemical composition, color and texture (**Table**
244 **1**).

245 In general, the acidity of the pickles varied depending on the preserving liquid
246 used. For cucumbers, whose original pH was around 6.0, the pH ranged
247 between 3.30 to 4.02 after pickling, whereas it varied between 3.22 to 3.95 for
248 the preserving liquids. For both cucumbers and preserving liquids, the most
249 acidic were samples VER 1:2, followed by VIN and VER1:10. These results are
250 in line with the quantity of the strongest organic acids present in the different
251 samples. Malic and tartaric acid concentrations were significantly higher in
252 VER1:2 for both samples, followed by VER1:10 and VIN. This is due to the high
253 quantity of malic and tartaric acid present in unripe grapes (Iland, Dry, Proffitt, &
254 Tyerman, 2011). Citric acid was added in the preserving liquid for all samples
255 and its final content (added + contained in vinegar or verjuice) did not
256 significantly differ among the two VER samples, both containing a significantly

257 higher amount than VIN. As expected, acetic acid was the main organic acid in
258 both VIN samples, while it was not detected in the VER samples as verjuice
259 does not undergo fermentation.

260 The highest quantities of glucose and fructose were found in the VER1:2
261 sample, followed by VER1:10 and, as expected, in VIN in which reducing
262 sugars are consumed during fermentation.

263 The content of potassium and sodium varied only slightly among the samples.

264 Regarding the instrumental color analysis, the values for all coordinates (L^* , a^* ,
265 b^*) were positive, with a significant difference observed only for the intensity of
266 the a^* coordinate, which was however very low for all the samples. Luminosity
267 (L^*) showed values ranging from 33.20 to 33.74 and b^* from 25.87 to 28.91 in
268 pickled cucumber samples.

269 The texture of the pickled cucumbers did not vary significantly between the
270 samples.

271

272 *3.2 Sensory evaluation*

273 *3.2.1 Descriptive analysis (DA) test*

274 DA was performed to determine the flavor characteristics of the pickled
275 cucumbers. Eight out of 15 terms of the DA were found to be significantly
276 different among the evaluated samples (**Fig. 1**).

277 Regarding the olfactory description, VIN was rated with a significantly higher
278 intensity for “vinegar smell” than the two VER samples, as expected, while the
279 “cucumber smell” attribute had significantly higher intensity for VER1:2 and
280 VER1:10 than VIN.

281 For “acidity”, VIN was evaluated as the most acidic, followed by VER1:2 and
282 VER1:10. Regarding the “salt” perception, judges evaluated VER1:2 and
283 VER1:10 as more salty, but VER1:10 did not differ significantly from VIN. On
284 the other hand, VIN was evaluated as the most “astringent” sample.
285 Judges rated VIN as the sample with the highest “pungency” and “vinegar
286 aroma”, followed by VER1:2 and VER1:10.
287 “Cooked vegetable aroma” was found to be more intense in VER1:2 and
288 VER1:10 in comparison to VIN sample.
289 Other attributes, including “color”, “brightness”, “crunchiness”, “firmness”,
290 “bitterness” and “cucumber aroma” were not reported to be different among the
291 different cucumbers.

292

293 *3.2.2 Temporal Dominance of Sensations (TDS)*

294 TDS was performed to evaluate which are the dominant in-mouth sensations for
295 11 predetermined attributes over 60 seconds. **Fig. 2** shows the TDS graphs for
296 the three pickled cucumber samples of the study, in which each curve
297 represents the evolution of the dominance rate of an attribute over time. To
298 assist with the interpretation of the results, a dashed line was added to the
299 graph indicating the significance level (5%) on TDS curves (Pineau et al., 2009).
300 For the VIN sample five major sensations dominated over the evaluation time,
301 with “crunchiness” being a significantly dominant sensation at the beginning,
302 followed by “acidity” and “vinegar aroma”, which dominated during almost the
303 entire evaluation. The “pungent” sensation made a timid dominance for a short
304 time after about 22 seconds. The “astringency” was significant towards the end
305 of the evaluation time.

306 For the VER1:2 sample the TDS curve shows that “crunchiness” also was a
307 significant dominant sensation at the beginning, along with “firmness”. “Acidity”
308 appeared a bit after “crunchiness” and was dominant until almost the middle of
309 the evaluation time, alternating with “cucumber aroma” and a little peak of “salt”
310 sensation. Differently to the VIN sample, here “cooked vegetable aroma” made
311 a timid but significant appearance at the middle of the consumption time. Also in
312 this case “astringency” was the dominant sensation in the second half of the
313 evaluation along with a discreet appearance of “salt” sensation.

314 The VER1:10 sample behaved quite differently from VIN and VER1:2.
315 “Crunchiness” was also the dominant sensation at the beginning of the test, but
316 lasted for longer than the other two samples. Differently from VIN and VER1:2,
317 “cooked vegetable aroma” dominated during almost the entire evaluation being
318 alternated with “salt” through two timid appearances (after 10 and 30 seconds of
319 the evaluation time). Also in this case “astringency” was the dominant sensation
320 at the end of the consumption time with but a lower dominance rate in
321 comparison the other samples.

322

323 *3.2.3 Consumers: Liking and JAR scales*

324 The overall liking of three pickled cucumbers with different acidifying agents
325 was evaluated by a 9-point scale for visual, olfactory and gustative aspects as
326 showed in **Fig. 3**. For visual and gustatory aspects, overall liking scores were
327 not significantly different between the three samples. For the olfactory aspect
328 VIN and VER1:2 liking scores were not significantly different, whereas a
329 difference was reported for VER1:2 and VER1:10.

330

331 The appropriateness of eight attributes by JAR 5-point scale was evaluated and
332 the penalties (or mean drops) were calculated for the gustatory aspect (**Fig. 4**).

337

338 The penalty analysis identified the following attributes as significant for the VIN
339 sample: not enough cucumber aroma, not enough sweet, too acidic, too
340 pungent, and too astringent; for VER1:2 sample: not enough cucumber aroma,
341 not enough sweet, and not enough acidic; for VER1:10: not enough cucumber
342 aroma, not enough sweet, not enough astringent, not enough bitter, not enough
343 salt, and too sweet.

344

345 **4. Discussion**

346 In this paper we explore the possibility to produce pickled vegetables by using
347 verjuice, an acidic liquid obtained from unripe grape berries. The chemical
348 characteristics of this product, and in particular its low pH, make verjuice a good
349 candidate for the substitution of vinegar as a preserving agent for vegetable
350 products. In addition, verjuice lacks in acetic acid (and therefore in volatile
351 acidity), a feature that can be interesting from a sensory point of view.
352 Therefore, the idea was to assess the possibility to obtain pickled vegetable
353 products in which the absence of acetic acid would enhance their sensorial
354 features at least for some consumers, thus opening the way for the production
355 of a new type of preserved vegetables.

356 To compare the effects deriving from the substitution of vinegar with verjuice as
357 the preserving agent, here the sensorial characteristics of cucumbers pickled
358 with verjuice, used in two concentrations (VER 1:2 and VER 1:10), are reported
359 and compared to those of cucumbers obtained by pickling with vinegar (VIN),

360 which are the classical products that can be found on the Italian market. To this
361 aim all the cucumber samples were prepared by following the procedure used
362 for the industrial processing, thus yielding samples representative of
363 commercial products.

364 In addition to the sensorial characterization of the pickled cucumbers, also their
365 basic chemical composition and some physical parameters were determined,
366 along with those of the preserving liquids (**Table 1**). These analyses highlighted
367 the compositional differences among products useful for the interpretation of the
368 sensorial results, and provided also useful information to assess the suitability
369 of verjuice for vegetable preservation.

370

371 *4.1 DA analysis*

372 The pickled cucumber samples were firstly studied by DA, a method based on
373 the panelist's ability to verbalize perceptions of a product in a consistent manner
374 allowing the survey, description and quantification of sensory attributes
375 (Alcantara & Freitas-Sá, 2018).

376 No significant differences in term of appearance (color and brightness, **Fig. 1**)
377 were found among the samples, indicating that no effects on color can be
378 perceived when verjuice is used in place of vinegar. However, the instrumental
379 color analysis (**Table 1**) indicated that the cucumbers pickled in vinegar had a
380 significantly higher a^* value then those pickled with verjuice. However, the a^*
381 value, which should be indicative of a reddish color, is very low for all the
382 samples considered. Conversely, the b^* value indicated that no differences in
383 the typical yellowish color of the pickled cucumbers arise when verjuice is used
384 in place of vinegar, confirming the results of the DA analysis. Also considering

385 the color differences by calculating the ΔE , a parameter incorporating the three
386 CIELab coordinates ($\Delta E = \sqrt{[(L_1 - L_2)^2 + (a_1 - a_2)^2 + (b_1 - b_2)^2]}$), the calculated
387 values were: $\Delta E_{VINvsVER1:2} = 3.4$, $\Delta E_{VINvsVER1:10} = 2.2$, $\Delta E_{VER1:2vsVER1:10} = 2.3$.
388 Some authors report the concept of Just Noticeable Difference (JND) with ΔE
389 values of around 2.3 (Sharma, 2003), thus justifying the DA results (**Fig. 1**).
390 From the olfactory point of view, cucumbers pickled with vinegar, as expected,
391 displayed the highest “vinegar smell” (**Fig.1**), indicating a main sensorial
392 difference which is certainly of interest as a distinctive feature for verjuice
393 pickled vegetables. This sensation is driven by the high acetic acid content of
394 the VIN sample (**Table 1**). Moreover, the low “vinegar smell” could also be
395 responsible for the enhancement of the “cucumber smell” detected in both
396 samples pickled with verjuice, where probably the lack of acetic acid did not
397 interfere with the “cucumber smell” perception. This indicates that the typical
398 aroma which is distinctive of the original plant food is likely to be better
399 maintained when verjuice is used in place of vinegar. The same masking effect
400 of the acetic acid was also probably present for the “cooked vegetable aroma”
401 attribute, possibly arising from the initial heat treatment applied to the
402 cucumbers, which was more prominent for the cucumbers pickled with verjuice
403 (**Fig. 1**).

404 Texture is an important characteristic for pickled vegetables. For this aspect,
405 crunchiness and firmness did not differ among the samples, indicating that
406 verjuice does not worsen the cucumber texture compared to vinegar. This is
407 also supported by the analytical data that did not indicate any change in term of
408 the shear force needed to cut the whole cucumber (**Table 1**).

409 Acidity is an important aspect for pickled vegetables to avoid microbial spoilage
410 and because it contributes to their typical sensorial properties. According to this
411 parameter, cucumbers pickled with verjuice showed pH values in the range of
412 those typical of vinegar-pickled vegetables, which is generally between 3 and 4.
413 However, the pH and the total acids content were dependent on the
414 concentration of verjuice in the preserving liquid, as expected, with the dilution
415 1:2 giving the most acidic cucumbers, also in comparison with those pickled
416 with vinegar (**Table 1**). Nevertheless, these latter had the highest perceived
417 acidity in DA (**Fig. 1**), a discrepancy between the chemical and sensorial values
418 that seems again attributable to the confounding effect that acetic acid present
419 in high quantities has for the perception of acidity by the judges. Moreover, to
420 explain the difference in perceived acidity between the cucumbers pickled with
421 the two verjuice the buffering activity of the saliva should be considered in
422 relation to the different concentration of acids to be neutralized, which is clearly
423 lower for the verjuice diluted 1:10.

424 In contrast with acidity, the “sweetness” attribute was evaluated at the same
425 level for the three samples (**Fig. 1**). Indeed, the total sugar content (glucose +
426 fructose) measured in the pickles was very low and, according to the
427 recognition threshold for sugars in water (Belitz, Grosch, & Schieberle, 2004), is
428 not likely to contribute to the perceived sweetness of the products (**Table 1**),
429 which can be further depressed by the presence of other attributes such as
430 acidity and bitterness (Stampanoni, 1993; Brannap, Setser, & Kemp, 2001).

431 Concerning “salt” perception, despite the small variations in salt content, judges
432 were able to perceive differences in saltiness, for which chemical and sensorial
433 data are in agreement (**Table 1** and **Fig. 1**).

434 Judges generally rated the attribute “bitterness” with low intensity scores,
435 without any significant difference between the samples. Since bitterness is
436 considered an important quality attribute for vegetables, this result again
437 indicates the suitability of verjuice as a substitute of vinegar.

438 Concerning “astringency”, cucumbers that were rated as the most astringent
439 were those pickled with vinegar, whereas pickling with verjuice had the same
440 scores for this parameter, regardless of the verjuice dilution. Therefore, despite
441 the fact that “astringency” is a descriptor for verjuice (Dupas de Matos et al.,
442 2017), it seems that pickling with this liquid results in cucumbers less astringent
443 than those in vinegar. The high astringency of the VIN sample is likely due to
444 the acetic acid content as it is known that organic acids have themselves a
445 direct effect on astringency perception. However, this effect is not linked to the
446 type of acid but to the pH determined by the presence of a particular acid
447 (Sowalski & Noble, 1998). When tasting the pickles it must be taken into
448 account that the pH in the mouth is affected by the interaction between the
449 bicarbonate ions in of the saliva and the acids introduced with the food (Bardow,
450 Moe, Nyvad, & Nauntofte, 2000). In turn, the pH affects the binding of the
451 salivary proteins to the phenolics present in the food (Lawless, Horne, & Giasi,
452 1996), which is the mechanism responsible for the astringency sensation.
453 Therefore, the presence of high quantity of acids, in particular acetic acid in the
454 sample pickled with vinegar should maintain a lower pH upon placing the
455 samples in the mouth, thus indirectly enhancing the astringency perception
456 **(Fig. 1)**.

457 The samples reported to have the higher “pungency” **(Fig. 1)** were the
458 cucumbers pickled with vinegar, results clearly driven by their acetic acid

459 content (**Table 1**). Interestingly, the two VER samples were rated differently
460 despite not having acetic acid. This can possibly be the result of the lower
461 buffering capacity of the more diluted preserving liquid (VER 1:10) when mixed
462 with the saliva. However, this hypothesis does not explain the results observed
463 for “vinegar aroma” which was found also in absence of acetic acid (**Fig. 1** and
464 **Table 1**). This is difficult to explain, but it might be the result of a psychological
465 association between pickled cucumbers and vinegar aroma due to the fact that,
466 at least in Italy, pickles always have vinegar taste. However, this result is also
467 clearly related to the total acids content that seems to be the driver for the ability
468 of judges in discriminating the two verjuice samples.

469 For the attribute “cucumber aroma”, judges did not notice any differences,
470 indicating that the preserving liquids did not interfere with its perceived intensity,
471 again indicating the suitability of verjuice as pickling agent. Interestingly, the
472 retronasal perception of the “cucumber aroma” does not show the differences
473 that were seen for the “cucumber smell”. This could be due to the physico-
474 chemical conditions existing in the mouth which can modify the perception of
475 this attribute.

476

477 *4.2 TDS test*

478 Given that the sensory perception is a dynamic phenomenon as the perceived
479 intensity of the sensory attributes changes along with the in-mouth
480 transformation of food (Sudre, Pineau, Loret, & Martin, 2012), the TDS test was
481 developed to describe the sequence of the dominant sensory perceptions
482 during the tasting of a product (Pineau et al., 2012). TDS experiments have
483 been conducted to describe the temporal sensory patterns of different foods

484 and beverages (Frost, Blackman, Ebeler, & Heymann, 2018; Charles et al.,
485 2017; Rodrigues et al., 2016; Sokolowsky, Rosenberger, & Fischer, 2013; Ng et
486 al., 2012). In this test, the dominant attribute in a given moment is not
487 necessarily the one with the highest intensity (Pineau et al., 2009), being rated
488 as the percentage of dominance among all attributes considered at a given time
489 point (Di Monaco, Su, Masi, & Cavella, 2014).

490 The perceptions varied greatly for most attributes (**Fig. 2**). All samples were
491 dominated by a “crunchiness” sensation at the very beginning of the trial. In
492 addition, in the two verjuice samples this was associated with “firmness”. This
493 might indicate that the replacement of vinegar with verjuice had an effect on the
494 physical properties of the cucumbers. It might be that the structure of the
495 cucumbers preserved in less acidic preserving liquids remain more intact, thus
496 inducing a longer dominance for the parameters related to mastication.

497 The dominance’s duration of the “acidity” sensation seemed linked to the total
498 acids content (**Table 1**). Also according to the duration it seems that the more
499 diluted verjuice sample did not yield the results that should be expected for
500 pickles, while this is not the case for the more concentrated verjuice sample.

501 For the vinegar sample, the observed TDS profile seemed mostly driven by the
502 “vinegar aroma” effect that was lacking in both verjuice samples (**Fig. 2**).

503 By looking at “acidity”, “vinegar aroma” and “pungency” profiles together, it
504 seems that these three attributes have a synergistic effect in the vinegar sample
505 resulting in a longer dominant acid sensation when compared to the two
506 verjuice samples.

507 The “cooked vegetable aroma” was never a dominant attribute for the vinegar
508 sample, probably because of the concurrent and long-lasting dominance of the

509 “vinegar aroma”. In contrast, the shorter period of dominance of the acidity in
510 the two verjuice samples probably allowed for the dominance of this attribute
511 that was, also in this case, linked to the acidity. Therefore, a certain level of
512 acidity seems to be necessary to reduce the dominance of a negative aroma as
513 the cooked vegetable one.

514 It is to be noted that the “cucumber aroma” did not emerge as dominant in the
515 vinegar sample but dominated at the same evaluation time, although for short
516 periods, for the verjuice samples. Again, this behavior can be due to the
517 masking effect of the vinegar aroma absent in the verjuice samples. This
518 confirms that verjuice can be a good choice for pickling cucumbers, if their
519 native aroma has to be maintained.

520 “Astringency” was the last attribute to emerge as the dominant sensation
521 towards the end of the evaluation time for all samples. Despite the time in which
522 judges swallowed the samples was not recorded, it is almost certain that
523 “astringency” became a dominant sensation after this moment and particularly
524 after the acidity is no longer significantly dominant, a phenomenon that has
525 already been described (Medel-Maraboli et al., 2017; Frost, Harbertson, &
526 Heymann, 2017).

527 Adopting a TDS approach highlighted differences among the samples that could
528 impact the appreciation of consumers, an aspect investigated in the next
529 section.

530

531 *4.3 Liking scores, JAR scale and penalty analysis*

532 Traditional sensory methods to evaluate consumer acceptability such as
533 hedonic scale (liking) in combination with JAR test have been widely used in

534 food science, especially for new product development and understanding
535 consumer needs (Viejo, Fuentes, Howell, Torrico, & Dunshea, 2018).
536 Normally, the 5-point JAR scale is reduced to three categories by combining the
537 two categories on the “not enough” side (1 and 2) of the midpoint (3) and by
538 combining the two categories on the “too much” side (4 and 5) of the midpoint
539 (Rothman & Parker, 2009).
540 Then, the mean overall liking (rating) was calculated as the differences between
541 the mean overall liking of each non-JAR category and the mean of the JAR
542 category. Further, the penalties were plotted versus the percentage giving each
543 response in a so-called mean drops (Gere, Sipos, & Héberger, 2015).
544 The three samples were all deemed similar for visual and gustative aspect,
545 while the olfactory evaluation showed that VER1:2 was liked at the same level
546 as that commercial standard VIN, and significantly more appreciated than
547 VER1:10 (**Fig. 3**). This indicates that cucumbers pickled with verjuice can
548 represent a valid choice for the consumers alongside those pickled with vinegar.
549 The penalty analysis is used to obtain clear indications on the attributes to be
550 considered for future reformulation as they are responsible for a drop in the
551 overall liking score. This methodology determines if those consumers
552 considering a particular attribute lower or higher than the midpoint (just-about-
553 right) dropped the overall liking more than those consumers finding the same
554 attribute just-about-right (Gere et al., 2015).
555 Penalty/mean drop analysis was then conducted on the JAR data obtained for
556 the three cucumber samples. In all samples, the significant drop in overall liking
557 was driven by the both the lack of some attributes (e.g. cucumber aroma,

558 sweetness, bitterness, acidity, astringency and salt) and by the excess of others
559 (e.g. acidity, astringency, pungency and sweetness, **Fig. 4**).

560 Despite cucumbers pickled in vinegar are the commercial reference, consumers
561 found several attributes responsible for a reduction in the overall liking of the
562 product and that deserve to be considered to find an alternative without this
563 drawbacks. Therefore, the search for an alternative preserving agent is justified.
564 However, VER1:10 does not seem to be the right alternative as it also presents
565 several penalizing attributes. Conversely VER1:2, having only 3 penalizing
566 attributes (“not enough sweet”, “not enough cucumber aroma”, and “not enough
567 acidic”) against the six of the other two samples (**Fig. 4**), seems to be a
568 promising acidifying agent for the production of a novel style of pickled
569 cucumbers with the potential to encounter the taste of consumers.

570 In particular, on the basis of the penalty analysis a few simple actions could be
571 applied to improve the sensorial quality of VER1:2 by adjusting, for example,
572 the sugar content and the acidity. It seems more complicated to improve the
573 “cucumber aroma” attribute that, on the other hand, penalizes all the products
574 examined here.

575

576 **5. Conclusion**

577 The use of different sensory methods (DA, TDS, Liking and Penalty analysis on
578 JAR) allowed for a comprehensive investigation on the sensorial characteristics
579 of cucumbers pickled a novel acidifying agent (verjuice) in comparison with a
580 traditional one (vinegar). In general, this type of studies are essential for the
581 development of a new food product. In our case, it was established that the use
582 of verjuice as preserving liquid for the production of pickled cucumbers

583 represents a viable alternative to vinegar conferring comparable characteristics
584 to the reference in terms of visual and textural characteristics, sweetness,
585 bitterness and cucumber aroma. However, some distinctive characteristics were
586 detected in the cucumbers pickled with verjuice, indicating the possibility of
587 developing a novel product different from the reference that, according to the
588 penalty analysis reported here, could be further improved by some simple
589 adjustments of the verjuice-containing preserving liquid. For example, the level
590 of acidity, a very important factor as shown here, can be adjusted either by
591 modulating the concentration of verjuice in the preserving liquid, or by using
592 verjuice produced at different stages of ripening thus having a different
593 sugar/acid balance. The absence of acetic acid seems to be the main
594 distinguishing feature of pickles prepared with verjuice, potentially conferring
595 them an additional value because acetic acid has a strong sensorial impact that
596 can be disliked by some consumers.

597 The data obtained here for cucumbers are likely to be valid also for pickling
598 other vegetables, and therefore this preserving liquid needs to be taken into
599 consideration as an alternative to vinegar in other food preparations, for
600 example to better maintain the smell of the original plant food more unaltered as
601 demonstrated here for cucumbers.

602 Verjuice presents the additional advantages of being produced from a
603 byproduct of the grape and wine industries (e.g. thinned grapes), and for its
604 simple method of production as it requires only a few processing steps.

605

606

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612

613 **Conflict of interest**

614 The authors have no financial conflicts of interest in this work.

615

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626

627 **Informed consent**

628 Informed consent was obtained from all individual participants included in the
629 study.

630

631

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758 **Figure captions**

759 **Fig. 1.** Sensory profiles of three pickled cucumbers prepared with vinegar (VIN),
760 verjuice 1:2 (VER1:2) and verjuice 1:10 (VER1:10) based on QDA test-fifteen
761 attributes by twelve judges. * Indicates significant differences in mean according
762 to Tukey's HSD test at $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

763

764 **Fig. 2.** Sensory profiles of three pickled cucumbers prepared with vinegar (VIN),
765 verjuice 1:2 (VER1:2) and verjuice 1:10 (VER1:10) based on TDS test-eleven
766 attributes by twelve judges. Dashed line represents the level of significance
767 (5%) on TDS curves.

768

769 **Fig. 3.** Overall liking scores of three pickled cucumber prepared with vinegar
770 (VIN), verjuice 1:2 (VER1:2) and verjuice 1:10 (VER1:10) based on consumer
771 test by 113 consumers.

772

773 **Fig. 4.** Mean drops in overall liking as a function of the percentage of
774 consumers. The vertical line represents 20% of consumers. Attributes in red
775 indicate significant differences in mean drop overall liking at $p < 0.05$

776 **Table captions**777 **Table 1.** Characteristics of the preserving liquid and of the pickled cucumbers.

		Pickled cucumber			Preserving liquid		
		VIN	VER1:2	VER1:10	VIN	VER1:2	VER1:10
pH		3.43 b	3.30 c	4.02 a	3.40 b	3.22 c	3.95 a
Organic acids^a	Tartaric	0.05 c	0.80 a	0.19 b	0.17 c	1.40 a	0.40 b
	Malic	0.67 c	3.02 a	1.62 b	1.03 c	5.03 a	2.68 b
	Citric	0.51 b	0.77 a	0.69 a	0.21 b	0.78 a	0.79 a
	Acetic	7.50	n.d.	n.d.	10.44	n.d.	n.d.
Total Acids^{a,b}		8.72 a	4.59 b	2.50 c	11.84 a	7.21 b	3.87 c
Sugars^a	Glucose	2.40 c	5.90 a	3.66 b	3.71 c	8.88 a	5.16 b
	Fructose	2.77 b	3.80 a	3.14 ab	4.01 b	5.15 a	4.35 ab
K^a		1.17 b	1.58 a	1.48 a	1.08 b	1.48 a	1.31 a
Na^a		6.31 b	7.46 a	6.71 ab	6.16 a	6.71 a	6.17 a
Color	L*	32.51 a	32.20 a	33.74 a	-	-	-
	a*	3.46 a	1.90 b	2.26 b	-	-	-
	b*	28.91 a	25.87 a	27.55 a	-	-	-
Shear force (N/cm²)		22.86 a	18.20 a	17.27 a	-	-	-

778 ^a Measured in mg/g for pickled cucumber, and in mg/mL for preserving liquid.779 ^b Sum of individual organic acid (tartaric, malic ,citric, acetic).

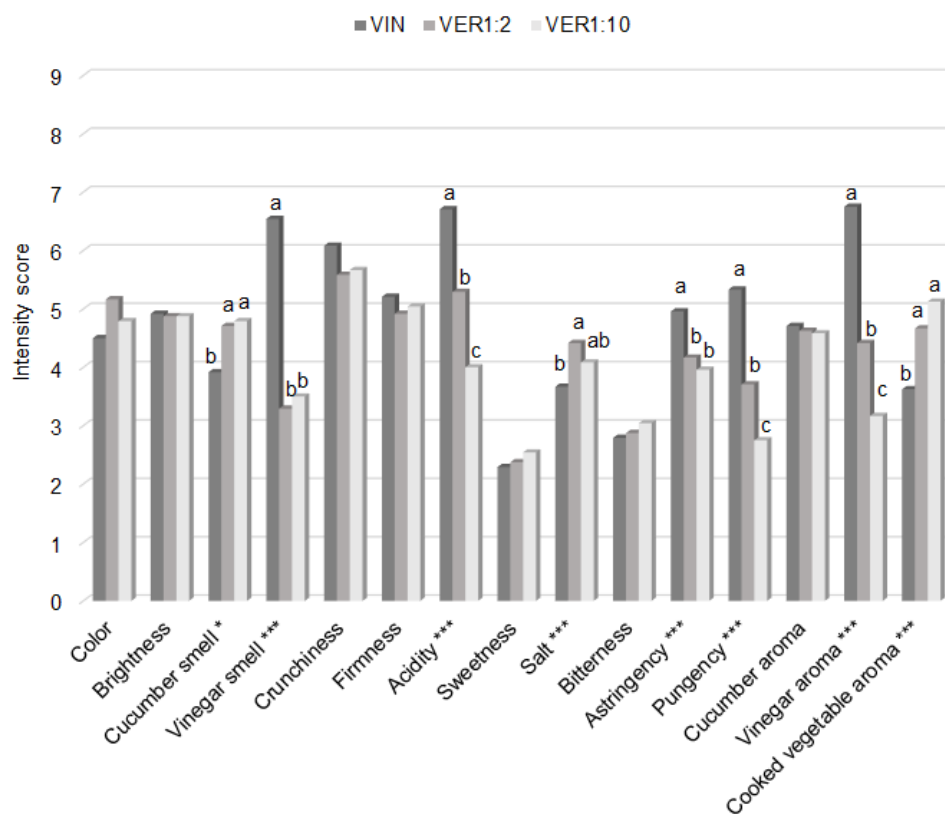
780 n.d.: not detected.

781 VIN: samples with vinegar; VER1:2: samples with verjuice 1:2; VER1:10:

782 samples with verjuice 1:10.

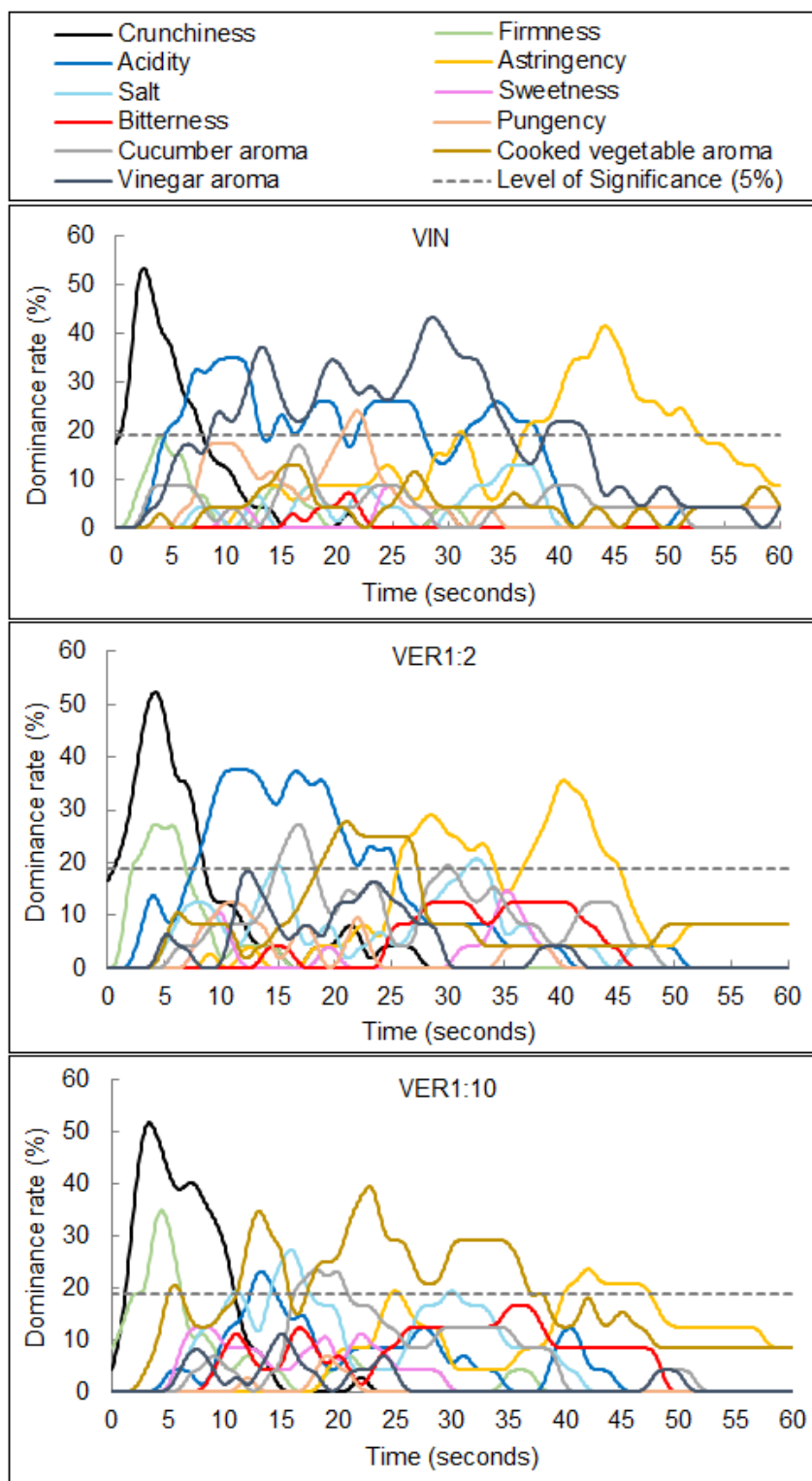
783 Means followed by the same letter in the row do not differ significantly by one-

784 way ANOVA followed Tukey's test ($p < 0.05$).



1

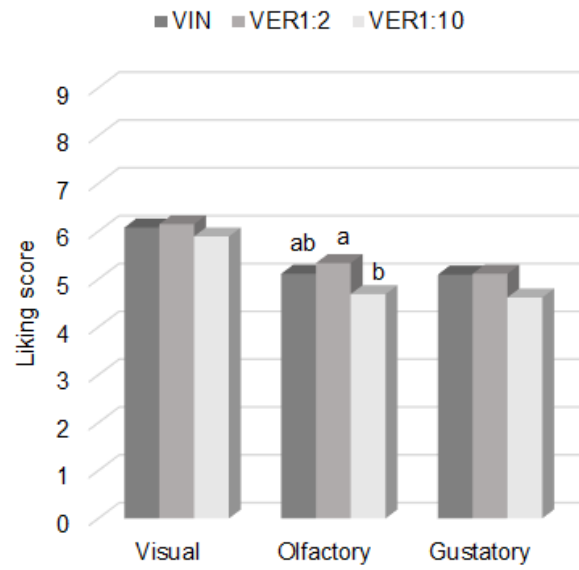
2 **Fig. 1.** Sensory profiles of three pickled cucumbers prepared with vinegar (VIN),
3 verjuice diluted 1:2 (VER1:2) and verjuice diluted 1:10 (VER1:10) based on
4 QDA test-fifteen attributes by twelve judges. * Indicates significant differences in
5 mean according to Tukey's HSD test at $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.



6

7 **Fig. 2.** Sensory profiles of three pickled cucumbers prepared with vinegar (VIN),
 8 verjuice diluted 1:2 (VER1:2) and verjuice diluted 1:10 (VER1:10) based on
 9 TDS test-eleven attributes by twelve judges. Dashed line represents the level of
 10 significance (5%) on TDS curves.

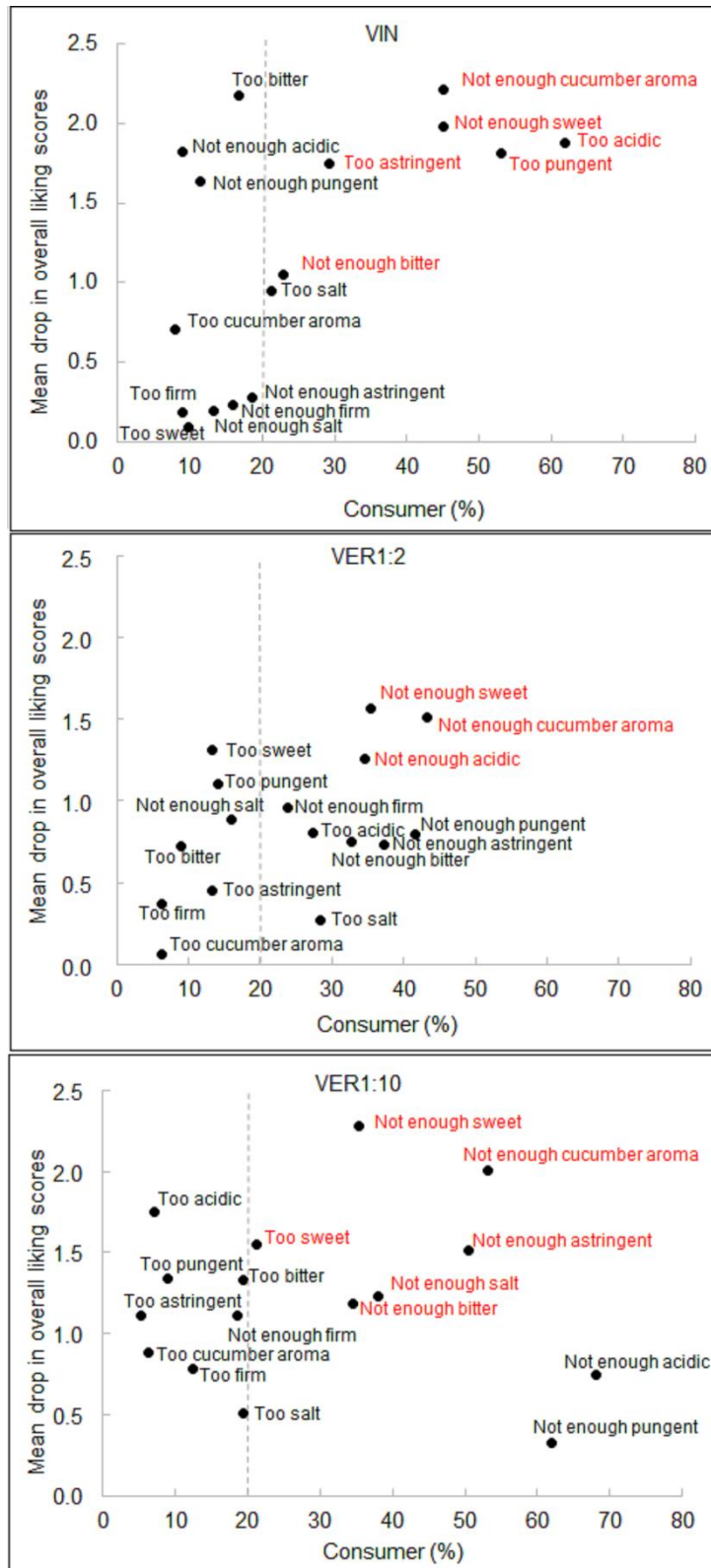
11



12

13 **Fig. 3.** Overall liking scores of three pickled cucumber prepared with vinegar
14 (VIN), verjuice diluted 1:2 (VER1:2) and verjuice diluted 1:10 (VER1:10) based
15 on consumer test by 113 consumers.

16



21

22 **Fig. 4.** Mean drops in overall liking as a function of the percentage of
 23 consumers. The vertical line represents 20% of consumers. Attributes in red
 24 indicate significant differences in mean drop overall liking at $p < 0.05$