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### ► To cite this version:

Amel Hedhili, Samuel Lubbers, Elias Bou-Maroun, Francine Griffon, Babatope Ebenezer Akinyemi, et al.. Moringa Oleifera supplemented biscuits: Nutritional values and consumer segmentation. South African Journal of Botany, 2021, 138, pp.406-414. 10.1016/j.sajb.2021.01.017 . hal-03187745

HAL Id: hal-03187745

<https://institut-agro-dijon.hal.science/hal-03187745v1>

Submitted on 13 Feb 2023

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1 ***Moringa Oleifera* supplemented biscuits: nutritional values and consumer**  
2 **segmentation**

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12

13 **Abstract**

14 *Moringa oleifera* is a nutrient-rich plant, known as a miracle tree, and commonly used  
15 as a food supplement. Despite its popularity, there are scant empirical findings on its  
16 real nutritional benefits and acceptance among consumers when added to food. To  
17 fill this gap, we added Moringa Dried Leaves (MDL) to biscuits. A consumer test was  
18 implemented to study the sensory properties of the biscuits. Nutritional properties,  
19 including in vitro protein digestibility, and physical properties were also investigated.  
20 Global liking indicates the segmentation of consumers into two clusters and  
21 confirmed heterogeneity. Cluster 1 consumers disliked the supplemented biscuits  
22 and cited dark green color and texture as the main reasons for dislike. Conversely,  
23 cluster 2 consumers, who were much younger, liked the supplemented biscuits due  
24 to their adventurous taste for novel products. Nutritional analysis revealed that the  
25 incorporation of MDL to biscuits increased their iron and proteins but led to  
26 incomplete protein digestion especially for biscuits with high supplementation. In  
27 addition, vitamin A derived from MDL was degraded during baking. The biscuit with  
28 the highest acceptability and digestion (C1) would increase protein intake only slightly  
29 but provide essential amino acids, which are deficient in wheat flour. These findings,  
30 confirm that there is a limit to further increasing the amount of MDL added to foods in  
31 order to provide more nutrients. In addition, it is recommended that Moringa  
32 supplemented products should be advertised with caution in order not to mislead the  
33 consumers.

34 **Keywords:** Moringa Dried Leaves (MDL); Hedonic test; Consumer segmentation;  
35 Open-ended questions; In vitro digestibility; Proximate analysis

## 36        **1. Introduction**

37    The *Moringa oleifera* tree is universally known as a miracle plant. This plant derived  
38    its name from the fact that all its parts (leaves, seeds, flowers etc.) can be used as  
39    nutritional supplement, medicine, water purifier and animal fodder (Daba, 2016).  
40    Native to northern India, *Moringa oleifera* Lam. (*Moringaceae*) is now cultivated in  
41    many subtropical regions in Africa, tropical America, Mexico, Malaysia, and the  
42    Philippines (Adeyemi et al., 2014; Fahey, 2005). Several studies have shown that  
43    *Moringa oleifera* leaves are a rich source of certain macro and micronutrients.  
44    Sánchez-Machado et al. (2010) reported that Moringa plant contains a high  
45    percentage of essential amino acids in green leaves. While Siddhuraju and Becker  
46    (2003) and Sreelatha and Padma (2009) have also reported antioxidant properties of  
47    *Moringa oleifera* leaves. The leaves of Moringa have also been found to contain  
48    important amounts of calcium, potassium, magnesium, vitamin A, C and E (Hekmat,  
49    2015). Witt's (2008) meta-analysis of 49 scientific articles summarizes the nutritional  
50    composition of Moringa dried leaves (MDL). It shows that in 100 g of MDL there are  
51     $24 \pm 5.8$  g of proteins,  $36 \pm 9.2$  g of carbohydrates,  $6 \pm 2.5$  g of fat, 20.6 to 28.6 g of  
52    fiber,  $32.5 \pm 10.8$  mg of iron, and  $3639 \pm 1980$   $\mu$ g of vitamin A.

53    Currently, the use of *Moringa oleifera* as a nutrient-rich plant for food  
54    supplementation is getting much attention. It was first used in India before it was later  
55    exported to African countries where it was incorporated in traditional foods such as  
56    amala, a starchy meal in Nigeria and Ghana (Oyeyinka and Oyeyinka, 2016). In  
57    developed countries, for instance in Europe, *Moringa oleifera* is marketed as a  
58    “superfood” for a wide range of health benefits such as high level of antioxidant  
59    activity (Bruins et al., 2018). The importation of Moringa to Europe was estimated

60 between 100 and 200 tons in 2017 and the main market destinations are the United  
61 Kingdom, Germany, and France (CBI, 2019).

62 Many scientific studies have shown that the supplementation of staple foods such as  
63 bread, cheese, yoghurt, and biscuits with MDL improve their nutritional properties  
64 (Dachana et al., 2010; Hekmat et al., 2015; Salem et al., 2013; Sengeev et al., 2013).

65 Biscuits is one of the most popular bakery products in the human diet due to many  
66 characteristics such as palatability, convenience, ready-to-eat, affordability and  
67 longer shelf-life in comparison to the other bakery products (Arshad, 2007; Nagi,  
68 2012). It is also widely accepted and consumed in many countries, which makes it a  
69 viable supplementation vehicle for nutritional improvement of vital nutrients like  
70 protein, dietary fibers and functional ingredients (Sulieman et al., 2019).

71 In previous studies, MDL were incorporated in biscuits with levels varying from 3% to  
72 15% and results showed that this supplementation increased protein, fiber, b-  
73 carotene (Dachana et al., 2010; Mouminah, 2019), and minerals (Fe, Ca, Mg, P and  
74 K) composition of biscuits (Abdel-Samie and Abdulla, 2014). The incorporation of  
75 Moringa also has an impact on the physical and sensory properties of biscuits.  
76 Physical properties such as diameter and thickness changed depending on the  
77 amount of MDL added and breaking force significantly increased with the  
78 concentration of Moringa (Dachana et al., 2010). Previous studies that focused on  
79 sensory evaluation showed that biscuits were acceptable up to 9% or 10% of  
80 Moringa supplementation respectively (Abdel-Samie and Abdulla, 2014; Dachana et  
81 al., 2010). Lee et al. (2014) revealed that the typical leafy flavor and slightly bitter  
82 taste decreased the consumer acceptance of a product supplemented with leaves.  
83 Furthermore, Dachana et al. (2010) observed that the addition of MDL to cookies  
84 resulted in darker samples, which can lead to a lower consumer acceptance. This

85 finding is in agreement with Ramota et al. (2013) who state that MDL has a deep  
86 green color, which may be attributed to its high chlorophyll content and may strongly  
87 mask the color of original foods.

88 In contrast, a study carried out by Ramaroson et al. (2014) in Madagascar showed  
89 that the Moringa green color is not always a problem when added to food. Cassava  
90 root cakes were supplemented with MDL and presented to children who had to score  
91 their overall liking of the samples. At the end of the experience, children chose one of  
92 the products as a reward for their participation. Results showed that the product  
93 containing the highest proportion of Moringa received high liking score and was  
94 chosen as reward by a majority of the children despite its greener color, which  
95 demonstrated that color was not a barrier to children acceptance in the study. This  
96 result suggests that there might be inter-individual difference between consumers in  
97 terms of acceptance.

98 Previous studies investigated consumer preferences without examining the  
99 underlining heterogeneity that might exist among consumers due to individual  
100 peculiarity (Abdel-Samie and Abdulla, 2014; Dachana et al., 2010; Rathnayake and  
101 Navarathna, 2017). Hence, the first objective of this study was to implement sensory  
102 analysis in order to gain understanding of heterogeneity in consumer preferences of  
103 Moringa supplemented biscuits by looking at inter-individual differences among the  
104 study participants.

105 Another potential limitation of the use of Moringa for food supplementation is that  
106 most of the reported evidence do not take into account the effect of processing on  
107 nutritional compounds and/or of the bio-availability of these compounds.

108 Furthermore, the few studies which explored this last point are far from being  
109 consensual. For example, a study carried out by Teixeira et al (2014), showed that

110 the in vitro protein digestibility (IVPD) of MDL was 33.29% whereas a previous study  
111 comparing the IVPD in thirteen plants species reported a value of 74.2% of IVPD of  
112 MDL (Ly et al., 2001). The second goal of this study was to investigate the level of  
113 nutritional improvement of biscuits due to Moringa supplementation.

114 To achieve these objectives, our study asked and offered answers to four questions,  
115 namely: (1) Did the incorporation of MDL in biscuits improve their nutritional  
116 properties and affect their IVPD? (2) What is the contribution of biscuits containing  
117 MDL in recommended daily requirement? (3) Did the incorporation of MDL in biscuits  
118 affect the physical properties of biscuits? (4) What is the effect of supplementation  
119 with MDL on sensory properties of biscuits and consumer acceptance?

## 120 **2. Materials and methods**

### 121 *2.1. Biscuit preparation*

122 All ingredients (Wheat flour T-45, sugar, shortening, salt and baking powder) used to  
123 produce the biscuits were purchased from local supermarket Carrefour®, Dijon,  
124 France. MDL were purchased in Dijon, France.

125 Three experimental biscuits (C1, C2, and C3) were prepared by supplementing a  
126 control biscuit (C0) with different amounts of MDL. The control biscuit was made of  
127 100 g wheat flour, 30 g sugar powder, 30 g shortening, 2.5 g baking powder, 0.5 g  
128 salt, and water. A proportion of 10%, 20% and 30% of MDL, respectively was added  
129 to the base recipe to obtain supplemented biscuits C1, C2 and C3, respectively.

130 Wheat flour and MDL were sieved before mixing with shortening until homogeneous  
131 lumps were formed. Then, sugar, salt and baking powder were added. The mixture  
132 was mixed at low speed with a pastry robot for 10 min with a stop every 2 min. Water

133 was added gradually and mixed for 2 min at low speed. The dough was wrapped in  
134 stretch film and refrigerated at 4 °C for 20 min. After 20 minutes, the dough was  
135 placed on a lightly floured biscuit sheet. The prepared dough was rolled to a  
136 thickness of 4mm and cut using a circular shape of 3 cm in diameter. The biscuits  
137 were baked at 180 °C for 20 min. After baking, the biscuits were cooled down to  
138 room temperature and kept in airtight food plastic boxes. Fig. 1 shows the control  
139 biscuit (C0) and the biscuits supplemented with the three levels of MDL (C1 to C3).

## 140 *2.2. Proximate analysis*

141 All reagents and chemicals used for the proximate analysis were purchased from  
142 Sigma Aldrich® (France) and were of analytical grade. Biscuit samples were  
143 analyzed in triplicate for moisture, proteins, vitamin A and iron. Moisture content was  
144 determined according to AOAC (2011). Crude proteins were determined according to  
145 *Kjeldahl* method. The proteins content was obtained using the conversion factor 4.4  
146 (factor used for tropical plant samples) (Ramaroson et al., 2014). Vitamin A content  
147 was determined using the method of vitamin A (Retinol) in foods (AOAC, 2001). Iron  
148 content was obtained following the method of determination of trace elements by  
149 atomic absorption spectrometry (French standard 14082, 2003).

## 150 *2.3. In vitro protein digestibility (IVPD)*

151 Pepsin (from porcine stomach mucosa, >2500 U/mg protein), pancreatin (from  
152 porcine pancreas, 4 x USP), casein sodium salt from bovine milk (C-8654) and TAME  
153 (p-Toluene-Sulfonyl-L-arginine methyl ester; T4626) were purchased from Sigma-  
154 Aldrich (France). In vitro digestibility was carried out according to the method of  
155 Minekus et al. (2014) with some modifications. Analysis was done in duplicate. In  
156 addition, 1 g of MDL or 1 g of biscuit was prepared in 10 mL of solution then 8 mL of  
157 simulated gastric fluid (SGF) electrolyte solution was added. To this mixture 1.6 mL



158 of porcine pepsin solution (25.000 U/mL) and 5  $\mu$ L of 0.3 M CaCl<sub>2</sub> were added. The  
159 pH of the mixture was adjusted to 3.0. The solution was incubated for 120 min at 37  
160 °C under continuous stirring at 170 rpm. After gastric digestion, gastric chime was  
161 mixed with 11 mL of simulated intestinal fluid (SIF). To this solution, 736 mg of the  
162 pancreatic enzyme and 40  $\mu$ L of 0.3 M CaCl<sub>2</sub> were added and the final volume was  
163 completed to 40 mL by SIF solution. The pH of the mixture was adjusted to 7.0. The  
164 final solution was incubated for 120 min at 37 °C with stirring. To determine the  
165 nitrogen release in the samples, the trichloroacetic acid (TCA) precipitation method  
166 was used. Aliquots of 10 mL of the withdrawn digestion mixture were mixed with 10  
167 mL of 10% TCA solution. The mixtures were mixed and then centrifuged (8000 g, 10  
168 min). The nitrogen content of the resulting supernatants was determined by the  
169 *Kjeldahl* method. The percentage of soluble nitrogen released during the digestion  
170 process was calculated using the following equation (Teixeira et al., 2014).

$$171 \quad \% \text{ Enzymatic Hydrolysis (\%EH)} = \frac{\text{amount of protein after digestion}}{\text{total amount of protein}} * 100$$

172 The percentage of digestibility of the proteins was deduced from the digestibility of  
173 the casein considered as standard (100%) (Teixeira et al., 2014).

## 174 *2.4. Physical characteristics of biscuits*

### 175 *2.4.1. Diameter, thickness and spread ratio*

176 Diameter (mm), thickness (mm) and spread ratio of samples were measured in  
177 triplicate following the method of Ganorkar and Jain (2014). To determine biscuit  
178 diameter, six biscuits were placed edge to edge and the total diameter was measured  
179 in mm by a Vernier caliper. Then biscuits were rotated at an angle 90° for triplicate  
180 measure. The average diameter was reported in mm. Six biscuits were placed on top

181 of one another to determine thickness. The total height was measured and the  
182 average was presented in mm. Spread ratio was calculated as: spread ratio =  
183 Average diameter / Average thickness \* 10.

#### 184 *2.4.2. Breaking force*

185 A three-point bend test was used to estimate the biscuits breaking force as an  
186 indicator of hardness using a texture analyzer TA-TX2 equipped with a 25 kg load  
187 cell. The peak breaking force (N) of biscuits using the force in compression was  
188 recorded. Biscuit samples were placed on base beams with a distance of 1.8 cm  
189 between the two beams. A three point-bending rig was used with an HDP/3PB, knife-  
190 edge probe. The analyzer was set at a return-to-start cycle with a pretest speed of 2  
191 mm/s, a test speed of 2 mm/s, and a post-test speed of 10 mm/s. The trigger was 20  
192 g and distance of 10 mm. Analysis was done in triplicate.

#### 193 *2.4.3 Color measurement*

194 Color attributes of biscuits supplemented with MDL ( $L^*$ ,  $a^*$  and  $b^*$ ) were measured in  
195 triplicate using Hunter Lab color analyzer (HunterLab Color Flex EZ, USA). The  $L^*$   
196 value (lightness index scale) ranges from 0 (black) to 100 (white) while,  $a^*$  value  
197 indicates the redness ( $+a^*$ ) or greenness ( $-a^*$ ) and the  $b^*$  value refers to the  
198 yellowness ( $+b^*$ ) or blue ( $-b^*$ ). The petri dish containing sample was placed directly  
199 on the colorimeter sensor.

#### 200 *2.5. Sensory evaluation*

##### 201 *2.5.1. Consumers*

202 The consumer panel consisted of 91 students and staff members from the University  
203 of Burgundy (73.63% Women – 26.37% Men; 74.63% <30 years and 25.27% >30

204 years). To ensure a larger generalizability of the results no constraint was placed on  
205 the nationality of consumers: 75.82% were French, 10.99% were African, 5.49%  
206 were European other than French and 7.69% Asian.

### 207 *2.5.2. Experimental conditions*

208 Consumers were provided with information about the study, following which they  
209 completed an informed consent form in keeping with French ethical requirements.

210 The consumers tasted the samples in a sensory evaluation room at Agrosup Dijon.

211 The biscuits were served in covered white plastic cups coded with a three-digit  
212 random number. Two series of four samples (C0, C1, C2, and C3) each were  
213 presented on a tray. Different random codes and different presentation orders were  
214 used for the two series. The presentation order followed Williams' Latin squares.

215 Consumers were not aware that the biscuits in the two series were the same. Bottled  
216 water was provided for palate cleansing. The questionnaire was written in French  
217 language and explained in English when necessary.

### 218 *2.5.3. Procedure*

219 The consumer test included two steps. During the first step, consumers were  
220 instructed to taste the first series of samples from left to right. They had to rate the  
221 overall liking of each sample on a structured scale ranging from 1 (I do not like at all)  
222 to 7 (I like it a lot) with 4 being anchored as neither like nor dislike. Before proceeding  
223 to the next samples, consumers were asked to write down what they liked and what  
224 they disliked about the sample (Symoneaux and Galmarini, 2014). During the second  
225 step, consumers were then asked to taste the second series of biscuits and evaluate  
226 the appearance, odor, taste and texture of the samples using 7-points hedonic  
227 scales. Consumers could answer either in French or in English as they wished.

228 Throughout tasting, consumers were told to drink the water provided to ensure palate  
229 cleansing.

## 230 *2.6. Statistical Analysis*

### 231 *2.6.1 Chemical composition*

232 Data were analyzed using one-way Analysis of Variance (ANOVA) with sample as  
233 fixed factor and protein, moisture, iron, vitamin A, thickness, diameter, spread ratio,  
234 breaking force,  $a^*$ ,  $b^*$ ,  $L^*$ , as dependent variables. When a significant sample effect  
235 was obtained, a Student Neuman-Keuls pair comparison test was applied ( $\alpha = 5\%$ ).

### 236 *2.6.2. Sensory evaluation*

237 Principal Component Analysis (PCA) was performed on the consumer x biscuit global  
238 liking scores to evaluate the agreement among consumers' judgments. Consumer  
239 segmentation was then obtained by performing a Hierarchical Cluster Analysis (HCA)  
240 of the coordinates of the consumers on the first two dimensions of the PCA. A  
241 Euclidean distance and a Ward aggregation criteria were used for the HCA.

242 For each consumer cluster generated by the HCA, an ANOVA was performed on all  
243 hedonic scales (global liking, appearance, odor, taste, texture) considering consumer  
244 as random factor and samples as fixed factor. When a significant effect of sample  
245 was obtained a Student Neuman-Keuls (SNK) pair comparison test was applied with  
246 an alpha risk of 5%.

247 The open-ended question was analyzed by grouping terms with similar meaning.

248 This was done by two researchers. For each cluster, we computed the number of  
249 consumers who used each term to describe what they liked and disliked in each  
250 biscuit. Only terms used by more than two consumers were considered in the rest of

251 the analysis. A correspondence analysis was performed to visualize the relationship  
252 between the samples and the descriptions.

### 253 **3. Results**

#### 254 *3.1 Biscuits characterization*

##### 255 *3.1.1 Proximate analysis*

256 Protein, iron, vitamin A content and moisture of raw MDL and MDL supplemented  
257 biscuits are presented in Table 1. The analysis of the raw MDL revealed a content of  
258 4.48 g of moisture, 20.7 g of protein, 11.77 mg of iron, 0.14 mg of vitamin A in 100 g  
259 of powder. These results are within the range of the percentage of protein (18.2 -  
260 29.8 g/100 g of MDL) reported in Witt's meta-analysis. However, the percentage of  
261 iron content is lower than the values given by this meta-analysis (21.72 - 43.28 mg /  
262 100 g of MDL). The vitamin A content in C1, C2 and C3 are below the detection limit.  
263 This result suggests that vitamin A was degraded during baking.

264 The ANOVA result showed a significant effect of MDL concentration on moisture (F  
265 (3; 8) = 129.99,  $p < 0.0001$ ), protein (F (3; 8) = 79.57,  $p < 0.0001$ ) and iron (F (3; 8) =  
266 708.63,  $p < 0.0001$ ) composition of biscuits. The pair comparison test indicates that  
267 the control biscuit C0 is significantly different in moisture content from the biscuits  
268 containing MDL. The amount of moisture increases with the concentration of MDL but  
269 the pair comparison test shows no significant difference between C1 and C2. The  
270 gradual increase of MDL concentration added to biscuits also caused a gradual  
271 increase in protein content. The pair comparison test, revealed a significant  
272 difference between the control C0 and all other biscuits. Thereby, a substitution of  
273 wheat flour with MDL (from 10 to 30%) increases the protein content in biscuit by plus

274 10, 17, and 21% (for C1, C2 and C3 respectively) based on the control biscuit.  
275 Finally, the addition of MDL increases significantly the biscuit iron content from 0.85  
276 mg/100 g (biscuit C1) to 2.46 mg/100 g (biscuit C3). The pair comparison shows a  
277 significant difference between all biscuits.

### 278 *3.1.2 In vitro protein digestibility (IVPD)*

279 The percentage of enzymatic hydrolysis (%EH) of casein is 92%, which corresponds  
280 to the expected 100% of IVPD of casein and thus validates our protocol. For raw  
281 MDL we obtained 57% of IVPD. The IVPD of biscuit C1 (84%) was higher than the  
282 control biscuit C0 (47%). Then, the IVPD decreased from C1, to C2 (58%), to C3  
283 (54%).

### 284 *3.1.3 Biscuit physical properties*

285 The analysis of the physical characteristics of the supplemented biscuits is presented  
286 in Table 1. The ANOVA indicates that there is a significant difference between  
287 biscuits for all parameters. A significant decrease in thickness was observed with the  
288 increase in MDL concentration ( $F(3; 8) = 162.88; p < 0.0001$ ). The biscuit containing  
289 the highest concentration of MDL (C3) exhibited the lowest thickness of  $6.72 \pm 0.19$   
290 mm, whereas the control biscuit (C0) showed the highest thickness of  $9.44 \pm 0.10$   
291 mm.

292 The diameter of the C3 biscuit was significantly higher ( $F(3; 8) = 35.45; p < 0.0001$ )  
293 than the diameter of the control sample while no significant difference was observed  
294 among C0, C1 and C2 samples. The spread ratio also increased significantly as the  
295 level of MDL added to the biscuit increased ( $F(3; 8) = 113.86; p < 0.0001$ ).

296 The breaking force increased significantly with the concentration of MDL ( $F(3; 8) =$   
297  $160.05; p < 0.0001$ ). A weight of 15.44 kg was necessary to break the biscuit with the

298 highest MDL concentration (C3), whereas, only 3.08 kg was necessary to break the  
299 biscuit without MDL. The pair comparison test showed no significant difference  
300 between samples C1 and C2.

301 The impact of incorporation of MDL on biscuit color parameters is presented in Table  
302 1. The ANOVA showed a significant effect of MDL on L\* values ( $F(3; 8) = 227.01$ ;  $p < 0.0001$ ). The SNK test showed that the lightness parameter significantly decreased  
303 ( $< 0.0001$ ). The SNK test showed that the lightness parameter significantly decreased  
304 (from 75.06 to 41.67) with increase in MDL concentration: The more the quantity of  
305 MDL added to the control sample, the darker the biscuits. The a\* value parameter  
306 also reflects a significant effect ( $F(3; 8) = 141.71$ ;  $p < 0.0001$ ) on biscuits containing  
307 MDL. The increase of MDL concentration moved biscuits from the red color area  
308 (+a\*) to the green color area (-a\*). For C0, an a\* value of +1.9 was observed, while  
309 the a\* values of C1, C2 and C3 were respectively -0.13; -0.27; -1.53. The pair  
310 comparison test shows no difference between C1 and C2 in term of a\*. The ANOVA  
311 showed that the b\* value decreased significantly ( $F(3; 8) = 192.37$ ;  $p < 0.0001$ ) from  
312 C0 to C3 (21.43 to 7.25) indicating that with the addition of MDL, biscuits had a dark  
313 blue color.

### 314 *3.2. Sensory evaluation results*

#### 315 *3.2.1. Consumer segmentation*

316 The first two Principal Components (PC) of the PCA explain 74.9% of the total  
317 variance of the overall liking scores (Fig. 2a and 2b). All four samples load positively  
318 on the first PC (53.4%). Consumers that are positively correlated with this component  
319 tend to like better the supplemented biscuits than other consumers. The second PC  
320 (21.5%) opposes samples C0 and C3. Consumers that are positively correlated with

321 this PC preferred sample C3 to sample C0 and conversely, consumers negatively  
322 correlated with this PC preferred sample C0 to sample C3.

323 The HCA separated the consumers into two clusters (Fig. 2b). Consumers from  
324 cluster 1 (N=49) tend to like the biscuits less than consumers from cluster 2 (N=42).  
325 The two clusters differ significantly in term of consumers' age ( $F(1; 89) = 5.53$ ;  $p$   
326  $< 0.0209$ ). Consumers in cluster 2 are much younger (median age = 24.5) than the  
327 consumers in cluster 1 (median age = 26). No significant difference was observed in  
328 term of nationality ( $\chi^2 = 1.33$ ) or gender ( $\chi^2 = 0.26$ ).

329 Fig 3a and b represent the global liking score distributions for cluster 1 and 2.  
330 Consumers from cluster 1 (Fig. 3a) accepted only the control biscuit C0 (median = 5).  
331 Seventy-five percent of consumers rated the C1 biscuit between 2 and 4 with a  
332 median of 4.3. For C2 and C3 products, all scores were below 4 with a median of 3  
333 and 2 respectively. Consumers from cluster 2 (Fig. 3b) liked better the biscuits  
334 containing MDL than consumers from cluster 1 (average score  $> 4$ ). For the C0  
335 product, 75% of consumers had liking scores greater than 5 with a median of 6. For  
336 product C1 and C2, 50% of consumers had scores between 5 and 6 and 4 and 5 with  
337 medians equal to 6 and 5 respectively. The liking scores for C3 were more variable  
338 with some consumers clearly disliking this biscuit (scores close to 1) and other liking  
339 it (scores equal to 6).

### 340 *3.2.2. Effect of Moringa content on overall, appearance, texture, smell and taste liking* 341 *scores*

342 ANOVA and pairwise comparison tests were carried out separately for each cluster  
343 and each rating scale (Table 2). A sample effect was observed in both clusters for  
344 each rating scale. This effect is, however, stronger in the first cluster than in the



345 second one. In both clusters, appearance is the factor that affects the liking of the  
346 biscuit the most ( $R^2 = 0.76$  and  $0.71$  for cluster 1 and 2 respectively). In the first  
347 cluster, texture is the factor that has least effects on the liking of the biscuit. The other  
348 scales have comparable effects. In the second cluster, texture influences the liking  
349 scores more than taste and odor, which is the least impacting factor.

350 The pair comparison indicates that for both clusters and all liking scales the control  
351 biscuit is significantly preferred to the MDL supplemented biscuits. In the first cluster,  
352 C2 and C3 are not significantly different for global liking, odor, and texture scale. A  
353 significant difference was observed for appearance and taste. In the second cluster,  
354 we observe significant differences between all samples for all scales but odors.

### 355 *3.2.3. Analysis of open-ended question*

356 We represented the frequencies of positive and negative terms as word clouds  
357 separately for each cluster (Fig. 4). For cluster 1, (consumers who liked less the  
358 Moringa supplemented biscuits) the most frequent word used to describe what  
359 consumers liked in the four samples is a texture term: crunchy. Consumers liked also  
360 the taste, especially the sweetness of the control sample as well as its color. On the  
361 negative side, they found it bland. Color was the most frequently used term to  
362 describe what consumers disliked in the samples supplemented with MDL. The term  
363 too hard was also mentioned for samples C2 and C3 followed by vegetal. For sample  
364 C3, terms frequently mentioned include odor, spinach, unpleasant, too strong, and  
365 bitter.

366 For Cluster 2, (consumers who liked more the MDL supplemented biscuits) crunchy  
367 was frequently used to describe the positive aspects of the four biscuits as for cluster  
368 1. Sweet and color were also mentioned frequently for C0 whereas new terms appear

369 to describe what consumers liked in the MDL supplemented biscuit such as taste,  
370 sweet, color and crispy for sample C1. Cluster 2 consumers also appreciated the  
371 originality of the MDL supplemented biscuits, especially in sample C3. Finally, the  
372 term vegetal was mentioned among the positive aspects of sample C2. For the  
373 negative side, as in cluster 1, consumers cited the term bland for sample C0. For the  
374 other samples, color was much less cited than in cluster 1. For sample C1 the most  
375 frequent term was vegetal. After-taste and spinach were also important for this  
376 sample. C2 was characterized mostly by the term too hard, followed by color, vegetal  
377 and bitter. C3 was perceived as having a taste that is too strong in addition to being  
378 vegetal and bitter. Color was also cited but in a lesser extent than for samples C2  
379 and C1.

380 Fig. 5 shows the first two dimensions (71.5% of the variance) of the CA carried out on  
381 the term frequency counts. For both clusters, the first dimension (55.5%) opposes the  
382 control sample to the MDL supplemented samples. In both clusters, the control  
383 sample is positively perceived except for the term bland whereas the MDL  
384 supplemented samples have negative global perception among consumers. The  
385 second dimension opposes the two clusters. The difference between the clusters  
386 increases with the proportion of MDL included in the biscuit. In cluster 1, the MDL  
387 supplemented biscuits attracted mostly negative terms, whereas in cluster 2 some  
388 positive terms were associated to these biscuits. Sample C1 attracted terms such as  
389 pleasant and balanced and samples C2 and C3 were positively associated with terms  
390 such as vegetal and original. Consumers in this group diverge somewhat in their  
391 appreciation of sample C3. While some consumers liked its originality and its vegetal  
392 character, others were less positive and associated it with negative surprise,  
393 bitterness and find its taste too strong.

## 394 **4. Discussion**

395 The first objective of this work was to implement sensory analysis in order to gain  
396 understanding of heterogeneity in consumer preferences of Moringa supplemented  
397 biscuits. Secondly, this study also investigated nutritional enrichment of biscuits  
398 supplemented with Moringa.

### 399 *4.1 Effects of MDL supplementation on biscuits nutritional properties and IVPD*

400 Although raw MDL contain vitamin A, this compound was degraded during the baking  
401 process. In order to avoid this loss, one solution could be to incorporate MDL after  
402 the cooking process (e.g. soup, sauce) or in raw products (e.g. salad). However, this  
403 solution may lead to nutritional or sensory related problems. Findings from the  
404 sensory analysis revealed that MDL had a pronounced vegetal taste, which is  
405 unpleasant when eaten raw. Previous studies showed that heat processing was an  
406 effective method to inactivate anti-nutritional factors of most tropical legumes. The  
407 inactivation of trypsin inhibitor, for example, makes protein more susceptible to  
408 enzymatic digestion (Akande and Fabiyi, 2010). Therefore, another solution could be  
409 to use cooking processes with low heating impact in order to find a trade-off between  
410 all these criteria.

411 Although, in agreement with previous work (Dachana et al., 2010; Mouminah, 2019;  
412 Rathnayake and Navarathna, 2017), the addition of MDL increased protein and iron  
413 content of biscuits, only a percentage of these proteins were digested. With our IVPD  
414 protocol (gastric digestion 2h; intestinal digestion 2h), 57% of the proteins of raw  
415 MDL was digested. This value is within the range of IVPD values reported by Teixeira  
416 et al. (2013) (33.3%) and Ly et al. (2001) (74.2%). This difference in percentage of  
417 IVPD can be explained by the difference in experimental conditions where, for

418 example, for Teixeira et al. (2013) the gastric digestion takes 3h and the intestinal  
419 digestion takes 1h.

420 Moreover, with respect to the IVPD of biscuits, the results showed that the biscuit  
421 sample which had the lowest concentration of MDL (C1), had the highest IVPD of  
422 84%. Beyond this concentration, the IVPD decreases when the protein concentration  
423 in biscuits increases. This decrease of digestibility could be explained by an  
424 incomplete enzymatic hydrolysis of proteins due to insufficiency of digestion time or  
425 enzyme concentration. Nevertheless, Boisen and Fernandez (1995) confirmed that in  
426 vitro digestibility can predict in vivo ideal digestibility. Thus, the appropriate solution is  
427 maybe to avoid incorporating a fairly large amount of MDL in the biscuit in order to  
428 optimize their digestibility and their benefits.

#### 429 *4.2. Contribution of supplemented biscuit to recommended daily requirement*

430 In order to determine the contribution of biscuits containing MDL to the recommended  
431 daily requirement, we chose the C1 biscuit, which is the best digested and the most  
432 widely accepted by consumers. So, 100 g of biscuit C1 (10%MDL; 84%IVPD) /day  
433 provide about 0.85 mg of iron and 5.11 g of proteins. Compared with the  
434 recommended daily requirement of adults (11 mg of iron /day and 0.83 g of  
435 protein/kg/day (ANSES, 2016), the consumption of 100 g of biscuits C1/ day would  
436 provide 7.7% of the iron. For protein, this consumption provides just 8.5% of the  
437 protein after digestion (for an adult weighting 60 kg). Despite this rather low  
438 percentage of protein intake, the leaves of Moringa contain the eight essential amino  
439 acids among which the most abundant is leucine and the least abundant is  
440 methionine (Ramaroson et al., 2014). Wheat protein have a lower nutritional quality  
441 since they are deficient in essential amino acids such as lysine and threonine

442 (Dewettinck et al., 2008; Ndife et al., 2011). Combining MDL with wheat flour  
443 products can improve essential amino acids composition.

#### 444 *4.3. Effects of MDL supplementation on physical properties of biscuits*

445 The incorporation of MDL in biscuits impacts also the physical properties of biscuits,  
446 as thickness decreases. This change is due to the reduction of the percentage of  
447 gluten contained in the blend. It has been reported by Sengev et al. (2013) that  
448 partial replacement of wheat flour with non-glutinous flour results in lower bread  
449 volumes. Gluten is the main structure-forming protein in flour. It is responsible for the  
450 elastic and extensible properties needed to produce good quality bread (Greene and  
451 Bovell-Benjamin, 2004). In addition, the breaking force increases when the  
452 percentage of MDL incorporated in the biscuits increases. This explains why the  
453 texture of the supplemented biscuit was affected by the addition of MDL. This result  
454 is in line with Galla et al. (2017) who show that the increase of leafy spinach in  
455 biscuits increases hardness and breaking strength. All of these changes and  
456 constraints confirm that there is a limit to further increasing the amount of MDL in the  
457 biscuit in order to provide more nutrients.

#### 458 *4.4. Effects of MDL supplementation on biscuit sensory properties and consumer* 459 *acceptance*

460 The results of this study show that the global liking of biscuits containing MDL was  
461 not homogenous. Some consumers (cluster 2) tended to like biscuits containing MDL  
462 whereas, other consumers did not accept them (cluster 1). When considering the  
463 different sensory modalities, the appearance was the factor that affected the most the  
464 global liking of biscuits for both consumer groups. The open-ended question  
465 suggests that color is the main visual driver of rejecting biscuits supplemented with  
466 MDL: color was clearly disliked by consumers of cluster 1 but less so by consumers

467 from cluster 2. Consumers in cluster 2 were younger than consumers in cluster 1.  
468 This age difference perhaps implies that younger consumers are more willing to  
469 accept the supplemented biscuit due to their curiosity to test novel products as  
470 attested by the high frequency of the term original in this cluster. This interpretation is  
471 in line with the study of Ramaroson et al. (2014) carried out in Madagascar.  
472 Concerning cluster 1, color hampers consumer's acceptance of MDL supplemented  
473 biscuit. This might be explained by the fact that consumers in this cluster were not  
474 familiar with the green color brought by MDL. The rejection of MDL supplemented  
475 biscuits by these consumers could thus be interpreted in terms of food neophobia,  
476 which is the avoidance of, or reluctance to consume novel foods (Aldridge et al.,  
477 2009). Texture was also one of the most important drivers of biscuit rejection. The  
478 main problem was the hardness of the biscuit: the force needed to break the biscuit  
479 sample that had the highest concentration of MDL (C3) was five times higher than the  
480 force needed to break the control biscuit. In line with this interpretation, the term too  
481 hard was frequently evoked in the open-ended question for MDL supplemented  
482 biscuits.

## 483 **5. Conclusion**

484 The present study showed that the incorporation of MDL in food products like biscuits  
485 would encounter several obstacles to consumer acceptance and affect physical  
486 properties especially in terms of color (green) and texture (hardness). For nutritional  
487 benefits, this fortification can enhance nutritional values such as iron and protein  
488 compounds but many constraints and negative effects appear following this  
489 incorporation. Protein provided by these biscuits were not completely digested.  
490 Besides, due to baking processes, some of the nutrients from Moringa such as

491 vitamin A can be lost. Therefore, incorporating a high concentration of MDL in  
492 biscuits is constrained by a lot of limits as previously explained.

493 In conclusion, *Moringa oleifera* could be a proper source to enhance nutritional  
494 compounds in foods. In addition, some advice can be given to food producers who  
495 are interested in food supplementation: it is essential to choose the appropriate  
496 product in which to incorporate Moringa leaves to benefit from these dietary  
497 advantages with the right quantity without influencing the technological and sensory  
498 properties of products, mainly texture and appearance.

499 **Declaration of Competing Interest:** The authors declare no conflict of interest.

## 500 **Acknowledgements**

501 The authors would like to thank AgroSup Dijon and Embassy of Tunisia in France for  
502 the financial support of this work. We gratefully thank Bernadette Rollin, Anne-Sophie  
503 Viaux and Christine Rojas for their assistance with the laboratory work. Catalina  
504 Onofrei is particularly acknowledged for English proofreading.

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657

658 **Tables**

659

660 **Table 1** Physico-chemical characteristics of MDL powder and MDL supplemented  
661 biscuits

	<b>MDL</b>	<b>C0 (0% MDL)</b>	<b>C1 (10% MDL)</b>	<b>C2 (20% MDL)</b>	<b>C3 (30% MDL)</b>
Moisture (g/100g)	4.48 ± 0.03	3.78 ± 0.11 <sup>b</sup>	2.71 ± 0.09 <sup>a</sup>	4.19 ± 0.05 <sup>c</sup>	4.64 ± 0.07 <sup>d</sup>
Proteins (g/100g)	20.70 ± 0.91	4.60 ± 0.09 <sup>a</sup>	5.11 ± 0.06 <sup>b</sup>	5.57 ± 0.20 <sup>c</sup>	5.96 ± 0.04 <sup>d</sup>
Iron (mg/100g)	11.77 ± 0.86	0.00 <sup>a</sup>	0.85 ± 0.07 <sup>b</sup>	1.72 ± 0.06 <sup>c</sup>	2.46 ± 0.10 <sup>d</sup>
Vitamin A (mg/100g)	0.14 ± 0.02	-	-	-	-
Thickness (mm)		9.44 ± 0.10 <sup>d</sup>	8.67 ± 0.17 <sup>c</sup>	7.39 ± 0.19 <sup>b</sup>	6.72 ± 0.19 <sup>a</sup>
Diameter (mm)		27.70 ± 0.10 <sup>a</sup>	27.47 ± 0.29 <sup>a</sup>	27.67 ± 0.15 <sup>a</sup>	28.83 ± 0.12 <sup>b</sup>
Spread ratio		29.33 ± 0.40 <sup>a</sup>	31.70 ± 0.92 <sup>b</sup>	37.46 ± 1.05 <sup>c</sup>	42.92 ± 1.34 <sup>d</sup>
Breaking force (Kg)		3.08 ± 0.40 <sup>a</sup>	7.78 ± 0.76 <sup>b</sup>	7.93 ± 0.41 <sup>b</sup>	15.44 ± 1.03 <sup>c</sup>
L*		75.06 ± 3.08 <sup>d</sup>	53.59 ± 1.29 <sup>c</sup>	44.92 ± 0.48 <sup>b</sup>	41.67 ± 0.74 <sup>a</sup>
a*		1.90 ± 0.03 <sup>c</sup>	-0.13 ± 0.01 <sup>b</sup>	-0.27 ± 0.01 <sup>b</sup>	-1.53 ± 0.41 <sup>a</sup>
b*		21.43 ± 1.17 <sup>d</sup>	16.78 ± 0.57 <sup>c</sup>	10.16 ± 0.88 <sup>b</sup>	7.25 ± 0.29 <sup>a</sup>

MDL: Moringa Dried Leaves. Values represent mean ± SD of triplicate assays. Means ± SD with the same letter are not significantly different at an alpha risk of 5%.

662

663 **Table 2** One-way repeated measure ANOVA with consumers as a random factor,  
 664 samples as a fixed factor and liking scores as dependent variables.

<b>Cluster 1</b>	<b>F</b>	<b>P</b>	<b>R<sup>2</sup></b>	<b>C0</b>	<b>C1</b>	<b>C2</b>	<b>C3</b>
Global liking	66.10	0.0001	0.65	4.9 <sup>a</sup>	3.7 <sup>b</sup>	2.6 <sup>c</sup>	2.4 <sup>c</sup>
Appearance	94.70	0.0001	0.76	5.6 <sup>a</sup>	3.9 <sup>b</sup>	3.0 <sup>c</sup>	2.3 <sup>d</sup>
Odor	45.69	0.0001	0.65	4.5 <sup>a</sup>	3.2 <sup>b</sup>	2.6 <sup>c</sup>	2.4 <sup>c</sup>
Texture	30.90	0.0001	0.60	5.3 <sup>a</sup>	4.7 <sup>b</sup>	3.4 <sup>c</sup>	3.4 <sup>c</sup>
Taste	69.20	0.0001	0.67	5.2 <sup>a</sup>	3.8 <sup>b</sup>	2.6 <sup>c</sup>	2.2 <sup>d</sup>
<b>Cluster 2</b>							
Global liking	24.60	0.0001	0.47	6.0 <sup>a</sup>	5.3 <sup>b</sup>	4.6 <sup>c</sup>	4.0 <sup>d</sup>
Appearance	54.97	0.0001	0.71	5.7 <sup>a</sup>	4.7 <sup>b</sup>	3.7 <sup>c</sup>	3.1 <sup>d</sup>
Odor	17.98	0.0001	0.48	5.2 <sup>a</sup>	4.0 <sup>b</sup>	3.7 <sup>bc</sup>	3.3 <sup>c</sup>
Texture	30.70	0.0001	0.61	6.0 <sup>a</sup>	5.4 <sup>b</sup>	4.1 <sup>c</sup>	3.9 <sup>c</sup>
Taste	27.39	0.0001	0.56	5.9 <sup>a</sup>	5.1 <sup>b</sup>	4.3 <sup>c</sup>	3.7 <sup>d</sup>

Means with the same letter are not significantly different at an alpha risk of 5%.

665 **Figure Captions**

666

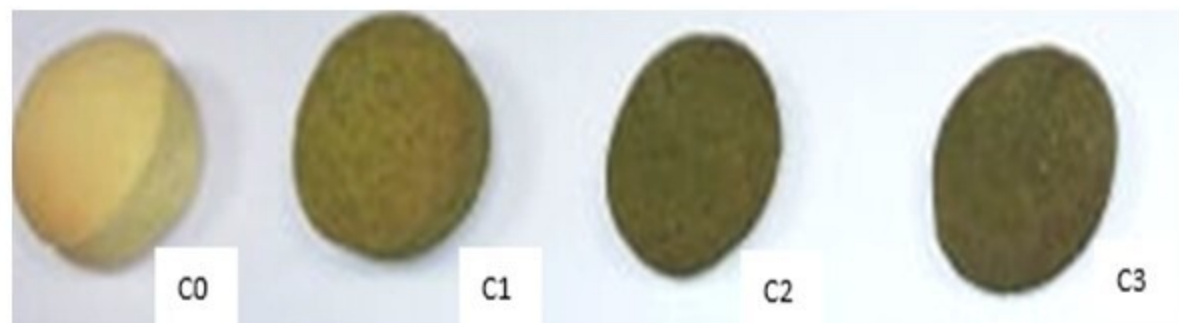
667 **Fig. 1** Photographs of control biscuit (C0) and Moringa supplemented biscuits (C1,  
668 C2 and C3)

669 **Fig. 2** Principal Components Analysis (PCA) of the consumers global liking scores.  
670 The left panel (a) represents the loading of the samples and the right panel (b) the  
671 projection of the consumers. The dot colors and the ellipses represent the two  
672 clusters yielded by the Hierarchical Cluster Analysis (HCA)

673 **Fig. 3** Boxplots representing the frequency counts of global liking scores for  
674 consumers from cluster 1(a) and cluster 2(b)

675 **Fig. 4** Word clouds of the terms generated by the consumers from the two clusters to  
676 describe what they liked and disliked in the samples. The size of the words in this  
677 representation is proportional to their use: the larger the word size, the higher the  
678 frequency of occurrence

679 **Fig. 5** First 2-dimensional space of the correspondence analysis of the terms  
680 generated by the consumers to describe what they like and dislike in the biscuits. The  
681 size of the diamonds is proportional to the quality of the representation of the terms in  
682 the subspace. Terms corresponding to what consumers like are followed by a sign  
683 (+) and terms corresponding to what consumers disliked are followed by a sign (-).  
684 Only terms used by at least 10% of consumers have been kept. Sample descriptions  
685 obtained for consumers from cluster 1 are denoted by CL1 before the code of the  
686 samples and sample descriptions obtained for consumers from cluster 2 are denoted  
687 by CL2 before the code of the samples

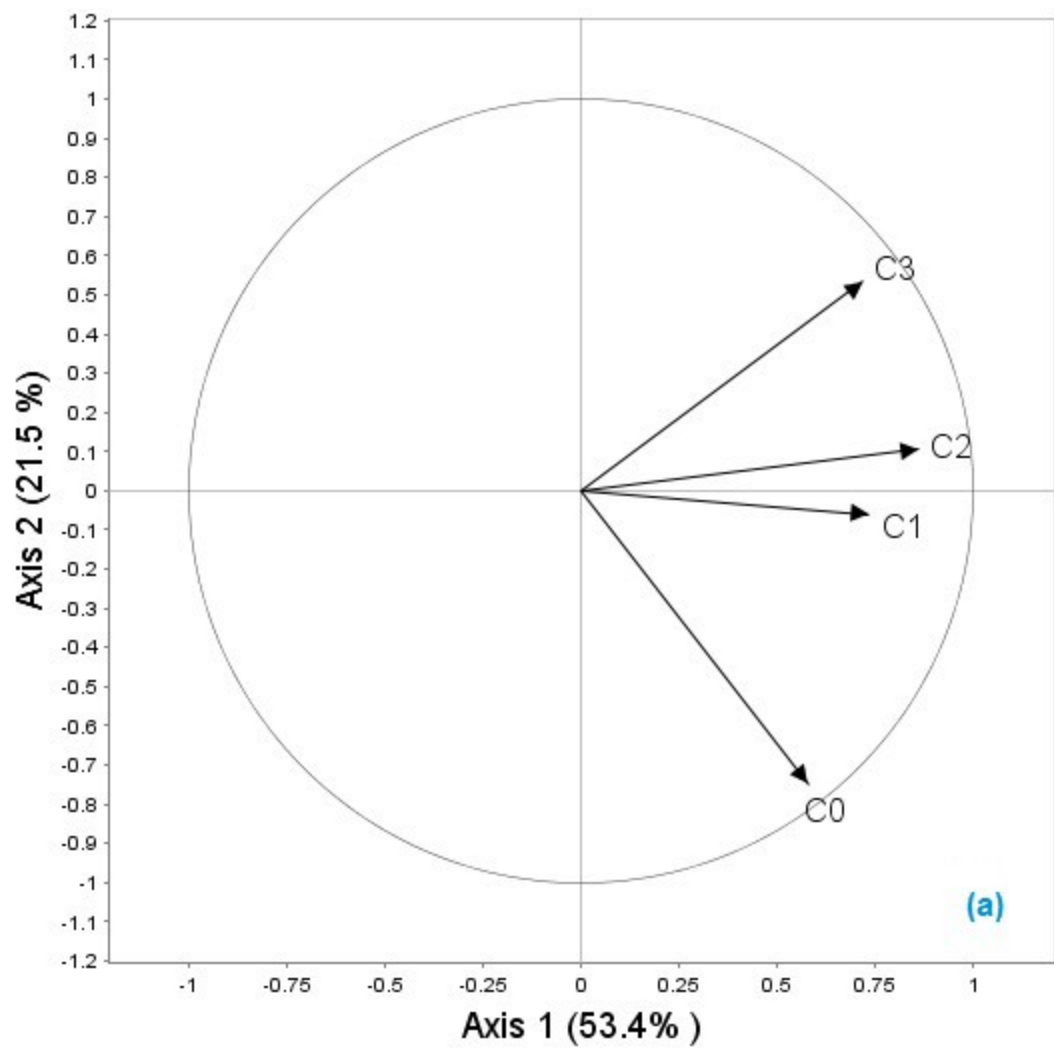


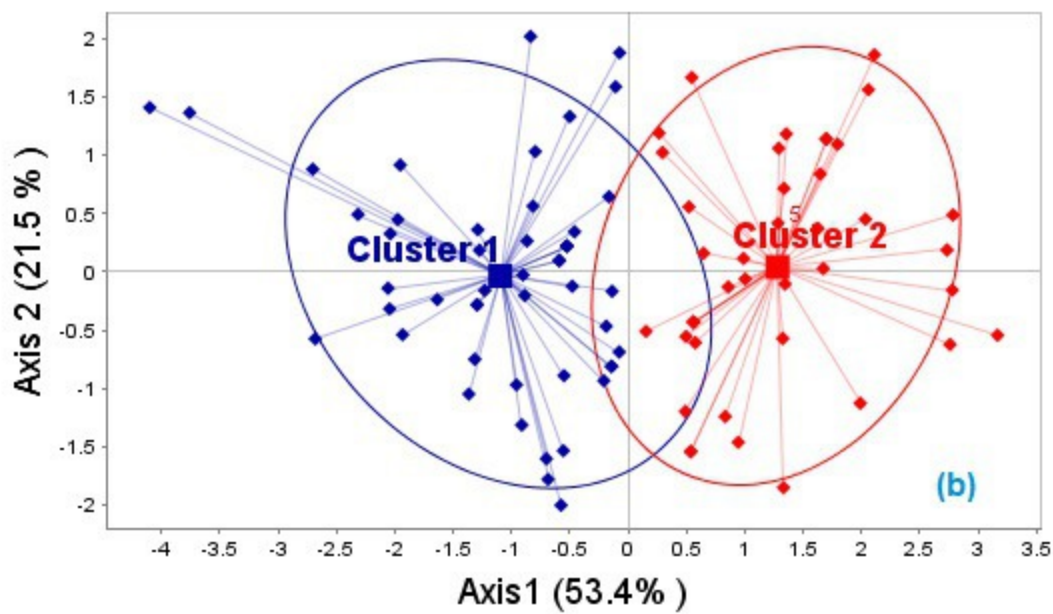
C0

C1

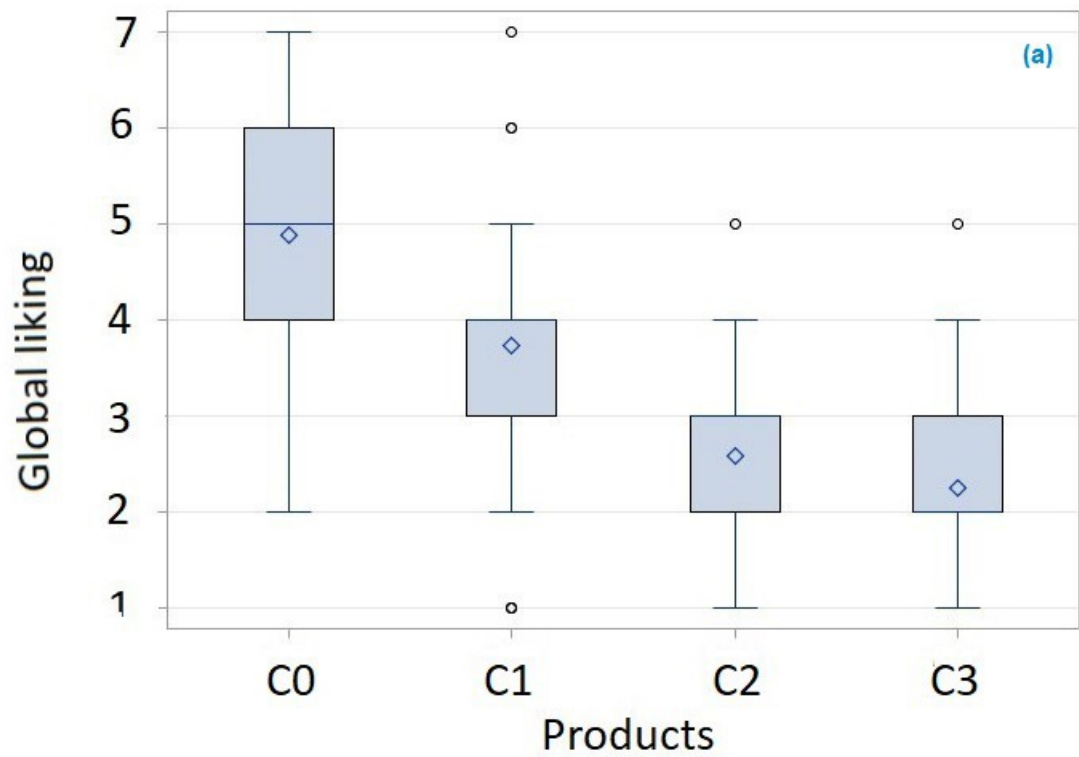
C2

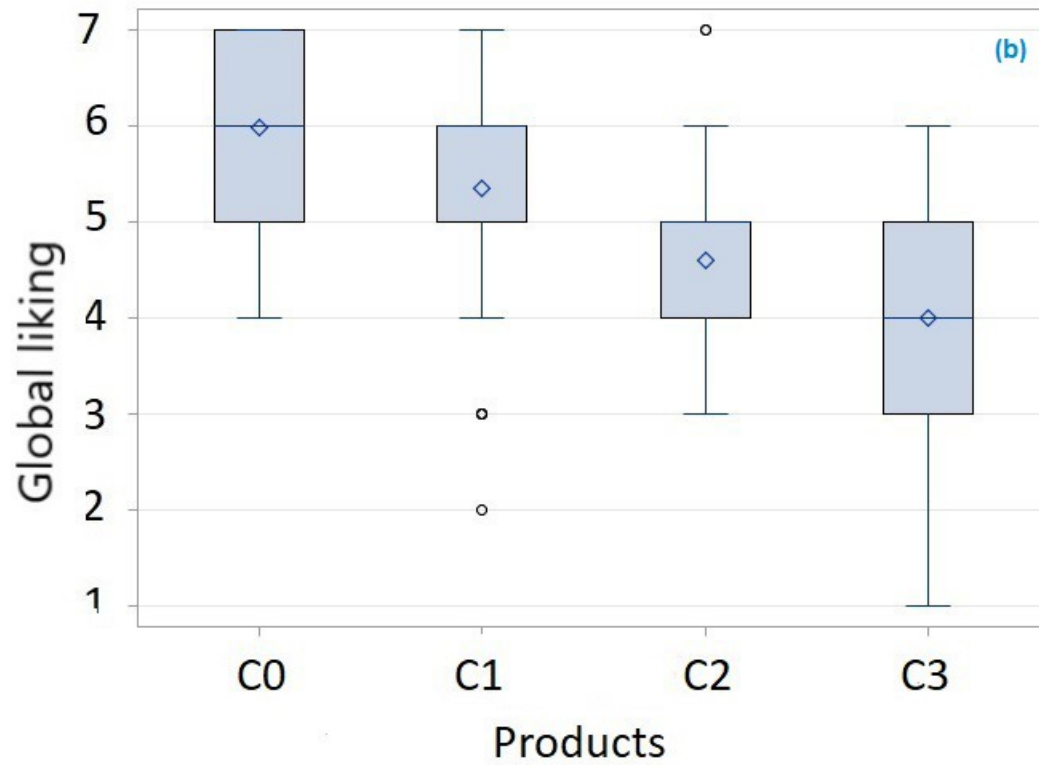
C3











Cluster1

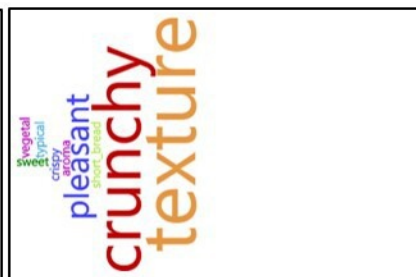
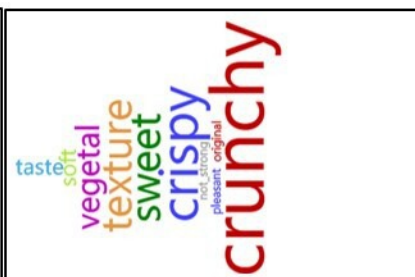
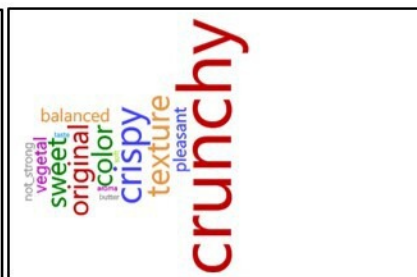
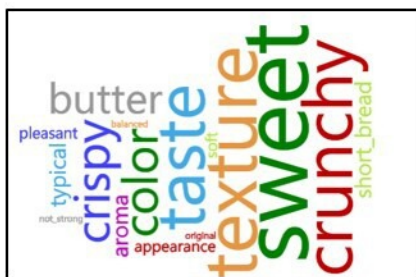
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C1

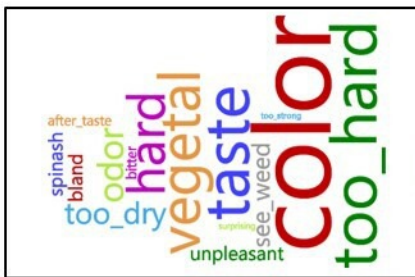
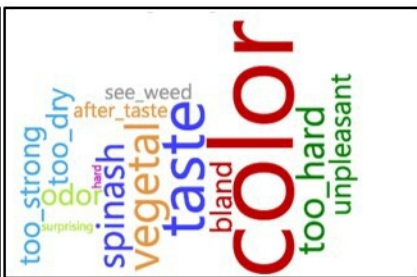
C2

C3

Like



Dislike



Cluster2

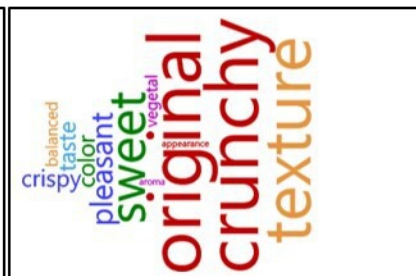
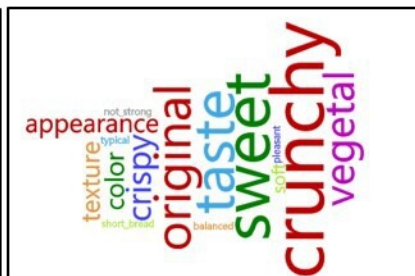
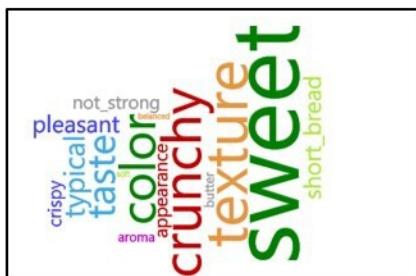
C0

C1

C2

C3

Like



Dislike

