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Moringa Oleifera supplemented biscuits: nutritional values and consumer segmentation

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Abstract

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

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

1. Introduction

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

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

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

Many scientific studies have shown that the supplementation of staple foods such as bread, cheese, yoghurt, and biscuits with MDL improve their nutritional properties (Dachana et al., 2010; Hekmat et al., 2015; Salem et al., 2013; Sengev et al., 2013). Biscuits is one of the most popular bakery products in the human diet due to many characteristics such as palatability, convenience, ready-to-eat, affordability and longer shelf-life in comparison to the other bakery products (Arshad, 2007; Nagi, 2012). It is also widely accepted and consumed in many countries, which makes it a viable supplementation vehicle for nutritional improvement of vital nutrients like protein, dietary fibers and functional ingredients (Sulieman et al., 2019).

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

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

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

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

Another potential limitation of the use of Moringa for food supplementation is that most of the reported evidence do not take into account the effect of processing on nutritional compounds and/or of the bio-availability of these compounds. Furthermore, the few studies which explored this last point are far from being 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

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

2.2. *Proximate analysis*

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

2.3. In vitro protein digestibility (IVPD)

Pepsin (from porcine stomach mucosa, >2500 U/mg protein), pancreatin (from porcine pancreas, 4 x USP), casein sodium salt from bovine milk (C-8654) and TAME (p-Toluene-Sulfonyl-L-arginine methyl ester; T4626) were purchased from Sigma-Aldrich (France). In vitro digestibility was carried out according to the method of Minekus et al. (2014) with some modifications. Analysis was done in duplicate. In addition, 1 g of MDL or 1 g of biscuit was prepared in 10 mL of solution then 8 mL of 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 µL of 0.3 M $CaCl_2$ 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 µL of 0.3 M $CaCl_2$ 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 $$\%\ \text{Enzymatic Hydrolysis (\%EH)} = \frac{amount\ of\ protein\ after\ digestion}{total\ amount\ of\ protein} * 100$$

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

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

2.4.2. Breaking force

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

2.4.3 Color measurement

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

2.5. Sensory evaluation

2.5.1. Consumers

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

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

2.5.2. Experimental conditions

Consumers were provided with information about the study, following which they completed an informed consent form in keeping with French ethical requirements. The consumers tasted the samples in a sensory evaluation room at Agrosup Dijon. The biscuits were served in covered white plastic cups coded with a three-digit random number. Two series of four samples (C0, C1, C2, and C3) each were presented on a tray. Different random codes and different presentation orders were used for the two series. The presentation order followed Williams' Latin squares. Consumers were not aware that the biscuits in the two series were the same. Bottled water was provided for palate cleansing. The questionnaire was written in French language and explained in English when necessary.

2.5.3. Procedure

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

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

2.6. Statistical Analysis

2.6.1 Chemical composition

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

2.6.2. Sensory evaluation

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

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

The open-ended question was analyzed by grouping terms with similar meaning. This was done by two researchers. For each cluster, we computed the number of consumers who used each term to describe what they liked and disliked in each biscuit. Only terms used by more than two consumers were considered in the rest of

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

3. Results

3.1 Biscuits characterization

3.1.1 Proximate analysis

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

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

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

3.1.2 In vitro protein digestibility (IVPD)

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

3.1.3 Biscuit physical properties

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

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

The breaking force increased significantly with the concentration of MDL ($F(3; 8) = 160.05$; $p < 0.0001$). 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 ($F(3; 8) = 227.01$; $p < 0.0001$).
303 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 ($F(1; 89) = 5.53$; $p < 0.0209$). 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 (khi2 = 1.33) or gender (khi2 = 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

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

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

4. Discussion

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

4.1 Effects of MDL supplementation on biscuits nutritional properties and IVPD

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

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

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

4.3. Effects of MDL supplementation on physical properties of biscuits

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

4.4. Effects of MDL supplementation on biscuit sensory properties and consumer acceptance

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

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

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

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

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Tables

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

	MDL	C0 (0% MDL)	C1 (10% MDL)	C2 (20% MDL)	C3 (30% MDL)
Moisture (g/100g)	4.48 ± 0.03	3.78 ± 0.11^{b}	2.71± 0.09^{a}	4.19 ± 0.05^{c}	4.64 ± 0.07^{d}
Proteins (g/100g)	20.70 ± 0.91	4.60 ± 0.09^{a}	5.11 ± 0.06^{b}	5.57 ± 0.20^{c}	5.96 ± 0.04^{d}
Iron (mg/100g)	11.77 ± 0.86	0.00 ^{a}	0.85 ± 0.07^{b}	1.72 ± 0.06^{c}	2.46 ± 0.10^{d}
Vitamin A (mg/100g)	0.14 ± 0.02	-	-	-	-
Thickness (mm)		9.44 ± 0.10^{d}	8.67 ± 0.17^{c}	7.39 ± 0.19^{b}	6.72 ± 0.19^{a}
Diameter (mm)		27.70 ± 0.10^{a}	27,47 ± 0.29^{a}	27.67 ± 0.15^{a}	28.83 ± 0.12^{b}
Spread ratio		29.33 ± 0.40^{a}	31,70 ± 0.92^{b}	37,46 ± 1.05^{c}	42.92 ± 1.34^{d}
Breaking force (Kg)		3.08 ± 0.40^{a}	7.78 ± 0.76^{b}	7.93 ± 0.41^{b}	15.44 ± 1.03^{c}
L*		75.06 ± 3.08^{d}	53.59 ± 1.29^{c}	44.92 ± 0.48^{b}	41.67 ± 0.74^{a}
a*		1.90 ± 0.03^{c}	-0.13 ± 0.01^{b}	-0.27 ± 0.01^{b}	-1.53 ± 0.41^{a}
b*		21.43 ± 1.17^{d}	16.78 ± 0.57^{c}	10.16 ± 0.88^{b}	7.25 ± 0.29^{a}

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%.

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.

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

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

Figure Captions

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

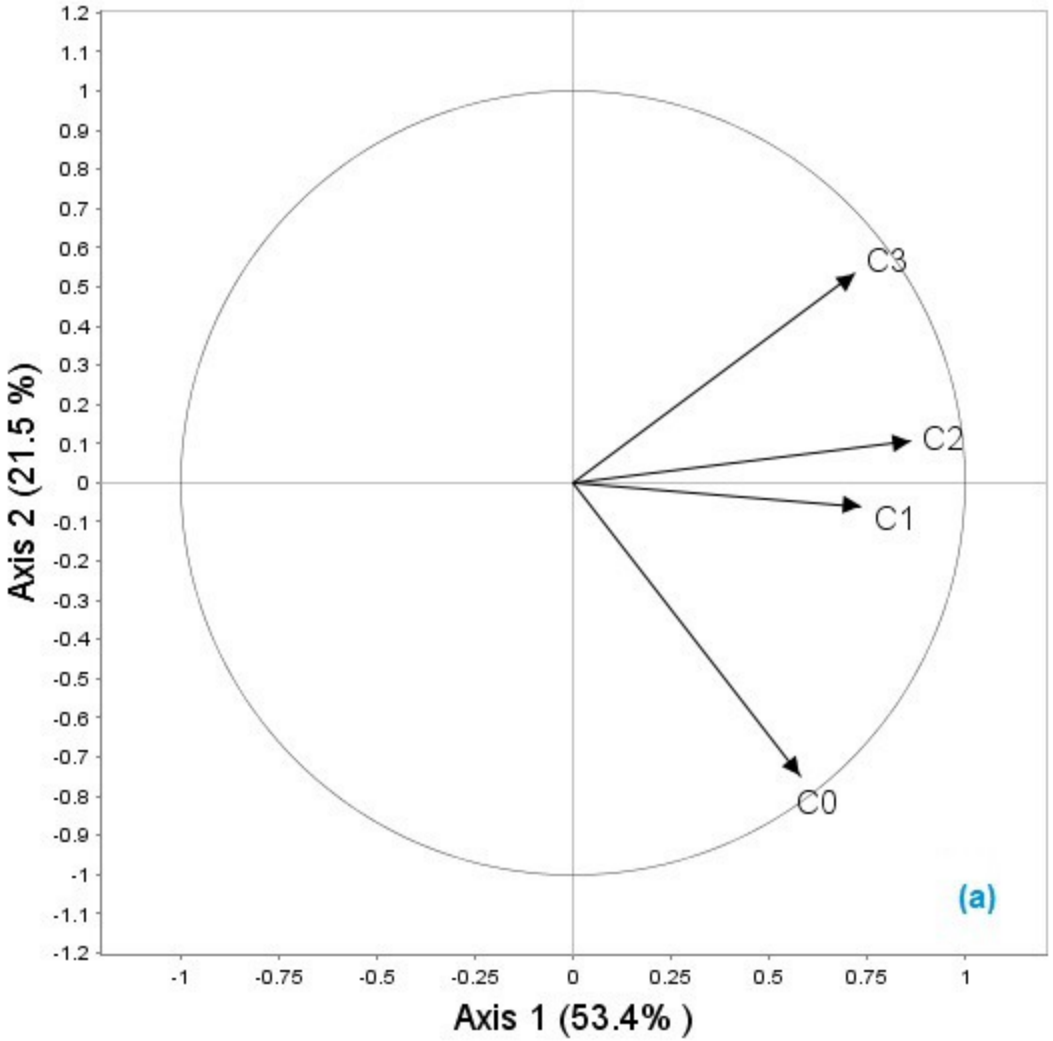
Fig. 2 Principal Components Analysis (PCA) of the consumers global liking scores. The left panel (a) represents the loading of the samples and the right panel (b) the projection of the consumers. The dot colors and the ellipses represent the two clusters yielded by the Hierarchical Cluster Analysis (HCA)

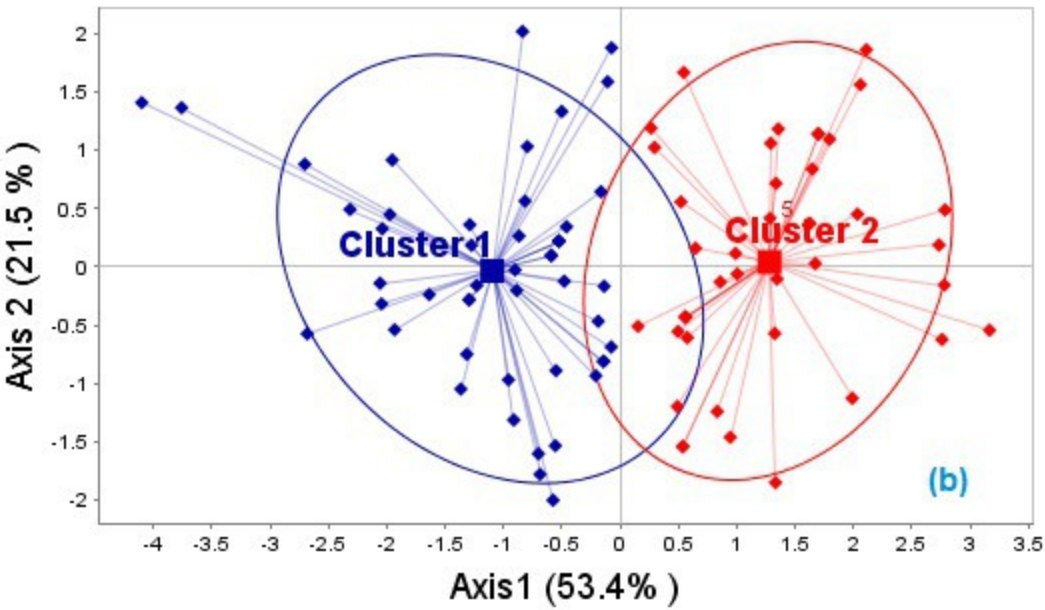
Fig. 3 Boxplots representing the frequency counts of global liking scores for consumers from cluster 1(a) and cluster 2(b)

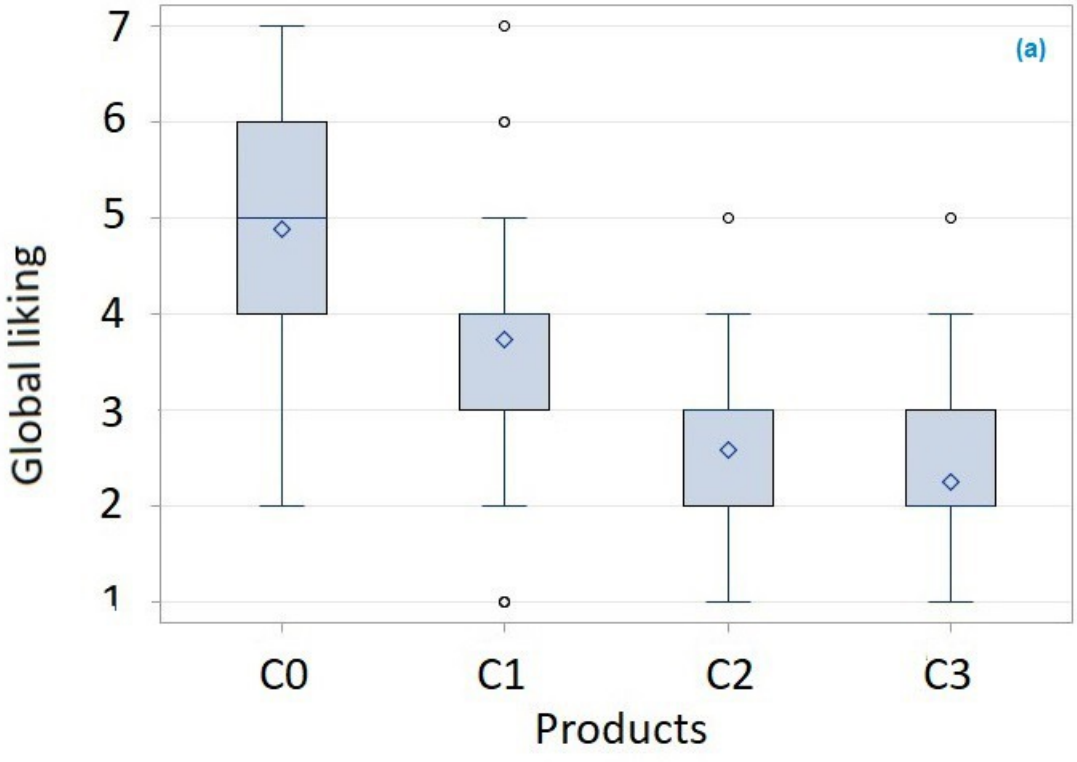
Fig. 4 Word clouds of the terms generated by the consumers from the two clusters to describe what they liked and disliked in the samples. The size of the words in this representation is proportional to their use: the larger the word size, the higher the frequency of occurrence

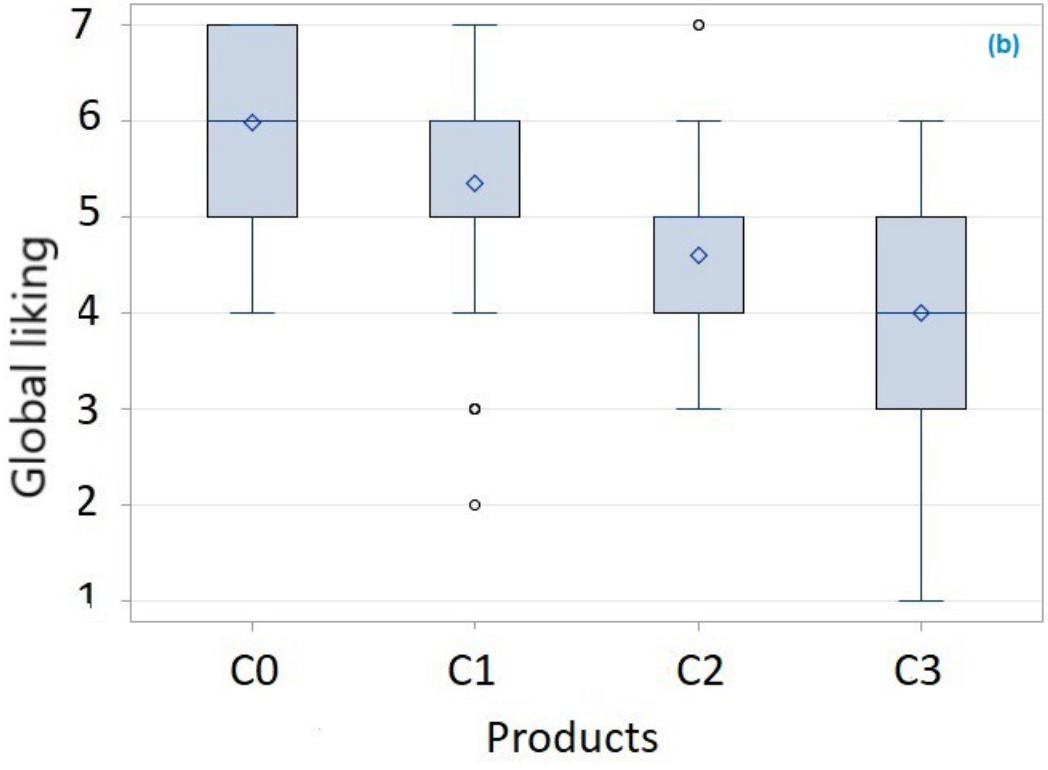
Fig. 5 First 2-dimensional space of the correspondence analysis of the terms generated by the consumers to describe what they like and dislike in the biscuits. The size of the diamonds is proportional to the quality of the representation of the terms in the subspace. Terms corresponding to what consumers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


C0
C1
C2
C3

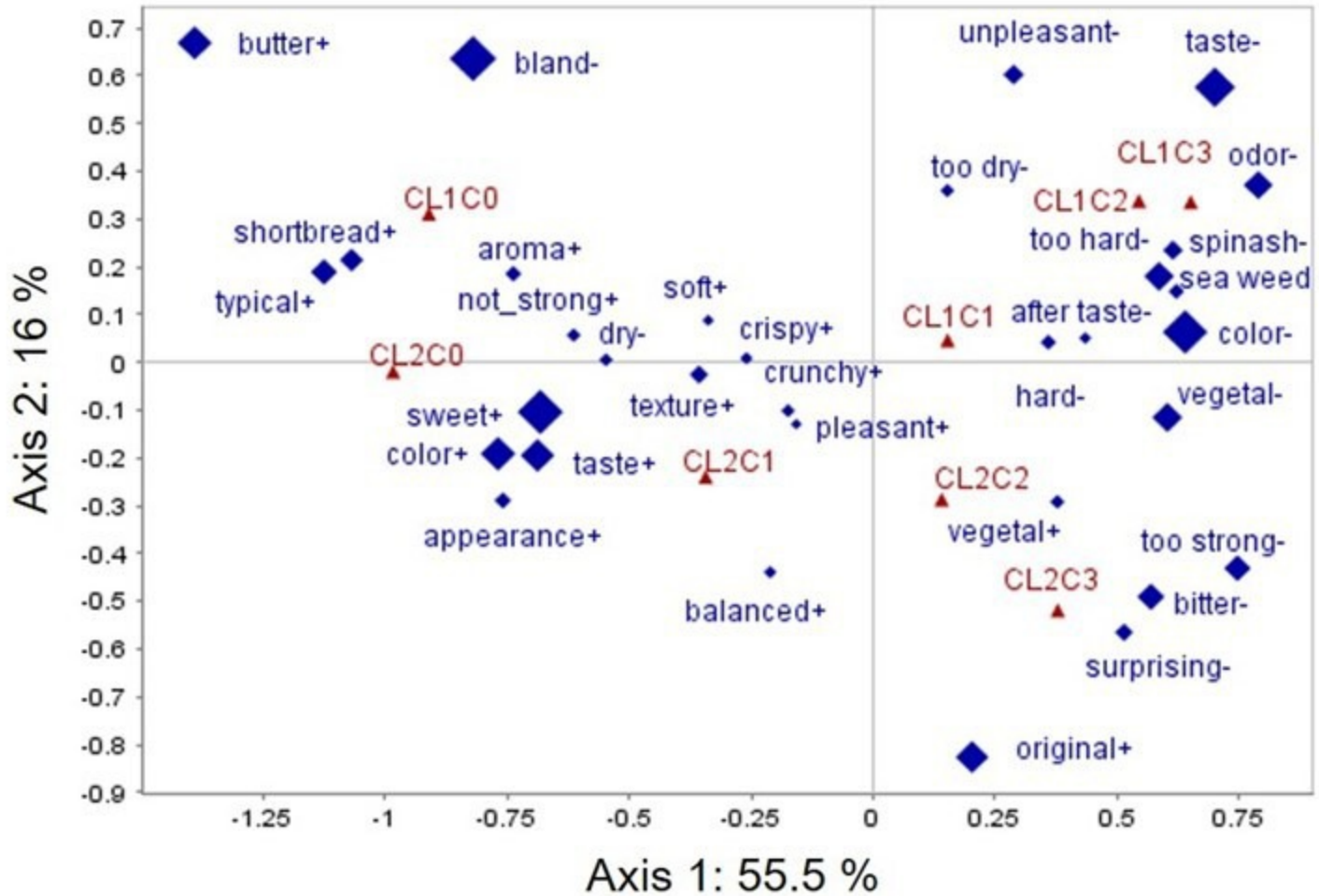

C3
C2
C1
C0
(a)
Axis 2 (21.5 %)
Axis 1 (53.4%)
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
-0.8
-0.9
-1
-1.1
-1.2
-1
-0.75
-0.5
-0.25
0
0.25
0.5
0.75
1


Cluster 1
Cluster 2
(b)
Axis 2 (21.5 %)
Axis1 (53.4%)
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-4
-3.5
-3
-2.5
-2
-1.5
-1
-0.5
0
0.5
1
1.5
2
2.5
3
3.5


(a)
Global liking
7
6
5
4
3
2
1
C0
C1
C2
C3
Products


(b)
Global liking
7
6
5
4
3
2
1
C0
C1
C2
C3
Products


Cluster1
C0
C1
C2
C3
Like
Dislike
Cluster2
C0
C1
C2
C3
Like
Dislike


butter+
bland-
unpleasant-
taste-
too dry-
CL1C3
odor-
CL1C0
CL1C2
shortbread+
too hard-
spinash-
aroma+
sea weed
typical+
not_strong+
soft+
CL1C1
after taste-
color-
dry-
crispy+
CL2C0
crunchy+
hard-
vegetal-
sweet+
texture+
pleasant+
color+
taste+
CL2C1
CL2C2
vegetal+
too strong-
appearance+
CL2C3
bitter-
balanced+
surprising-
original+
Axis 2: 16 %
Axis 1: 55.5 %
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
-0.8
-0.9
-1.25
-1
-0.75
-0.5
-0.25
0
0.25
0.5
0.75