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Catherine Rébufa, Nathalie Dupuy, Isabelle Bombarda. AComDim, a multivariate tool to highlighting impact of agroclimatic factors on *Moringa oleifera* Lam. leaf's composition from their FTIR-ATR profiles. *Vibrational Spectroscopy*, 2021, 116, 10.1016/j.vibspec.2021.103297. hal-03452451v1

**HAL Id: hal-03452451**

**<https://amu.hal.science/hal-03452451v1>**

Submitted on 26 Jan 2022 (v1), last revised 26 Nov 2021 (v2)

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# AComDim, a multivariate tool to highlighting impact of agroclimatic factors on *Moringa oleifera* Lam. leaf's composition from their FTIR-ATR profiles

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## A B S T R A C T

The quality control of the chemical composition leaves of *Moringa oleifera* Lam. is fundamental for the dietary treatment of diseases or for any quantitative study on human nutrition. To highlight the impact of climatic and edaphic factors on the chemical composition of *Moringa oleifera* Lam. leaves, a multivariate chemometric method, ANOVA (ANalysis Of Variance) Common Dimensions (AComDim) was used to interpret the changes in their Fourier Transform Infrared Attenuated Total Reflectance (FTIR-ATR) spectra. Leaves were collected from five geographical areas of Cameroon, recognized for their climatic and soil diversity. The factor "agroclimatic zone" was the most significant, followed by "month/year" (January, April and September between 2015 and 2017), "year" and "maturity" factors. Three slightly significant interactions between "year" and "agroclimatic zone" or "maturity" or "month/year" were observed. For a given agroclimatic zone, January samples were richer in polysaccharides than April samples which were characterized by a highest amount in proteins and aromatic compounds. The "maturity" factor revealed that young leaves contained fewer proteins and antioxidant compounds. The "year" factor was involved in all significant interactions and confirmed the temporal character of the studied factors. The diversity of climate and soils in Cameroon must be taken into account if a certain typicity of collected *Moringa Oleifera* leaves, or a constant quality may be assured before their marketing phase in powder form as a dietary supplement.

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## 1. Introduction

Physiological characteristics and metabolism of plants are impacted by environmental factors such as photoperiodism, wind, salt stress, temperature, humidity, chemical and microbiological quality of the soil. Plants adapt to their environment by modifying their organic molecules content (polysaccharides, lipids, peptides and proteins) and minerals such as calcium, the secondary messenger most regularly involved in plant responses to environmental variations.

When plants leaves are used in the manufacture of food supplements, the variability of their chemical composition may be a problem and particularly when they are intended for populations' health and well-being. They are consumed because they are interesting sources of proteins, vitamins, minerals and antioxidants but no criteria are proposed to assure a constant nutritional quality in relation with their geographic origin. A monitoring of the chemical composition of marketed leaf

powders, eaten locally or not, would allow a better quality-control of the latter and their use could be better monitored and appropriate to the needs of the people consuming them. Thus, before all transformation processes, it is necessary to know how the climatic and edaphic parameters impact leaf chemical composition.

The maturity stage of the leaves affects their nutritional status [1]. Mature leaves are richer in cellulose, hemicellulose, pectin and lignin than young leaves which use their reserves to grow. Mature leaves may also contain more calcium because of its role in the gelling of pectin which ensure the rigidity of the cell wall. The soil nature also impacts the chemical content of plant leaves [2], knowing that the soil composition varies from one geographical place to another, upstream or downstream of a slope, according to the vegetation which covers it, according to the climate or according to the human use. A nitrogen deficiency can result in a decrease in the protein and leaf chlorophyll content [3]. The pH of the soil can influence the assimilation and

availability of mineral elements by plants which will then show differences in their mineral status [4–6].

*Moringa oleifera* Lam. (MOL) leaves are eaten fresh (boiled like spinach) or dried (in decoction or powder form), in the traditional way in many African countries. Various humanitarian associations use it to fight against malnutrition of vulnerable populations (pregnant women, very young children and the elderly) and to improve their living conditions by the development of value chains for MOL leaves to provide them additional income. It is recognized that MOL leaves are rich in nutrients, minerals as well as metabolites with biological activity of interest (antidiabetic, anticancer and other medicinal virtues) [7,8]. Nevertheless, more and more MOL leaf powder is sold on the market (especially on the internet) and sometimes without being subject to strict control of their chemical composition. Various studies reported that the chemical composition of MOL leaves depended on different environmental parameters such as soil, climate, season, and plant age [9,10]. The variability of the leaf chemical composition is due to the various geographic origins of trees even if leaves are collected from trees cultivated on the same plot [11]. Phytochemical analyses of MOL, collected monthly from the same tree's branches during a whole year, showed that the highest chlorophyll content, phenol content, and the greatest scavenging activity were obtained during the spring season [12]. Even if, in certain works, analytical data have been treated using chemometric methods such as Principal Component Analysis (PCA) and Partial Least Squares (PLS) Regression, few studies identified factors that might explain the different chemical compositions of MOL leaves [10, 13–15].

The objective of this study was to seek to correlate the impact of various abiotic factors with the overall chemical composition of MOL leaves in terms of primary metabolites (lipids, proteins, polysaccharides) in order to better control their quality when they are used as dietary supplements. This was achieved by using ANOVA Common Dimension (ACoMDim) method to prioritize the significant factors and their interactions from Fourier Transform Infrared (FTIR) signatures of MOL leaf samples, harvested over three years and in different agroclimatic zones of Cameroon. This multi-block method has shown its effectiveness in different fields [16–19] and is especially interesting because it uses all the spectral variance for each level of factors studied. In this study, five factors with different sub-levels were considered such as the collection year and month, the agroclimatic zone of harvest (defined according to Liéno et al. [20], the soil nature (defined according to the classification of Segalen [21]) and the maturity of MOL leaves.

## 2. Materials and methods

### 2.1. Sampling

*Moringa oleifera* leaves were collected in Cameroon, between September 2015 and April 2017 from 5 different localities representing 5 agroclimatic zones following the map entitled “agroecological zones of Cameroon” dated for the year 2000 from the Center of Agricultural Research for Development (CIRAD). The 5 agroclimatic zones are the Sudano-Sahelian zone (SS), the High Savannah zone (HS), the Bimodal Forest zone (BF), the Monomodal Forest zone (MF) and the Highlands zone (HL). For a given location, only one tree was considered for collecting leaves. Young leaves (Y) were separated from the mature leaves (M) when it was possible. MOL leaves were collected in 13 sites spread across the different agroclimatic zones for which the soil nature was established from the “French classification of soils” [21]. The different harvest sites were presented on the map (Fig. SM-1). Information about the harvest location (agroclimatic zone and soil nature), harvest month and year are given in Table 1 as well as the number of collected samples.

The soils sampled in this study are classified as yellow ferrallitic, red ferrallitic, halomorph and hydromorph soils according to the major soil classification [21]. This classification reflects different physico-chemical properties and fertility of soils. The ferrallitic soils have a light texture

**Table 1**  
Number of MOL leaf samples by locality and month of harvest between 2015 and 2017.

	Harvest locations in Cameroon												
	Bafut	Batoké	Bertoua	Dibombati	Douala	Doumé	Dschang	Ebolowa	Garoua	Maroua	Mbalmayo	Ngaoundéré	Pendja
Agro-climatic zone	HL	MF	BF	MF	MF	BF	HL	BF	SS	SS	BF	HS	MF
Soil nature	RF	YF	RF	YF	HAL	RF	RF	RF	HYD	HYD	RF	RF	YF
2015													
September		1								2			2
2016													
January	1						4						6
April	1	1	2		2	2	2	4	1	2	2	1	2
August/September													7
2017													
January	1	1	2	2	2	2	2	4	5	5	2	5	4
April	1	2	4	2	4	4	15	8	6	9	4	6	6
<b>Total</b>	<b>4</b>	<b>5</b>	<b>4</b>	<b>2</b>	<b>4</b>	<b>4</b>	<b>15</b>	<b>8</b>	<b>6</b>	<b>9</b>	<b>4</b>	<b>6</b>	<b>27</b>

BF : Bimodal Forest zone, HAL : halomorph soil, HL : Highlands zone, HS : High Savannah zone, HYD : hydromorph soil, MF : Monomodal Forest zone, RF : red ferrallitic soil, SS : Sudano-Sahelian zone, YF : yellow ferrallitic soil.

which makes them highly susceptible to erosion, but these soils are very fertile. The yellow color of yellow ferralitic soils is due to goethite presence, while the red color of red ones is due to amorphous products overlying crystallized iron products of various colors (illite and kaolinite mainly). Halomorph soils are a grouping of several soils dominated by the presence of various salts in their profile. Their surface horizon is compact and has a massive structure (without pores or biological activity), hence their very low permeability and low fertility. Hydromorphic ones are soils, whose characteristics are due to an evolution dominated by the effect of an excess of water due to a temporary or permanent waterlogging of a part or of the whole profile. Some of these soils are found along the coast where they are characterized by the presence of sulphates in the soil solution, making them extremely infertile.

## 2.2. Pre-treatment of samples

The harvested leaf samples were air dried, in the shade, then ground to powder (Moulinex, type 843). The powders were stored in airtight jars and placed in the dark prior to analysis. Before each analysis, the powder samples were lyophilized for 48 h to remove the available water. The water presence modifies the appearance of the leaf spectral fingerprint by the overlapping of its IR bands with the characteristic bands of leaf metabolites [22].

## 2.3. Recording of FTIR-ATR signature of MOL

Lyophilized MOL leaf samples were directly deposited onto a Specac's Golden Gate™ ATR accessory. Fourier Transform Infrared Attenuated Total Reflexion (FTIR-ATR) spectra were collected at room temperature using a Thermo Nicolet IS10 spectrometer equipped with a MCT detector, an Ever-Glo source and a KBr/Ge beam-splitter. Spectra were acquired between 4000 and 650  $\text{cm}^{-1}$ , with a 4  $\text{cm}^{-1}$  nominal resolution. For each spectrum, 100 scans were co-added with a background spectrum in air. Ten spectra were recorded for each sample to reduce the possible impact of powder heterogeneity.

## 2.4. AComDim method

AComDim method, based on the same concept as ANOVA-PCA [23] (also called APCA) has been used for different industrial applications to provide information about the importance of specific factors and their interactions. Its theory is detailed by Gaston et al. [18] and Guisset, Martin and Govaerts [24]. The method computes at first a decomposition of the original data matrix in different effect matrices corresponding to each term of an ANOVA model and then realizes a multivariate analysis of each effect matrix separately with Principal Components Analysis (PCA). These matrices are built by using the levels of the various studied factors. AComDim method uses a Common Dimension (ComDim) approach which analyses simultaneously these different data matrices (or data block) by finding a common space of representation, with each matrix having a specific weight, called salience,  $\lambda_i$  in each of the orthogonal common dimensions or common components (CCi of  $i^{\text{th}}$  block) of this space. The technique relies on the weighted sum of sample variance-covariance matrices or association matrices. An iterative procedure calculates a series of score vectors for each successive common dimension. Once the first CC calculated (CC1), the matrices are all deflated, and the process repeated until the required number of CCs extracted. The number of common components is defined by the sum of the number of factors, their interactions and the noise (first common component, CC1). Since residual matrix is added to factor and interaction matrices, all blocks contain then a contribution from the residual matrix. Then all blocks are compared to CC1 and the blocks that contribute significantly to CC1 (those with high saliences) contain mainly noise. The block significance is afterward estimated with a Fisher test (student Fisher F-test with  $n-1$  degrees of freedom, where  $n$  is the

number of blocks and an alpha level equal to 0.05) applied on the F-values (Fi) equal to the ratio of the salience of the residual block on CC1 and the salience of the  $i^{\text{th}}$  block on CC1. The blocks for which Fi is greater than the critical value (Fc) of the Fisher table, are considered as being related to influential factors or interactions. The effect of the most important factors is visualized on scores scatter plot (linking common dimensions with factor levels) on the informative CCs vs. CC1 while their importance and significance is quantified from loadings scatter plot (linking common components with the original variables).

AComDim approach was applied on 98 FTIR-ATR profiles of MOL leaf samples. The different studied factors (Table 2) were the harvest year (2015, 2016 and 2017), the harvest month/year (September 2015 & 2016, January 2016 & 2017 and April 2016 & 2017), the leaf maturity (young, mature or unknown), the agroclimatic zone (Bimodal Forest zone, Highlands's zone, High Savannah zone, Monomodal Forest zone and Sudano-Sahelian zone) and the soil nature (yellow ferralitic, red ferralitic, halomorph and hydromorph).

## 2.5. Spectral pre-treatments and software

OMNIC 8.1 (Thermo Nicolet) was used to record FTIR-ATR spectra. Spectral zone between 4000 and 1800  $\text{cm}^{-1}$  was withdrawn to keep region with the most significant information and Multiplicative Scatter Correction (MSC) was used to compensate for additive and/or multiplicative effects in spectral data [25]. These pre-treatments were realized by the help of Unscrambler version 10.3 from CAMO (Computer Aided Modeling, Trondheim, Norway). AComDim computations were performed using Matlab 7.14 (R2012a) and a procedure adapted from the ComDim function of the free toolbox SAISIR [26]. The code (Matlab) of the ComDim (CCSWA) method and examples of applications, are available on the web of the French group of chemometrics: <http://www.chimie.fr/comdim.html>.

## 3. Results

### 3.1. FTIR-ATR profile of Cameroonian MOL leaf powder

FTIR-ATR spectroscopy is used to obtain a global fingerprint of plant material (fresh or dehydrated) because it is a simple and non-destructive method. Fig. 1 shows the characteristic FTIR-ATR corrected spectra of mature and immature MOL leaf powders. Small variations were observed between mature and immature leaves. The fingerprint shows characteristic bands corresponding to the main compound's families present in the leaves including polysaccharides (cellulose, hemicellulose and pectin), proteins and lipids (aliphatic polyesters, cutin, suberin, waxes). The assignments of FTIR-ATR spectral bands were also done according to spectroscopic characterizations of plant leaves [27–30] and Socrates tables [31]. Between 3000 and 3600  $\text{cm}^{-1}$ , the broad band is characteristic of OH stretching vibrations of alcohol or acid groups of polysaccharides, cutin and lignin, overlapped with bands of asymmetric and symmetric stretching vibrations of  $-\text{NH}_2/\text{NH}$  group of amino/amido acids. At 2915 and 2848  $\text{cm}^{-1}$  appear principally bands originating from the acyl chain, those due to  $\text{CH}_3$  and  $\text{CH}_2$  asymmetric and symmetric stretching vibrations of lignin and lipids including waxes and cutin. The  $\text{C}=\text{O}$  stretching vibration at 1735  $\text{cm}^{-1}$  is observed for acetyl and carboxyl groups of lipids, proteins pectin and hemicelluloses.

The spectral region between 1700 and 1450  $\text{cm}^{-1}$  is attributed to OH deformation vibrations of polysaccharides, amide bands of protein and peptides such as amid I bands (1663  $\text{cm}^{-1}$ , due to  $\sim 80\%$  CO stretching,  $\sim 10\%$  CN stretching,  $\sim 10\%$  NH bending vibrations) and amid II band (1550  $\text{cm}^{-1}$ , due to  $\sim 60\%$  NH bending,  $\sim 40\%$  CN stretching vibrations), overlapped with C–C stretching conjugated with  $\text{C}=\text{C}$  stretching vibrations of aromatic structures found in lignin and phenolic compounds principally.

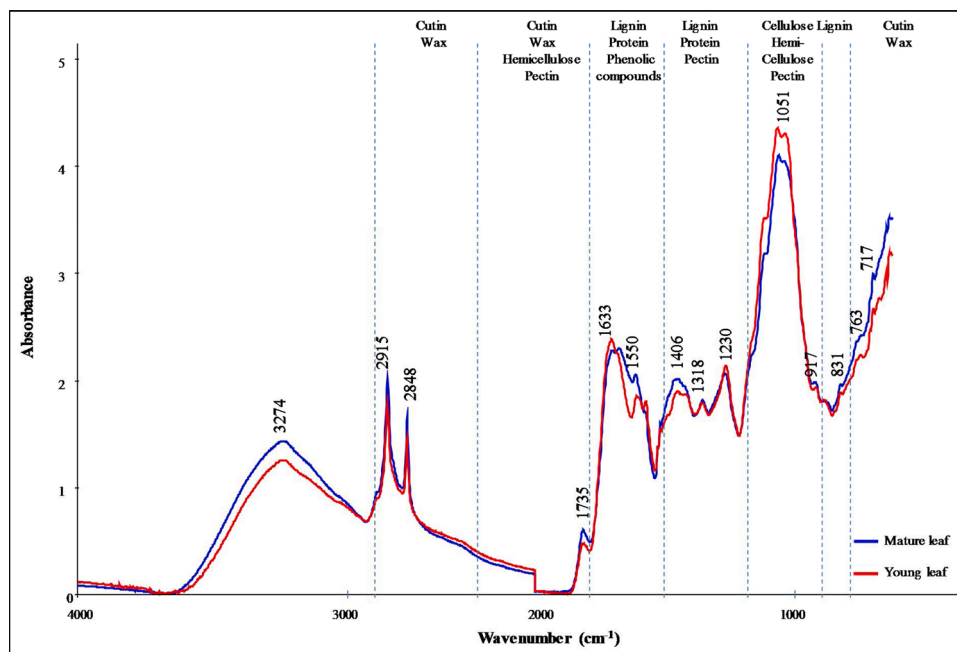
Bands pointed in 1450–1100  $\text{cm}^{-1}$  region are assigned to different groups such as  $\text{CH}_3$  symmetric deformation, wagging vibration of  $\text{CH}_2$

**Table 2**

Studied factors, level factors and number of MOL sample used for AComDim method.

STUDIED FACTORS									
Year		Month/year		Leaf maturity		Agroclimatic Zone		Soil nature	
Levels	Nb	Levels	Nb	Levels	Nb	Levels	Nb	Levels	Nb
2015	5	September15	5	Young	42	BF	20	Yellow ferralitic	34
2016	40	January16	6	Mature	44	HL	19	Red ferralitic	45
2017	53	April16	7	Unknown	12	HS	6	Halomorph	4
		September16	27			MF	38	Hydromorph	15
		January17	10			SS	15		
		April17	43						

BF : Bimodal Forest zone, HL : Highlands zone, HS : High Savannah zone, MF : Monomodal Forest zone, Nb: sample number, SS: Sudano-Sahelian zone.



**Fig. 1.** FTIR-ATR corrected profiles of MOL leaf powders with assignment of spectral zones *versus* the metabolites the more representative of bands (MSC correction with elimination of the CO<sub>2</sub> absorption zone between 2400 and 1800 cm<sup>-1</sup>).

(in lignin, proteins, pectin and lipids), CH ring, C–O stretching vibration and OH deformation (in polysaccharides, pectin), amid III band (due to ~ 30 % CN stretching, ~ 30 % NH bending, ~ 10 % CO stretching, ~ 10 % O=C–N bending and rest other vibrations) of proteins.

The spectral region (1100–900 cm<sup>-1</sup>) is characteristic of polysaccharides. Bands at 1160, 1050 and 1030 cm<sup>-1</sup> were attributed to C–O–C glycosidic stretching vibration, C–C stretching, C–O deformation and C–O stretching vibration in hydroxyl group of hemicelluloses and cellulose principally. Also, they may be characteristic of other polyoses and pectin. Under 950 cm<sup>-1</sup>, appear characteristic bands of C–O stretching, C–H aromatic deformation (lignin), ring vibration and CH<sub>2</sub> rocking (polysaccharides, cutin and waxes).

### 3.2. Determination of influential agroclimatic factors from AComDim approach

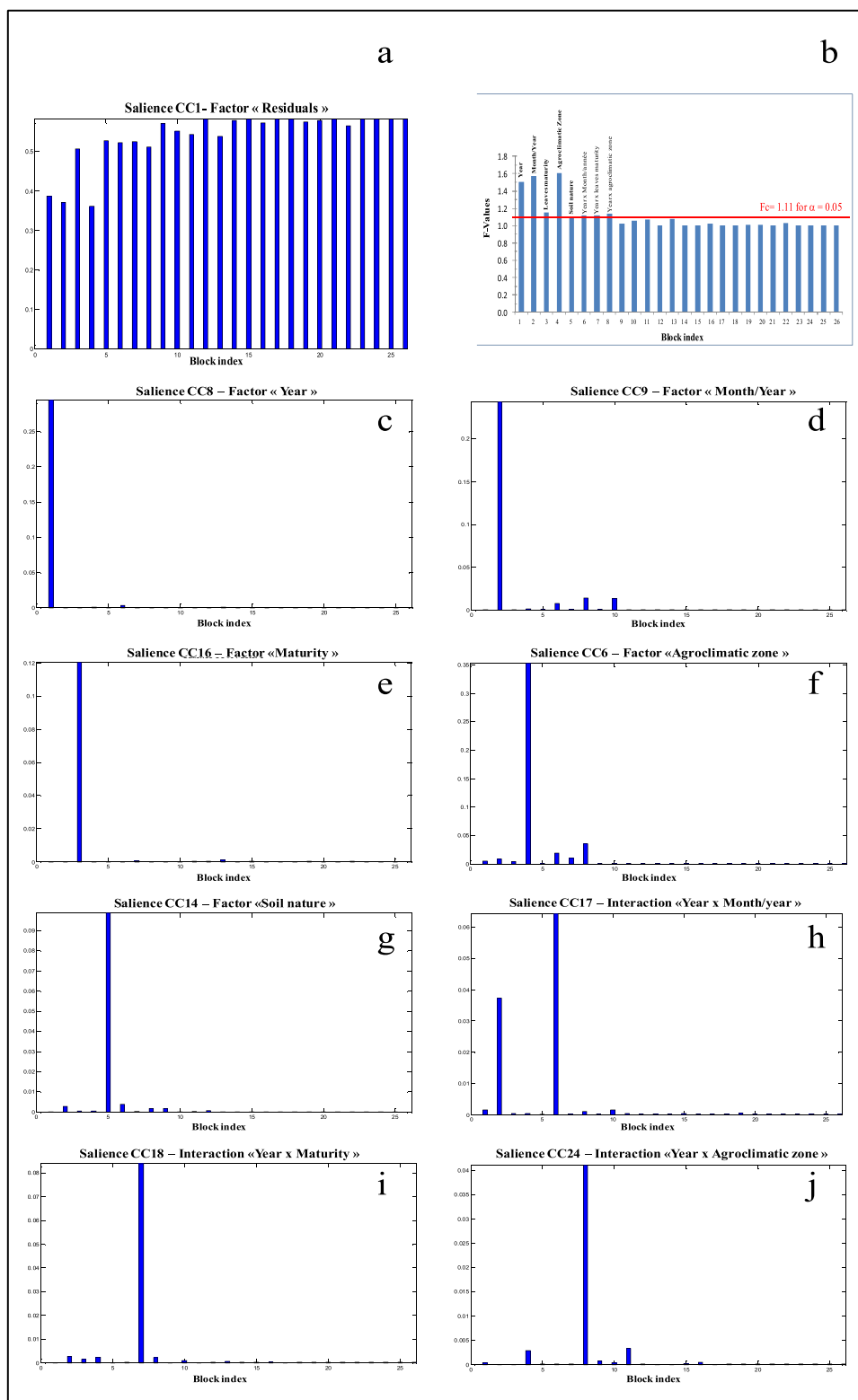
Considering a data matrix of 980 FTIR-ATR spectra, this multiblock method allowed to calculate 30 common components (only 26 CCs were shown on Fig. 2), the first five of which are attributed to the studied factors, the next twenty to multiple interactions between factors and the last one for the noise. The F-values (computed from the saliences on CC1, Fig. 2a) of the different blocks (Fig. 2b) were close to 1. Only blocks with a F-value upper to the critical F-value ( $F_c = 1.11$ ) of the Fisher table will be considered as significant factors or interactions because they are

significantly different from the noise. It's the case for the factors and the interactions “year” (block 1), “month/year” (block 2), “maturity” (block 3), “agroclimatic zone” (block 4), “year x month/year” (block 6), “year x maturity” (block 7) and “year x agroclimatic zone” (block 8). According to the intensity of their F-value, the factor 4 “agroclimatic zone” is the most significant, followed by factor 2 “month/year”, factor 1 “year” and factor 3 “maturity”. The factor 5 “soil nature” is not significant but it can be considered that this parameter is already taken into account through the factor “agroclimatic zone” which encompasses the soil classes. Three interactions are slightly significant; in order of priority, these are “year x agroclimatic zone”, “year x month/year” and “year x maturity”. The important salience value of common component (CCi) associated to each significant factor or interaction (Fig. 2c-j) confirmed that the components are therefore significantly influenced by these blocks, which are the cause of the clusters observed on the score plots, CCi *versus* CC1.

For each factor, the score plots and their associated loading are presented and commented by order of greater importance.

- Impact of the “agroclimatic zone” factor

The CC6 (Fig. 3a) corresponds to the block 4 which is related to the differentiation of the agroclimatic zone. The score projection associated to the CC6 shows a discrimination of MOL leaf samples according to the harvested zone. The greatest discrimination was observed between



**Fig. 2.** AComDim applied on FTIR-ATR data of MOL leaves: Saliency for CC1 (a), F-values compared with critical F-value (b), saliencies for significant CCS: (c) factor 1 “year”, (d) factor 2 “month/year”, (e) factor 4 “maturity”, (f) factor 4 “agroclimatic zone”, (g) factor 5 “sol nature”, (h) interaction “year x month/year”, (i) interaction “year x maturity”, (j) interaction “year x agroclimatic zone”.

samples from High Savannah zone (placed in the negative part of CC6) and the Highlands zone (located in the positive side of CC6). Sudano-Sahelian zone is located on the negative side of CC6 (like High Savannah zone), whereas Bimodal Forest and Monomodal Forest zones contribute slightly to the sample differentiation because of their central

location along the CC6 axis. The corresponding loading (Fig. 3b) shows that samples collected in Highlands’s zone are richer in aromatic compounds and proteins in the positive part of CC6 loading. Bands observed between 1623 and 1400  $\text{cm}^{-1}$  are attributed to amide I band or stretching vibrations of C=C bonds of lignin, phenolic and aromatic

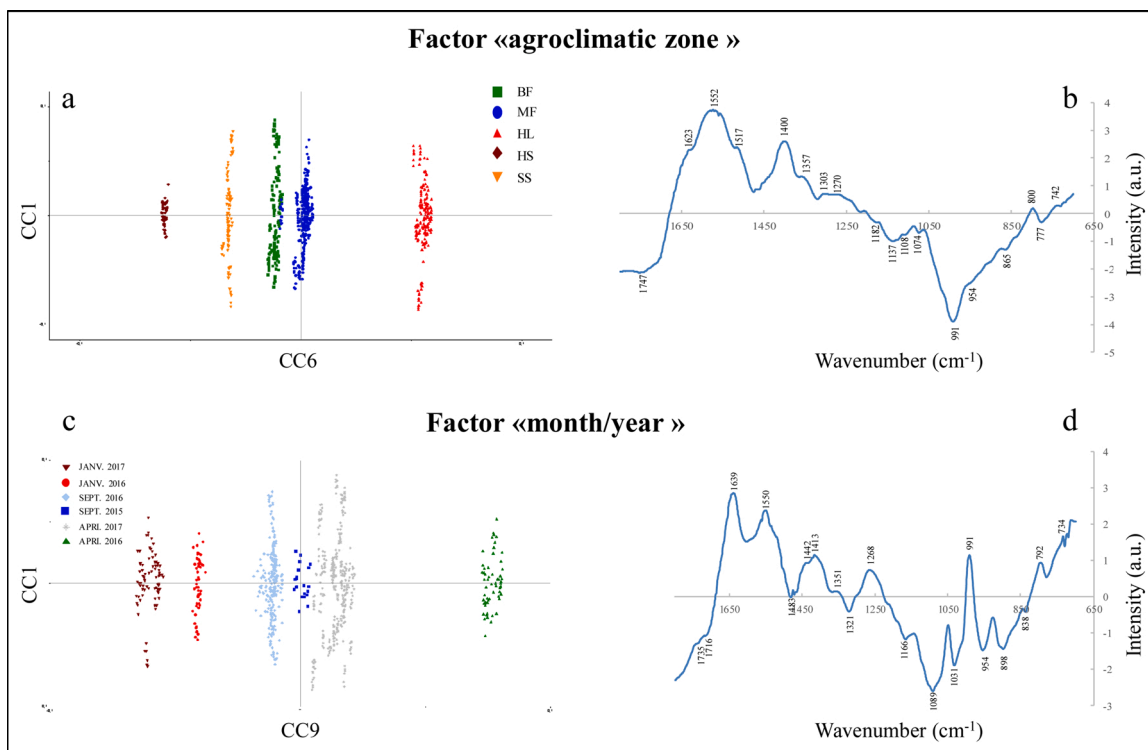


Fig. 3. Scores on CC6 (a), CC9 (c) and their associated loadings (b, d) for the significant factors "agroclimatic zone" and "month/year".

compounds. Bands pointed at around 1550 cm<sup>-1</sup> characterize the amide II band of proteins. Bands between 1270 and 800 cm<sup>-1</sup> are assigned to the C–O stretching and to C–H out-of-plane bending of lignin or phenolic compounds. FTIR-ATR profiles of samples from High Savannah and Sudano-Sahelian zones present more intense bands in the negative part of CC6 loading, around 991 cm<sup>-1</sup>, characteristic of the C–O stretching

vibration (glycosidic linkage) of carbohydrates and at 777 cm<sup>-1</sup> describing the C–H bending deformation of polysaccharides.

- Impact of temporal factors: "month/year" and "year"

On the basis of the F-values, factor "month/year" is the second factor

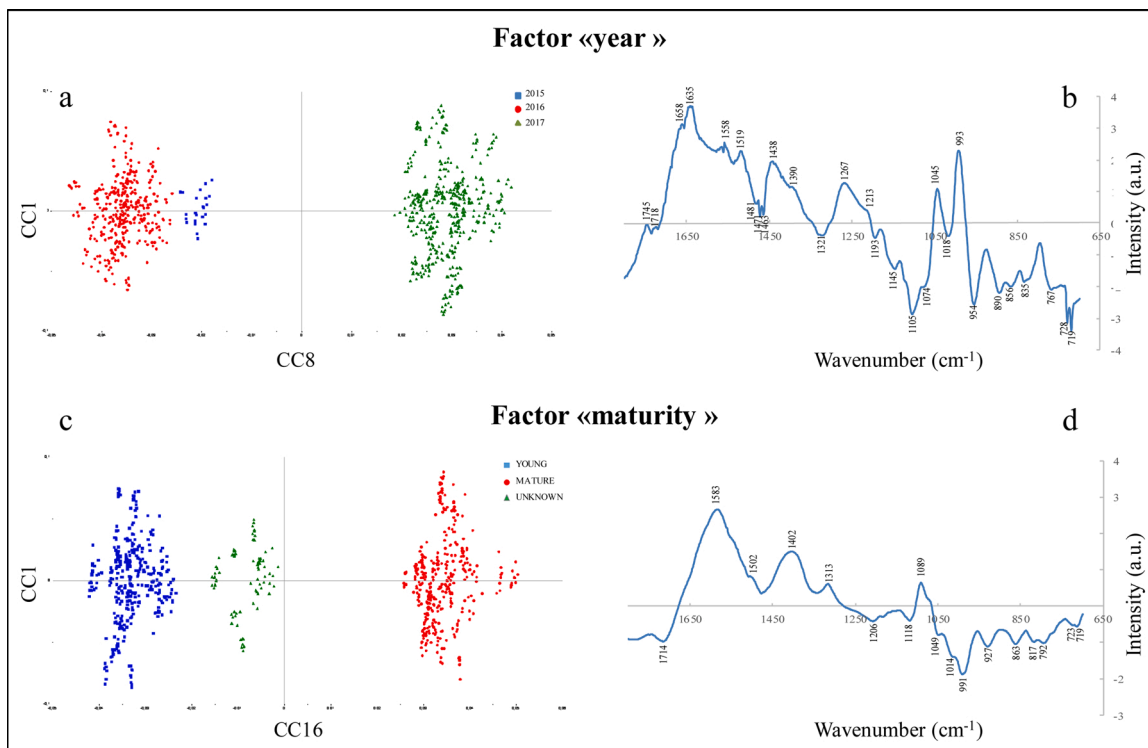


Fig. 4. Scores on CC8 (a), CC16 (c) and their associated loadings (b, d) for the significant factors "year" and "maturity".

the most significant. The score projection associated to the CC9 (Fig. 3c) shows a discrimination of MOL samples along CC9 axis according to harvest month as well as the harvest year. Three distinct groups are observed: the samples collected in January (2016 and 2017) (on the negative side of the CC9 axis), samples collected in September (2015 and 2016) and in April (2017) (in central position of the CC9 axis), and samples collected in April (2016) (on the positive side). A clear distinction is observed between the harvest months January vs April. Associated samples are on each side of the CC9 axis, whatever the year (2016 and 2017). Low difference of behavior is noted for samples collected in September 2015 as they are placed in the center of CC9 axis. A greater difference in behavior is observed between the months January vs April for the year 2016 compared to 2017. From the associated loading (Fig. 3d), we notice that samples collected in January are characterized by the spectral negative bands pointed at 1735, 1716, 1321, 1189, 1166, 1089, 1031, 954, 898 and 836  $\text{cm}^{-1}$  which are characteristic of polysaccharides (such as cellulose, hemicellulose and pectin). Samples collected in April 2016 are characterized by positive bands found at 1639, 1652, 1550, 1519, 1475, 1442, 1413, 1351, 1268, 991, 792, 734  $\text{cm}^{-1}$  which suggest that they may possess a higher amount of lignin, proteins and phenolic compounds (that can be found within the leaf cells wall).

The study of the “year” factor impact shows a clear differentiation for the harvest year 2017 isolated on the positive part of CC8 score axis (Fig. 4a). Samples collected in 2015 (in September) and in 2016 (in January, April and September) are clustered together on the negative side of the CC8, in opposition with the samples collected in 2017 (in January and April) (placed in the positive side of the CC8). Samples collected in 2015 and 2016 are characterized by bands at 1321, 1193, 1145, 1105, 1074, 1018, 954, 890, 865, 767 and 728  $\text{cm}^{-1}$  (Fig. 4b) which characterized pyranose compounds. It cannot be cellulosic derivatives as the bands at 1735, 1716  $\text{cm}^{-1}$  are not found on the CC9 loading contrary to CC8 loading. Samples collected in 2017 are characterized by a greatest amount in lignin, proteins and phenolic compounds as suggested by the spectral bands found at 1658, 1635, 1614, 1564, 1558, 1519, 1438, 1417, 1390, 1267, 1213, 1045 and 993  $\text{cm}^{-1}$ .

- Impact of “maturity” factor

The third most significant factor is the maturity. For some MOL leaf samples collected, the state of maturity could not be identified (young and mature leaves were not separated before analysis). A clear differentiation of young and mature leaves is expressed according to CC16 scores axis, grouping young leaves in the negative part and the mature ones in the positive part while undetermined samples are classified with the young leaves (Fig. 4c). Compounds associated to the state of maturity are identified from spectral bands of CC16 loading (Fig. 4d). The negative spectral bands pointed at 1780 and 1714  $\text{cm}^{-1}$  (C=O stretching vibration), between 1049 and 1090  $\text{cm}^{-1}$  (C–O–C stretching vibration), between 1205 and 927  $\text{cm}^{-1}$  (corresponding to the stretching of C–O–H), between 863 and 771  $\text{cm}^{-1}$  (corresponding to the bending of C–H bond) suggest that young samples are richer in polysaccharides compound such as pectin or simple sugars such as glucose or fructose [32] known to have a role in plant leaf maturity. Bands at 723 and 719  $\text{cm}^{-1}$  are characteristic of  $\text{CH}_2$  bending, found in lipids. This could be related to leaf membrane development which happens during their growth and maturation. Mature leaf samples (found on the positive side of the CC16 axis) are characterized by bands at 1583, 1506, 1402, 1313, 1089 and 1070  $\text{cm}^{-1}$  which could suggest the presence of pectin usually found in highest quantities in mature leaves.

The advantage of AComDim approach is to access to factors interactions, reflecting the real relationships found between the agroclimatic conditions and phenological parameters of MOL leaves.

- The interaction “year  $\times$  agroclimatic zone”

The score plot of CC24 (Fig. 5a) with a codification according to agroclimatic zones shows that samples from Monomodal Forest zone react differently according to the year. A first batch is placed on the negative part of CC24, another at the center and the last one on the negative part, independently of a group according to the years, knowing that the samples were taken in different cities for a same agroclimatic zone. A difference of behavior is observed for Bimodal Forest samples: samples for the year 2016 are placed in the negative part and those collected in 2017 are projected positively; an identical classification is observed for samples of High Savannah zone, while it is the opposite situation for samples coming from Highlands’s zone. Whatever the year, samples of the Sudano-Sahelian zone are placed in positive part of CC24 axis. According to CC24 loading (Fig. 5.b), it has been shown that samples collected in Sudano-Sahelian zone are characterized by their high amount in polysaccharides (in cellulose in particular) whatever the year, while the negative bands of CC24 loading characterize aromatic compounds which are predominantly present in the MOL leaves collected in other agroclimatic zones vs the harvest year.

- The interaction “year  $\times$  maturity”

The projection of young and mature leaves on CC18 axis (Fig. 5c) shows that le factor “year” impacts samples of known maturity in the same way. Globally, the young or mature samples of 2016 are placed in the negative part and those of 2017 in the positive part of CC18. But as they are placed in the centre of CC8, the impact of this interaction is low. The difference is mainly made on samples whose maturity is unknown; the richest in polysaccharides have been collected in 2017 according to the spectral bands observed in the negative part of CC18 loading (Fig. 5d).

- The interaction “year  $\times$  month/year”

According to the sample projection on CC17 axis (Fig. 5e), MOL leaves collected in January and in September for the years 2016 and 2017 are grouped on each side of the CC17 axis while samples harvested of April are distributed both in the negative part (year 2017) and in the positive part (year 2016). The year factor also influences the composition of the leaves, in addition to the harvest month factor. It was observed a higher concentration of polysaccharides in January (2016 and 2017) and in April (only 2016) according to the spectral bands assigned from CC17 loading (Fig. 5f).

#### 4. Discussion

FTIR-ATR spectroscopy appeared as a good technique to analyze easily and rapidly the chemical composition of plant response to environmental factors. Significant changes in FTIR spectral profile of plant were observed if they were exposed to either water or temperature stress [33] or to pollution during their growth [34]. Other sources of spectral variability such as maturity and sunlight exposure were also examined [27]. To our knowledge, few studies related the impact of agroclimatic factors on the chemical composition of Moringa leaves using spectroscopic approach. Here the FTIR-ATR chemical signature of a large number of MOL samples from different Cameroonian areas were used to achieve this objective.

- Impact of agroclimatic conditions

According to various studies, it is recognized that climatic parameters (temperature, rainfall and relative humidity) as well as soil characteristics are important factors for the leaf development and therefore which impact the leaf chemical content [35]. These parameters depend on the geographical origin. Some studies [2,36] suggested that temperature and precipitation were the most important climatic parameters in the construction and distribution for most vegetation of the world.



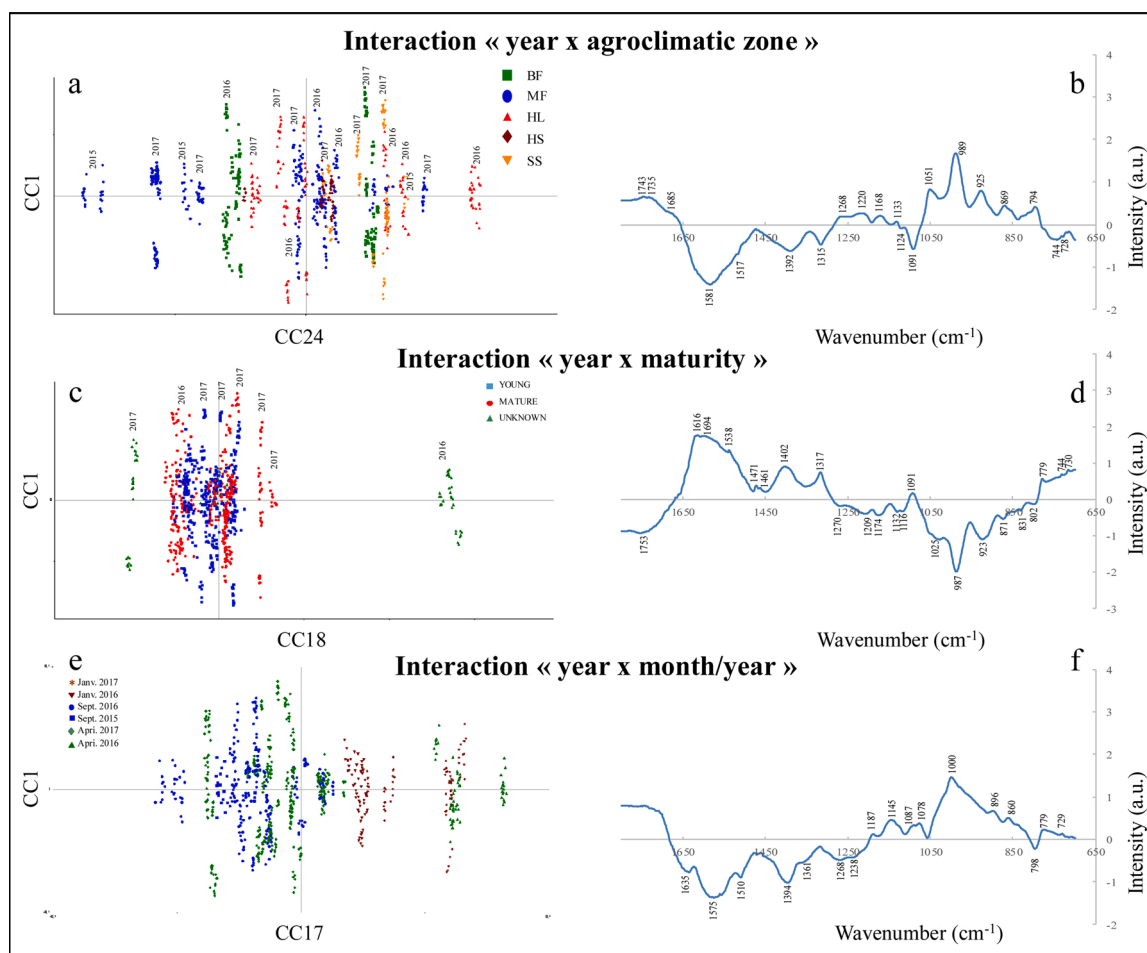


Fig. 5. Scores on CC24 (a), CC18 (c), CC17 (e) and their associated loadings (b, d, e) for the significant interactions.

Differences in seasonal temperatures (thermoperiodism) have a significant impact on the absorption balance of the plant, its growth and fructification. When exposed to light for a long time, the leaves tend to produce more polyphenols than chlorophylls and proteins. Overexposure to light can also lead to a decrease in the chlorophyll/carotenoid ratio and a reduction in photosynthetic activity [37,38]. Leaves exposed to the sun, subjected to water stress (absence of water) or at low temperatures would have the same physiological response, *i.e.* an increase in the thickness of the cuticle [27,39]. When leaves were exposed under sunlight, spectral ester band increased reflecting a higher content of cutin [27] while heat stress significantly modified differently the area of C=O ester band for different plant varieties because of their different behavior to environmental stress [40].

In literature, some physico-chemical properties of soils, where MOL sampling was realized, were given but no correlation between soil quality and leaves composition were found. In this work, we were unable to correlate the variations observed between the FTIR spectral of the MOL samples to the soil quality (ferrallitic, halomorph or hydromorph); "soil nature" factor was not significant using AComDim approach. Nevertheless, soil type is embedded in "agroclimatic zone" factor. In this study, the discrimination between High Savannah and Highlands zones is not due to the soil nature because the localities Bafut and Dschang (Highlands's zone) and Ngaoundéré (High Savannah zone) are locations characterized by a red ferralic soil on the basis of Segalen's classification [21]. However, these localities are subject to different climates, that could explain the distribution of the agroclimatic zones on CC6 scores plot. Monomodal Forest and Bimodal Forest are grouped together because they are characterized by an equatorial climate (short dry season centered on the month of July, with a very little temperature

contrast and high permanent humidity). The difference between the two regions is mainly due to the total annual amount of precipitation. The Monomodal Forest harvested zones (Batoké, Douala, Dibombari and Pendja) are rather subject to a north coast equatorial climate with two seasons and abundant precipitations while Bimodal Forest harvested zone (Bertoua, Doumé, Ebolowa and Mbalmayo) have four well-marked seasons with abundant precipitations. On the basis of weather station data reported by Sighomnou [41], rainfall days could not differentiate these Cameroonian zones because rainfall varies with proximity to the sea, altitude and latitude. Rainfall decreases from the sea inland and from south to north. The Sudano-Sahelian zone has the longest dry period. But the discrimination of the FTIR-ATR MOL signatures according to agroclimatic zones along CC6 axis is not due to their rainfall profile. The CC6 distribution of these agroclimatic zones was not due to temperature effect because the maximum average temperatures are recorded around Garoua in Sudano-Sahelian zone. Moreover, the average annual temperatures in Cameroon range from 18 °C in the Highlands region of the West of the country to more than 28 °C in the North (in Sudano-Sahelian zone). The average annual daytime amplitudes are around 7–10 °C at the seaside in Monomodal and Bimodal Forest zones respectively. They exceed 10 °C in the North-East (15–16 °C in Highlands and Sudano-Sahelian areas), then increase with latitude to reach 14 °C in High Savannah zone. The last parameter is the atmospheric humidity which is one of the key elements of climatology in tropical regions due to the relationships established between temperature and precipitation. The average of the annual relative humidity decreases from south to north of the country. The amplitudes of the monthly variations are greater in the North (40 % at Ngaoundéré) than in the South (10 % at latitude 4°) which benefits from the stabilization

ensured by the forest regions [41,42]. Then lower amplitude of relative humidity and higher average annual daytime amplitude could explain the position of High Savannah zone in opposition with the Highlands zone having these climatic parameters which evolve in the opposite direction. The consideration of UV index recorded in these different locations from the World Weather Online website [43] (not shown here) was not a significant selection criterion to explain the differentiation of MOL spectra according to the agroclimatic zones, but it was already shown in literature that infrared spectroscopy has the potential to confirm the effect of sunlight on the cellulose content. No spectral differences according to the salinity effect were observed here for MOL samples of monomodal forest zone (MF) located in different cities near Atlantic Ocean while FTIR-ATR spectroscopy was able to detect salinity response in leaf tree [44]. Thus, FTIR spectral fingerprints of MOL showed that they were a more important source of polysaccharides where they are subject to extreme climatic conditions (very high or very low average annual temperatures, long dry periods but a low amplitude of relative humidity).

- Impact of MOL leaf maturity

At each stage of maturity, the chemical content of leaves was different. It was observed that the richness in some nutrients in young leaves was often linked to their photosynthetic activity. Young leaves thus produced more available carbohydrates than mature leaves [10]. Few publications have investigated FTIR technique to demonstrate the impact of maturity on leaf signatures [27,45]. The stages of development (young or mature) were observed from the modifications of the characteristic bands of polysaccharides such as cellulose, hemicellulose and pectin, located at 1050, 1032, 1008  $\text{cm}^{-1}$  while the band at 1050  $\text{cm}^{-1}$  could be also attributed to the accumulation of silica during the development of the leaves. Authors observed variations in the intensity and the position of absorption bands in this spectral region (1100–100  $\text{cm}^{-1}$ ) due to the presence of various sugars from polysaccharides and different pyranoside linkages, explained by modification in microfibril orientation, caused by cell elongation during its development. Here, AComDim approach exalted the bands with higher intensity between the different levels of the maturity factor. Thus, the presence of a peak at 1714  $\text{cm}^{-1}$ , a shoulder at 1049  $\text{cm}^{-1}$  at the base of a strong band around 991  $\text{cm}^{-1}$  confirmed that the young MOL are very rich in an ester fraction that can be assimilated to cutin, rich in cellulose and hemicellulose. These bands became less significant in mature MOL due to the stiffening of the leaf cell wall where a no ester fraction became more abundant due to lignification. IR broad bands at 1583, 1402  $\text{cm}^{-1}$  could overlap the aryl ring stretching and O–CH<sub>3</sub> deformation bands of lignin and also the stretching and bending vibrations of amide bands I associated with proteins as identified in literature (Stark et al., 2016). Then, the presence of a band at 1089  $\text{cm}^{-1}$  could be associated to glycosidic bonds of others pectic polysaccharides or ring stretching of aromatic rings and also, C–O bond in lignin. Nevertheless, no band at 840  $\text{cm}^{-1}$  (linked to the deformation vibrations of the C–H aryl bond) was exalted by AComDim method while the intensity of this lignin bands decreases with leaf maturity according to Ribeiro da Luz [27]. Our results are in agreement with previous studies which confirmed that mature leaves contained more fibers, proteins, and bioactive compounds including phenolic compounds than young leaves [10]. The antioxidant activity and the total phenols contents were found to be higher in mature MOL leaves (with total phenol values ranging from 8.4–11.8 / 100 g of dried matter for young leaves and from 9.0–13.6 mg / 100 g of dried matter for mature leaves [46,47] but according to other studies, the levels of total phenols and total tannins in Cameroonian samples were not influenced by the maturity stage of the leaves [10]. In our case, CC16 loading not allowed to differentiate young and mature leaves from specific spectral bands assigned to phenolic compounds. In addition, no specific bands of lipids could discriminate young or mature MOL leaves. For Agamou et al. [10], maturity had no influence on the lipid and vitamin C content

of MOL leaves. CH<sub>2</sub> bands at 719 and 723  $\text{cm}^{-1}$  in the CC16 loading characterized essentially pyranose ring. Thus, FTIR technique could be a rapid approach to estimate the maturity stage of MOL powders used in different fields in order to differentiate them according to their content of polysaccharides, compounds with numerous bioactivities, and to target their application in fields of pharmacology and biological medicine [48,49].

- Spatio-temporal impact

This is the first report of the spatio-temporal effect on leaves chemical composition using a spectroscopic approach considering leaf harvest on several years. Climate data, monthly and yearly averages (temperature, rainfall and humidity) recorded over three years (data from World Weather Online website [43]) showed a cyclic comportment of the seasons (humid and dried seasons), characteristic for each agroclimatic zone. FTIR spectroscopy made it possible to visualize the impact of seasonal climatic variations, on the MOL chemical composition through significant spectral differences according to month or year harvest. Differences in the intensity of climatic parameters (not shown here) were observed depending on the year. This behavior was confirmed by the presence of interactions with year factor, which appeared significant in AComDim approach. Thus, the obtained FTIR classification of MOL samples supposed that agroclimatic variations were more important during the years 2016 and 2107 for HS, HL and BF areas while a lower impact is recorded in SS area. In MF area, no trend emerges, in the same way that less variation in climatic parameters was observed for this area during the 3 years studied. The chemical composition of the leaves seems to be less varied in January from year to year (2016–2017). Greater variations can be observed when MOL harvests are made at the end of August-beginning of September (2015–2016). However, there was no consistency in the compositional quality of the leaf powders in April over the two collection years.

## 5. Conclusion

AComDim method applied on a large collection of FTIR-ATR profiles of MOL leaf powders allowed the ranking of factors influencing the leaf chemical composition. It was the first application of AComDim tool to a vegetal material but an additional one demonstrating the spectroscopy power to follow the chemical composition evolution of a plant material exposed under different environmental conditions. The FTIR-ATR MOL signatures have showed intensity variations of spectral bands of the primary metabolites which content could differ according to various parameters linked to the leaf harvest conditions. Differences were observed in assignation of polysaccharide IR bands showing that different polyose, protein and aromatic structures were identified according to its stress response and its development stage. Thus, the “agroclimatic zone” was the most significant factor explaining differences in primary metabolites content of MOL leaves. That was explained by the fact that Cameroon is characterized by one of the most variable climates on the planet, and this variability increases as one moves north through sub-humid and semi-arid areas. This factor was followed by temporal factors: firstly “month/year” and secondly “year” of collection. Climate variability manifests itself in a spatio-temporal dynamic and more particularly with seasonal rainfall regimes. Results reported here showed that MOL leaf samples from north Cameroon (High Savannah and Sudano-Sahelian zones) were richer in polysaccharides than those collected in other parts of the country. It observed that for a given agroclimatic zone, January samples were richer in polysaccharides than April ones; the latter were characterized by the highest amount in proteins and aromatic compounds (lignin and phenolic compounds) in comparison with samples collected in January or September. The maturity factor had the least significant impact, but it confirmed that young MOL leaves contained less proteins and antioxidant compounds such phenolic ones because these last were used for their growth and

their defense. The "year" factor was involved in all significant interactions.

Moreover, this study, focused on samples from Cameroon, aims ultimately to be able to make recommendations for the use of these MOL powders according to their region of origin and their collection period. On a world market still very little controlled, mainly dominated by India and little penetrated by the countries of sub-Saharan Africa, the development of a nutritional and chemical typicity of the MOL can constitute an original angle to enter the market with a significant competitive advantage. The possibility to select MOL leaves according to their chemical composition will also allow better planning of therapeutic diets and, above all, contribute to better nutrition for African populations. This approach could be transposed to the quality control of other agrifood products.

#### Author statement

C. Rébua and I. Bombarda have designed and supervised the experiments and the analyses.

C. Rébua, I. Bombarda and N. Dupuy have analyzed the data.

C. Rébua wrote the article with the contributions of all the authors and agrees to serve as the author responsible for contact and ensures communication.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Acknowledgement

We thank Inès Pany for collecting leaves in the different harvest locations of Cameroon.

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