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

3 Amel Hedhili^{a,b,*}, Samuel Lubbers^b, Elias Bou-Maroun^b, Francine Griffon^a, Babatope
4 Ebenezer Akinyemi^c, Florence Husson^b, Dominique Valentin^a

5 ^a: Centre des Sciences du Goût et de l'Alimentation, AgroSup Dijon, CNRS, INRAE,
6 Univ. Bourgogne Franche-Comté, F-21000 Dijon, France

7 ^b: Univ. Bourgogne Franche-Comté, AgroSup Dijon, PAM UMR A 02.102, F-21000
8 Dijon, France

9 ^c: Department of Agricultural Economics & Extension. University of Fort Hare, Alice,
10 5700, South Africa

11 *Corresponding author. E-mail address: Amel.Hedhili@inrae.fr (A. Hedhili)

12

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; Sengeev 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

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

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

2. Materials and methods

2.1. Biscuit preparation

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

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

Wheat flour and MDL were sieved before mixing with shortening until homogeneous lumps were formed. Then, sugar, salt and baking powder were added. The mixture 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

of porcine pepsin solution (25.000 U/mL) and 5 µL of 0.3 M CaCl₂ were added. The pH of the mixture was adjusted to 3.0. The solution was incubated for 120 min at 37 °C under continuous stirring at 170 rpm. After gastric digestion, gastric chime was mixed with 11 mL of simulated intestinal fluid (SIF). To this solution, 736 mg of the pancreatic enzyme and 40 µL of 0.3 M CaCl₂ were added and the final volume was completed to 40 mL by SIF solution. The pH of the mixture was adjusted to 7.0. The final solution was incubated for 120 min at 37 °C with stirring. To determine the nitrogen release in the samples, the trichloroacetic acid (TCA) precipitation method was used. Aliquots of 10 mL of the withdrawn digestion mixture were mixed with 10 mL of 10% TCA solution. The mixtures were mixed and then centrifuged (8000 g, 10 min). The nitrogen content of the resulting supernatants was determined by the *Kjeldahl* method. The percentage of soluble nitrogen released during the digestion process was calculated using the following equation (Teixeira et al., 2014).

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

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

2.4. Physical characteristics of biscuits

2.4.1. Diameter, thickness and spread ratio

Diameter (mm), thickness (mm) and spread ratio of samples were measured in triplicate following the method of Ganorkar and Jain (2014). To determine biscuit diameter, six biscuits were placed edge to edge and the total diameter was measured in mm by a Vernier caliper. Then biscuits were rotated at an angle 90° for triplicate 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

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

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

3.2. Sensory evaluation results

3.2.1. Consumer segmentation

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

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

The HCA separated the consumers into two clusters (Fig. 2b). Consumers from cluster 1 (N=49) tend to like the biscuits less than consumers from cluster 2 (N=42).

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 consumers in cluster 1 (median age = 26). No significant difference was observed in term of nationality ($\chi^2 = 1.33$) or gender ($\chi^2 = 0.26$).

Fig 3a and b represent the global liking score distributions for cluster 1 and 2.

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

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

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

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

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

3.2.3. Analysis of open-ended question

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

For Cluster 2, (consumers who liked more the MDL supplemented biscuits) crunchy was frequently used to describe the positive aspects of the four biscuits as for cluster 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.

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

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

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

4.2. Contribution of supplemented biscuit to recommended daily requirement

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

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

5. Conclusion

The present study showed that the incorporation of MDL in food products like biscuits would encounter several obstacles to consumer acceptance and affect physical properties especially in terms of color (green) and texture (hardness). For nutritional benefits, this fortification can enhance nutritional values such as iron and protein compounds but many constraints and negative effects appear following this incorporation. Protein provided by these biscuits were not completely digested. 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 oleifera* 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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658 **Tables**

659

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

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.

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}	3.3 ^c
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

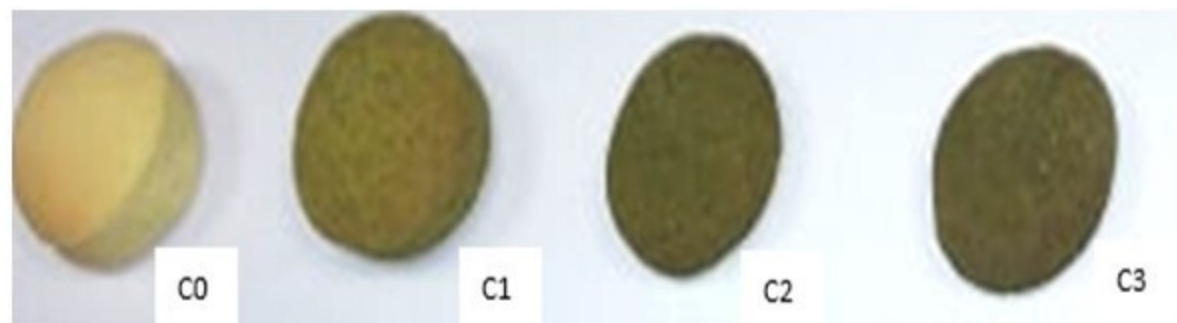
Fig. 1 Photographs of control biscuit (C0) and Moringa supplemented biscuits (C1, C2 and C3)

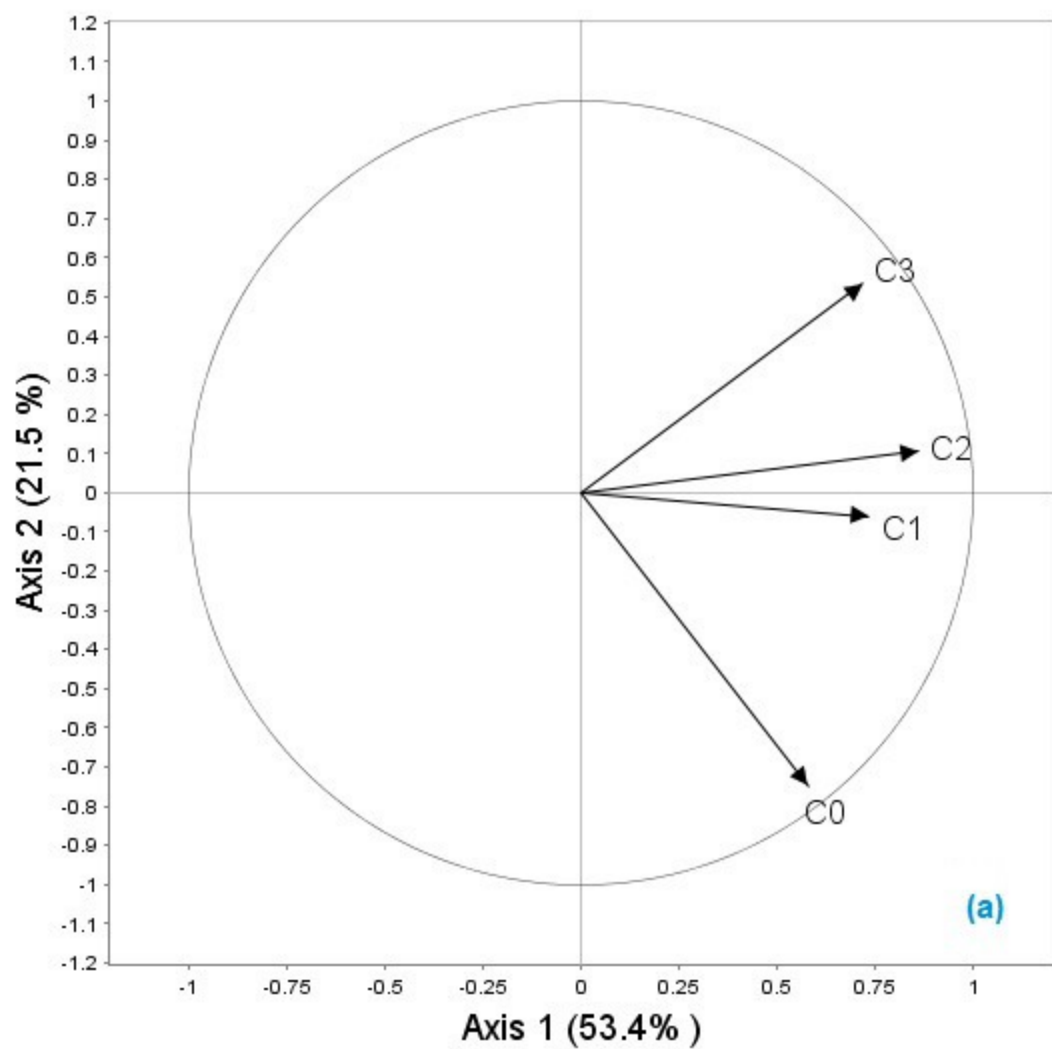
Fig. 2 Principal Components Analysis (PCA) of the consumers global liking scores. The left panel (a) represents the loading of the samples and the right panel (b) the projection of the consumers. The dot colors and the ellipses represent the two clusters yielded by the Hierarchical Cluster Analysis (HCA)

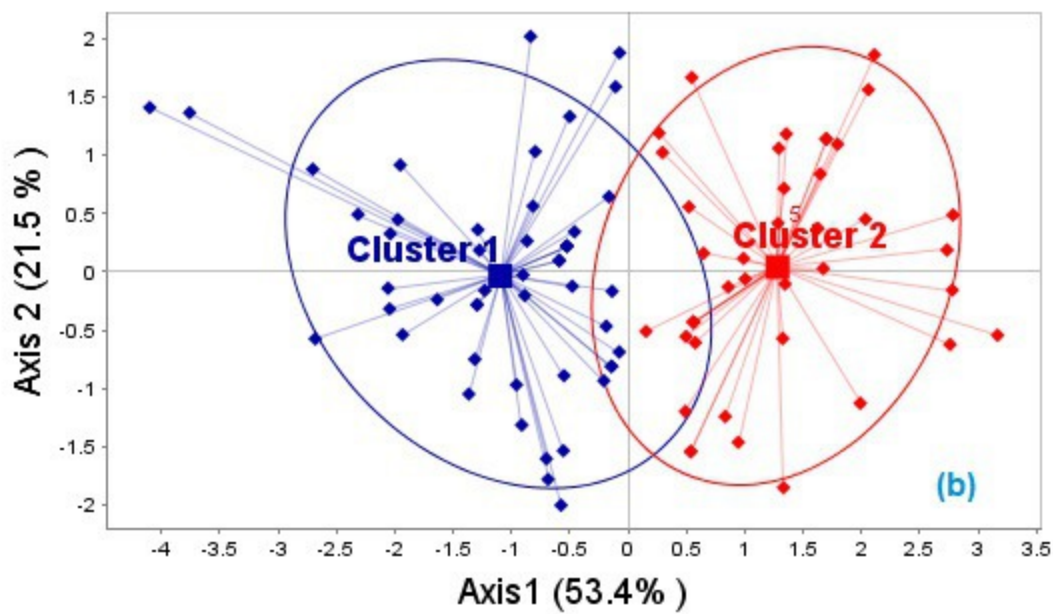
Fig. 3 Boxplots representing the frequency counts of global liking scores for consumers from cluster 1(a) and cluster 2(b)

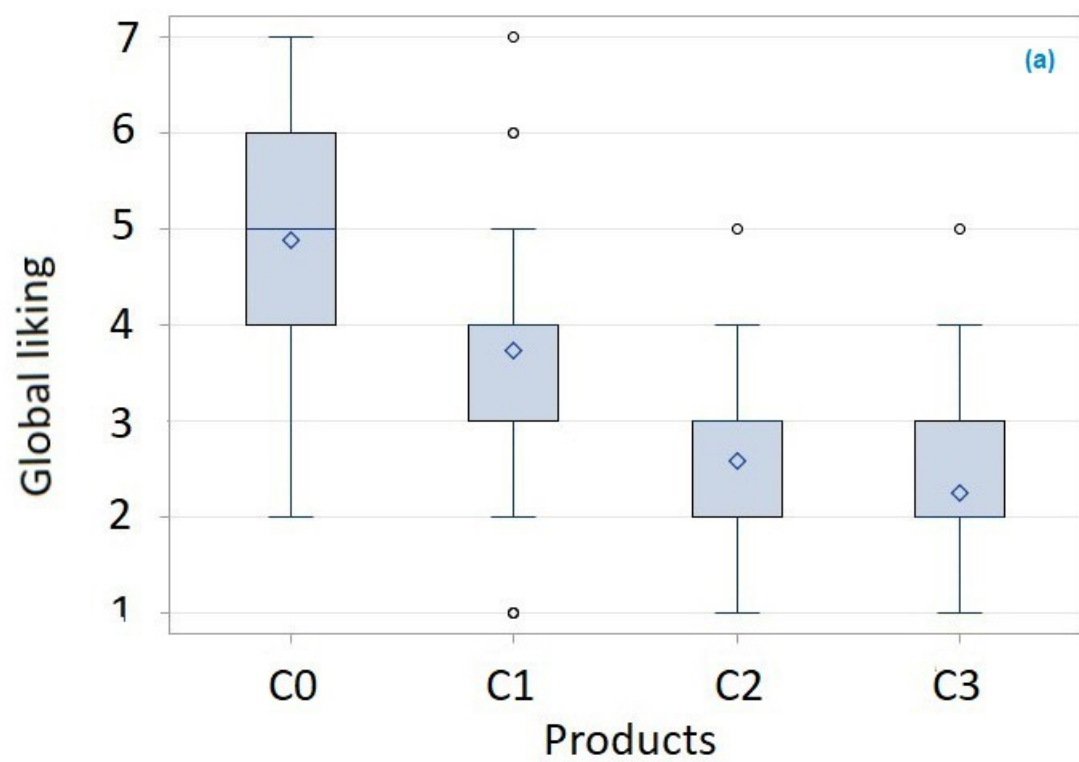
Fig. 4 Word clouds of the terms generated by the consumers from the two clusters to describe what they liked and disliked in the samples. The size of the words in this representation is proportional to their use: the larger the word size, the higher the frequency of occurrence

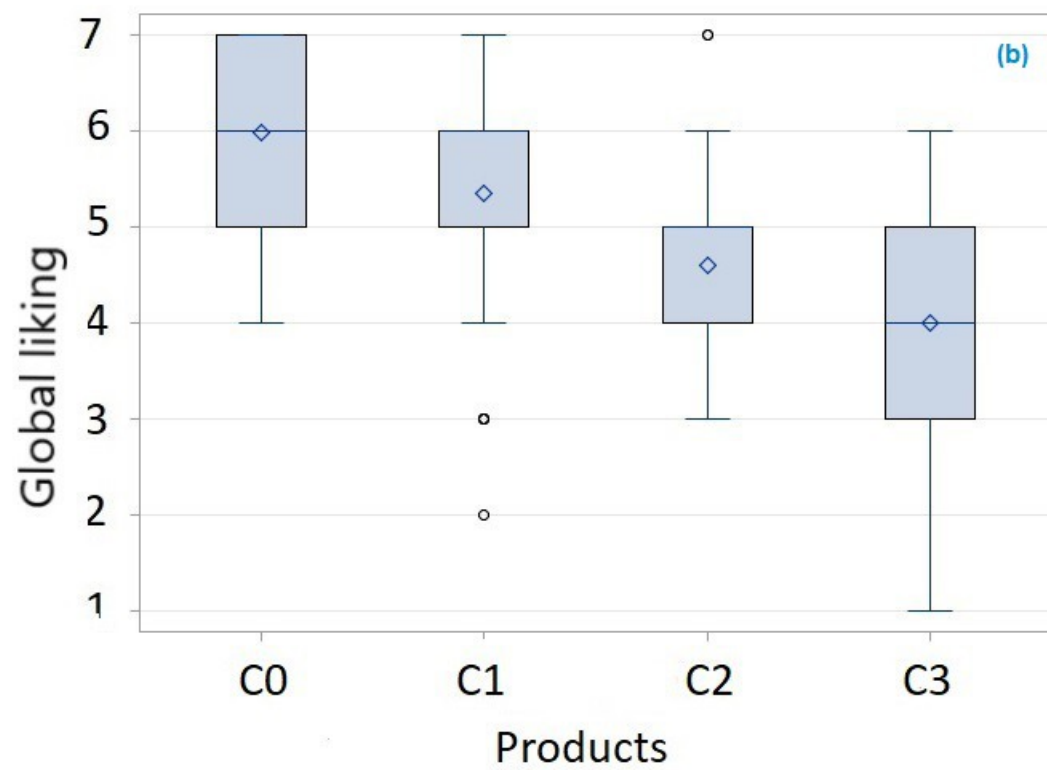
Fig. 5 First 2-dimensional space of the correspondence analysis of the terms generated by the consumers to describe what they like and dislike in the biscuits. The size of the diamonds is proportional to the quality of the representation of the terms in the subspace. Terms corresponding to what consumers 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











Cluster1

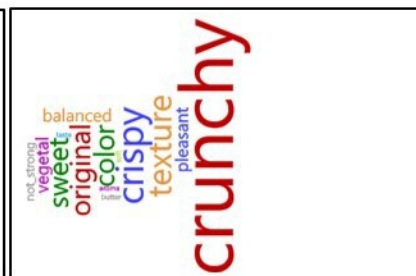
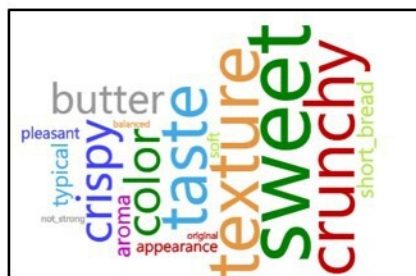
C0

C1

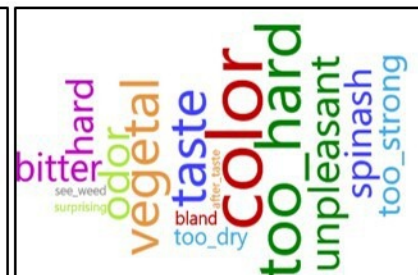
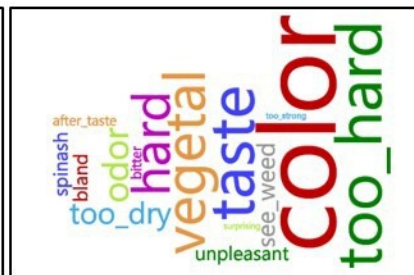
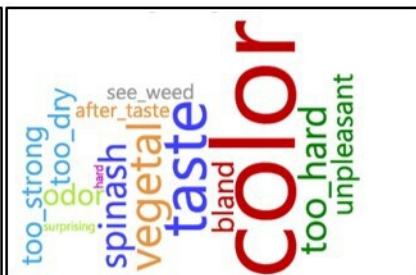
C2

C3

Like



Dislike



Cluster2

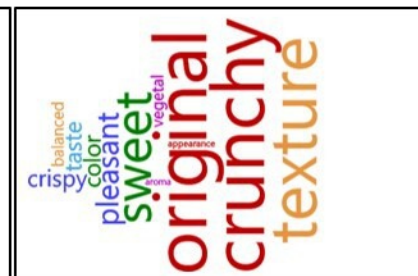
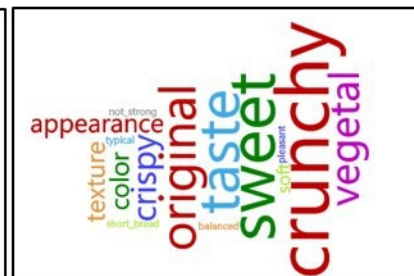
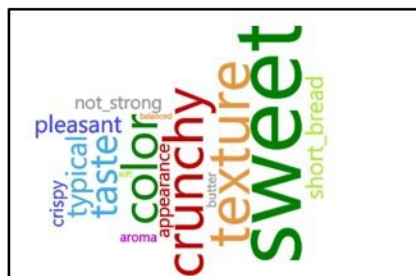
C0

C1

C2

C3

Like



Dislike

