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HPLC Fingerprinting of Glucosinolates during Fermentations Assisted by Chemometric Analysis

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Abstract

Glucosinolates, bioactive phytochemicals found in Brassica vegetables proved to be associated with multiple health benefits. Therefore, the food processing of these vegetables need to consider how glucosinolates content can strongly be affected. The HPLC analysis of glucosinolates, from four different Brassica vegetables (broccoli, cauliflower, white and red cabbage) was done before and after natural home-made fermentation. Two different lactic fermentations were used, in salt and acetic acid or with salted water. The average content of individual glucosinolates differed among Brassica vegetables. The highest total GLS content was noticed in broccoli, followed by red and white cabbage, and cauliflower (4.93, 3.35, 3.15 and 2.07 µmol/g dw, respectively). After lactic fermentation, the most affected were indole glucosinolates, excepting the white cabbage, where total aliphatic glucosinolates decreased strongly. Among individual glucosinolates, 4-hydroxy-glucobrassicin increased by 27% and 39% during fermentation in salt/acetic acid and with salted water, respectively. This study has shown that individual glucosinolates content decreased substantially after lactic fermentation and as demonstrated by Multivariate Statistical Analysis, glucosinolates profiles can be considered as significant discrimination factors to evaluate the different fermentation processes of Brassica vegetables.

Keywords: *glucosinolates, homemade fermentation, Brassica vegetables, principal component analysis, canonical variant analysi*

1. Introduction

Glucosinolates (GLS) are plant secondary metabolites, which are present in all Cruciferous vegetables, mainly in *Brassica* vegetables which includes a large variety of horticultural crops [1], appreciated by their nutritional properties and potential health benefits. Among them, broccoli (*Brassica oleracea* var. *italica*), cauliflower (*Brassica oleracea* var. *botrytis*), white and red cabbage (*Brassica oleracea* convar. *capitata* var. *alba* and *Brassica oleracea* convar. *capitata* var. *rubra*) are used for human consumption as fresh and under different processing technologies. Recent studies [2-5] show that ingestion of *Brassica* vegetables may decrease the incidence of different types of cancer.

During the food processing of *Brassica* vegetables, the total GLS content decreases, three mechanisms being involved: (i) enzymatic breakdown involving myrosinase, a thioglucoside glycohydrolase (EC 3.2.3.1), (ii) leaking of GLS into the cooking water and (iii) thermal degradation.

The GLS profiles from *Brassica* vegetables have been studied during different cooking processes, such as blanching and treatment with acetic acid [6] and thermal degradation [7-10].

Glucosinoates and myrosinase, are located in separate cell compartments of the plant cell, the damage of vegetable tissues, release this enzyme, and therefore the hydrolysis of GLS begins. The chemical structure of GLS, the temperature, the pH value, the ascorbic acid content and the Fe^{2+} ions determine which end products (isothiocyanates, thiocyanates, nitriles or oxazolidine-2-thiones) are formed [11].

The lactic fermentation of vegetables is one of the oldest preservation technologies, which yields palatable food, which either retains a part of the original nutrients or new ones are formed, in a probiotic environment (lactic microorganisms). The fermented vegetables can be preserved for long periods of time, without refrigeration, especially when herbs, spices and other ingredients are added to improve the stability, aroma and flavor.

Fresh vegetables may contain a variety of microorganisms, including aerobic spoilage microflora, yeast and molds, but during fermentation, organic acids diffuse into the brine and the pH decreases (from 7.0 to 3.4-3.7) favoring the proliferation of lactic acid bacteria [12]. Sodium chloride (NaCl), when added before and during fermentation, acts as a traditional preservative, reducing the water activity preventing the texture softening, as an inhibitor of endogenous pectolytic enzymes and increasing the osmotic pressure which release the cellular juice [13]. Meanwhile, high intake of NaCl is a risk factor in cardiovascular diseases, some studies being focused on the reduction or partial replacement of NaCl in the fermentation process [13-15].

Under enzymatic hydrolysis during fermentation, glucoraphanin is converted to sulforaphane [11], while glucobrassicin, in indole-3-carbinol. As the pH decreases, indole-3-carbinol reacts non-enzymatically with L-ascorbic acid to yield ascorbigen, a chemical with anticarcinogenic properties [16].

Few informations approaching the fate of individual GLS during natural homemade fermentation are available. The majority of these studies were conducted in laboratory, under controlled conditions [13, 16, 17]. In Romania, fermented foods are often prepared and used, by traditional recipes without starter cultures, the fermented cabbage being the major product of this category, followed by cauliflower and, in recent years, broccoli.

Although the content of GLS and their breakdown products from *Brassica* products were intensively investigated, there are no data about the fate of GLS from broccoli and cauliflower under the lactic fermentation. Therefore, the purpose of the present paper was to investigate the impact of homemade lactic fermentation on the level of individual GLS, from four different *Brassica* vegetables: broccoli, cauliflower, white and red cabbage, using advanced chromatographic separation by HPLC-PDA (High Performance Liquid Chromatography coupled with Photo Diode Array) assisted by chemometry analysis.

2. Materials and methods

Plant material and the homemade fermentation process

All samples of *Brassica* vegetables (broccoli, cauliflower, white and red cabbage) were obtained from a micro-farm located in the North Western region of Romania. All of edible parts of the vegetables were harvested at the end of October, 2012. Aliquots from each of the vegetables (ca. 100 g) were immersed in liquid nitrogen and lyophilized using a freeze dryer Alpha 1-2 Christ (Martin Christ, Osterode am Harz, Germany), in order to obtain powder samples of non-fermented tissue, as a reference (FRH). The powder samples were stored at -20^{o}C until the extraction of GLS was performed, and were labeled as BRCL_FRH,

CLF_FRH, WHITE CBG_FRH and RED CBG_FRH for non-fermented broccoli, cauliflower, white and red cabbage, respectively. Another tissue portion (ca. 1kg) from each vegetable was used for the homemade fermentation process, using two different traditional treatments. The first treatment (codified FM_SL_AC) referred to an acidic fermentation (salt/acetic acid combination) in a glass container (10 L), where all raw material was placed together in a boiled aqueous solution containing commercial vinegar (8%), salt (1.5%), sugar (0.5%) with dried dill, celery, horseradish, bay leaves, pepper and mustard. The fermentation lasted 6 weeks under ambient conditions (19-20^{o}C for two weeks and 6-10^{o}C for the last 4 weeks). Samples were collected after fermentation, freeze-dried and stored at -20^{o}C, before monitoring the GLS contents. The second treatment, codified FM_SL (salted water) was applied only for cauliflower and white cabbage, using the same quantities for each aliquot. The fermentation was carried out in separate vessels (glass vessel for cauliflower and wooden barrel for white cabbage), using an aqueous solution containing only salt (1.5%). The samples were collected after 6 weeks (in the same conditions described above), freeze-dried and stored at -20^{o}C, before the evaluation of GLS contents.

HPLC-PDA Analysis of glucosinolates

Extraction of glucosinolates from Brassica vegetables

The GLS content in *Brassica* vegetables was assessed according to the EU official method (EEC Regulation N1864/90). Duplicate freeze-dried samples of about 200 mg were extracted in 5 ml of boiling methanol 70% for 5 min at 80^{0}C, and centrifuged at 2500 x *g* for 20 minutes. The extraction was repeated in the same way on the solid residues. Supernatants were then combined and the total volume was measured, each type of extract was analyzed twice by loading 1 ml onto a mini-column filled with 0.6 ml DEAE-Sephadex A-25 anion-exchange resin conditioned with 25 mM acetate buffer pH 5.6. After washing the column with 3 ml of buffer, a volume of 200 µl purified sulphatase (Sigma-Aldrich) was loaded onto the column and left overnight at room temperature. The second day, the desulfo-GLS were eluted with 3 ml of ultra pure water and the collected fraction was finally injected into HPLC. A known amount of glucotropaeolin (200 µl from a solution containing 1mg/ml) was added to each *Brassica* samples before the first extraction, as an internal standard for the HPLC analysis.

HPLC quantification of glucosinolates from Brassica vegetables extracts

The desulfo-GLS extracted and eluted from each vegetable tissue were analyzed using a Shimadzu HPLC system using a Platinum (C18) 100 A column (250 x 4.6 mm, 5 µm), thermostated at 30^{o}C, using a diode array as detector. The separation was performed at a flow rate of 0.5 ml/min with a mobile phase consisting of a gradient of water (A) and acetonitril (B) following the program: 0-1 min 1% B; 1-22 min linear gradient from 1% to 22 % B, 22-32 min linear gradient down to 1% B. The desulfo-GLS peaks were monitored at 229 nm and identified by retention time, using in parallel the standards. The content of each GLS, expressed in µmol/g dry weight (dw) was calculated using glucotropaeolin as an internal standard and considering the response factors of the other desulfo-GLS relative to the desulfo-glucotropaeolin.

Statistical analysis

All data were obtained from two samples and analyzed in duplicates. At the first stage, for every GLS and all *Brassica* vegetables, one-way analysis of variance with the factor type and treatment type (fresh and FM_SL_AC), at $P = 0.05$ significance level, was performed. In order to correctly interpret the differences (fresh *vs*. FM_SL_AC), the Bonferroni-corrected multiple comparison post-hoc tests, were performed when these differences resulted to be statistical significant (i.e. $P < 0.05$). The comparisons between fermentation with salted water and both, non-fermented and fermented with salt/acetic acid types, were also subjected to one-way analysis of variance in the same manner as formerly described for salt/acetic acid

fermentation. For all analysis of variance tests, the GraphPad Prism version 5.00 software was used (GraphPad Software, San Diego, CA, www.graphpad.com). The principal component analysis (PCA) and the canonical variable analysis (CVA) were performed in order to compare groups and to identify the clusters, in multivariate way [18].

3. Results and discussions

Quantification of GLS from fresh *Brassica* vegetables

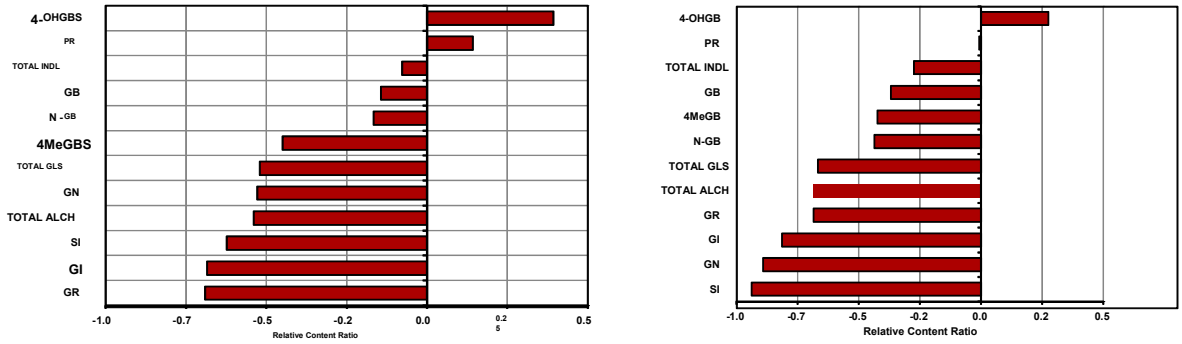
Table 1 presents the GLS types, according to their chemical structure. Nine different GLS were detected in the four different *Brassica* vegetables before fermentation, namely: glucoiberin (GIB), progoitrin (PRO), sinigrin (SIN), glucoraphanin (GRA), gluconapin (GNA), 4-hydroxy glucobrassicin (4-OHGBS), glucobrassicin (GBS), 4-methoxy glucobrassicin (4-MeGBS) and neoglucobrassicin (N-GBS). In fresh broccoli, the main GLS were aliphatic (59%), the major representatives being PRO and GRA (1.51 μmol/g dw, respectively 1.37 μmol/g dw) and 41% from indole GLS, the predominant compound being GBS (1.44 μmol/g dw). In fresh cauliflower, the aliphatic GLS were also predominant (87%), the major GLS being SIN (0.60 μmol/g dw). In the white cabbage the main GLS were also aliphatic (96%), while in the red cabbage the percentage are almost equal between aliphatic and indole class (52%, respectively 48%) (**Table 1**). The pattern of GLS in the fresh *Brassica* vegetables were the same as reported in the other studies [6, 16, 19-22].

Change in the GLS fingerprint and content during fermentation

According to the data showed in **Table 1**, after the pickling procedures (FM_SL_AC and FM_SL) one can identify changes in the GLS quantitative profiles. Almost all individual GLS decreased significantly after fermentation processes, excepting GIB in broccoli, N-GBS in cauliflower, PRO and 4-OHGBS in white cabbage and SIN and GRA in red cabbage. Also, the total GLS, total aliphatic and total indole GLS, were significantly decreased in all vegetables ($P<0.001$) after fermentation processes (**Table 1**).

In order to highlight which of the GLS are more affected by the type of fermentation, a relative ratio of the individual GLS was calculated between fresh and fermented samples (FRH *vs*. FM_SL_AC and FRH *vs*. FM_SL). A drastic decrease was considered to occur only for the GLS that have the relative ratio more than 50%. For example, in the broccoli case, after salt/acetic acidic fermentation, in a decreasing order, the most affected GLS were: GBS (91%) > 4-OH GBS (83%) > GRA (73%) > 4-MeGBS (69%) > N-GBS (68%), and in the red cabbage case were N-GBS (100%) > GBS (92%) > 4-Me GBS (89%) > PRO (76%) > 4-OH GBS (54%). In both types of cauliflower fermentation, the indole GLS decreased and during FM_SL fermentation the aliphatic GLS were affected: SIN (87%) > GIB (79%) > PRO (58%). Instead, in the white cabbage case, both fermentations decreased only aliphatic GLS: GIB (81%) > GRA (69%) for FM_SL_AC and GRA (70%) > GIB (68%) > SIN (62%) for FM_SL. Surprisingly, in the white cabbage case, for both fermentation types (FM_SL_AC and FM_SL), a slight increase in the amount of 4-OH GBS, were found (**Figure 1a,b**). Compared with fresh samples (0.020 μmol / g dw (FRH) *vs.* 0.026 μmol / g dw (FM_SL_AC) and 0.028 μmol / g dw (FM_SL), respectively), the increase is statistically significant in both cases ($P < 0.01$). The increase of 4-MeGBS, after the souring of blanched cabbage, was also reported by WENNBERG, & al. [6]. Up to now, the mechanisms involved in the indole GLS formation in the presence of salt and/or acetic acid is unknown.

By plant tissue destruction, the endogenous myrosinase is released and attacks GLS, in particular GBS forming indole-3 carbinol, which in the presence of vitamin C yields ascorbigen during the fermentation process.



a. **b.**

Figure 1. White cabbage GLS relative content ratios between: **a** – fermented with salted water and non-fermented values (WHITE CBG_FM_SL / WHITE CBG_FRH); **b** – fermented with salt/acetic acid and non-fermented values (WHITE CBG_FM_SL_AC / WHITE CBG_FRH)

Table 1. Content of different GLS (µmol/g dw) separated from fresh (FRH) and fermented *Brassica* vegetables, using two treatments (FM_SL_AC and FM_SL)

GLS	BRCL FRH	BRCL FM_SL_AC	CLF FRH	CLF FM_SL_AC	CLF FM_SL	WHITE CBG_FRH	WHITE CBG_ FM_SL_AC	WHITE CBG_ FM_SL	RED CBG_FRH	RED CBG_ FM_SL_AC
GIB	0.04^{a} ±0.01	0.03^{a} ±0.00	0.56^{a} ±0.01	0.11^{b} ±0.01	0.12^{b} ±0.01	0.93^{a} ±0.01	0.17^{b} ±0.01	0.30^{c} ±0.00	0.43^{a} ±0.01	0.22^{b} ±0.03
PRO	1.51^{a} ±0.08	1.09^{b} ±0.02	0.34^{a} ±0.00	0.18^{b} ±0.00	0.14^{b} ±0.02	$0.45^{a,b}$ ±0.03	0.45^{b} ±0.03	0.52^{a} ±0.01	0.56^{a} ±0.04	0.13^{b} ±0.00
SIN	nd	nd	0.60^{a} ±0.00	0.02^{b} ±0.00	0.08^{c} ±0.00	0.56^{a} ±0.00	0.04^{b} ±0.00	0.21^{c} ±0.01	0.030^{a} ±0.00	0.04^{a} ±0.01
GRA	1.37^{a} ±0.06	0.37^{b} ±0.02	0.29^{a} ±0.01	0.21^{b} ±0.01	0.27^{a} ±0.01	0.86^{a} ±0.04	0.27^{b} ±0.00	0.27^{b} ±0.01	0.41^{a} ±0.01	0.34^{a} ±0.07
GNA	-	-	-	-	-	0.21^{a} ±0.00	0.02^{c} ±0.00	0.10^{b} ±0.00	0.32^{a} ±0.01	0.08^{b} ±0.01
4OHGBS	0.12^{a} ±0.01	0.02^{b} ±0.01	0.06^{a} ±0.01	0.03^{b} ±0.01	0.03^{b} ±0.00	0.02^{b} ±0.00	0.03^{a} ±0.01	0.03^{a} ±0.00	0.18^{a} ±0.00	0.08^{b} ±0.01
GBS	1.44^{a} ±0.01	0.12^{b} ±0.02	0.18^{a} ±0.02	0^{b} ±0	0.02^{b} ±0.00	0.10^{a} ±0.01	0.06^{b} ±0.00	0.08^{c} ±0.00	1.37^{a} ±0.04	0.11^{b} ±0.01
4MeGBS	0.08^{a} ±0.01	0.03^{b} ±0.00	0.01^{a} ±0.00	0.00^{b} ±0.00	0^{c} ±0	0.01^{a} ±0.00	0.00^{b} ±0.00	0.00^{b} ±0.00	0.05^{a} ±0.00	0.01^{b} ±0.00
N-GBS	0.37^{a} ±0.04	0.12^{b} ±0.01	0.02^{a} ±0.01	0.01^{a} ±0.01	0^{c} ±0	0.01^{a} ±0.00	0.00^{b} ±0.00	$0.01^{a,b}$ ±0.00	0.01^{a} ±0.00	0.00^{b} ±0.00
TOTAL GLS	4.93^{a} ±0.04	1.78^{b} ±0.03	2.07^{a} ±0.04	0.56^{b} ±0.02	0.65^{b} ±0.04	3.15^{a} ±0.01	1.05^{c} ±0.03	1.51^{b} ±0.01	3.35^{a} ±0.01	1.01^{b} ±0.08
TOTAL ALPH	2.91^{a} ±0.00	1.50^{b} ±0.01	1.79^{a} ±0.02	0.52^{b} ±0.01	0.61^{c} ±0.04	3.02^{a} ±0.01	0.95^{c} ±0.02	1.39^{b} ±0.01	1.75^{a} ±0.03	0.82^{b} ±0.09
TOTAL INDL	2.02^{a} ±0.05	0.29^{b} ±0.02	0.27^{a} ±0.02	0.05^{b} ±0.01	0.04^{b} ±0.00	0.13^{a} ±0.01	0.09^{c} ±0.01	0.12^{a} ±0.00	1.61^{a} ±0.03	0.19^{b} ±0.01

Note: Values represent means ± SD expressed as µmol/g dw content. The same superscript in the same lines means no significant difference ($P < 0.05$). For each GLS from broccoli (BRCL), cauliflower (CLF), white cabbage (WHT CBG) and red cabbage (RED CBG) the mean values comparisons were done by Bonferroni-corrected multiple comparison post-hoc test ($P = 0.05$), after the two-way ANOVA ($P = 0.05$) prescribed statistical significant factors interactions in every case.

The ascorbigen is able to induce phase I and II enzymes that are involved in the detoxification of xenobiotics [23]. Generally, we observed a decreased content of GLS. In our experimental study, considering that fermentation was realized on intact tissues, the mechanisms of GLS loss cannot be attributed to enzymatic degradation. Future studies are needed to identify the mechanisms which may explain the loss of GLS from the intact vegetable matrix during the fermentation. Recently, FERNÁNDEZ-LEÓN, & al. [20], studied the influence of a gas mixture containing 10% of O_2 and 5% of CO_2, with 85-90% relative

humidity, combined with cool storage (1 and 2^{o}C) on broccoli quality parameters after harvesting, including GLS. According to their results, aliphatic GLS remained constant during controlled atmosphere, while glucobrassicin was the predominant indole GLS at the end of storage. The increase of GLS levels by biosynthesis in controlled atmosphere is the result of stress response due to the increased CO_2 and decreased O_2 concentrations [20, 24]. The level of 4OH GBS increased with 27% and 39% in FM_SL_AC and FM_SL respectively, only in white cabbage during both fermentation conditions. From aliphatic GLS, only PRO concentration was increased with 14%. These effects are due to the specific fermentation conditions, when vegetables are immersed in the brine and low oxygen availability. HARBAUM & al. [25] investigated the impact of fermentation on the quantitative polyphenol composition of Chinese *Brassica* vegetables. Their results have shown that the fermented process leads to enzymatic (esterase and glycosidase) degradation of the phenolic derivatives due to microorganisms involved in the fermentation process.

Multivariate Statistical analysis

The Principal component analysis (PCA) considered as variables the five aliphatic GLS (GIB, PRO, SIN, GRA and GNA) and four indole GLS (4-OHGBS, GBS, 4-MeGBS and N-GBS) for all *Brassica* vegetables. These nine variables are continuous type and have the same units by with very different variances (**Table 1**), thus the PCA using the pre-processing of data correlation matrix, was done. In addition was considered a SVD (Single Value Decomposition) with N = 9999 bootstrap. First, PCA used the samples values grouped in ten groups respecting the fermentation types and also the *Brassica* types, simultaneously, as presented in **Table 1**. The biplot representation (**Figure 2a**) of factor scores for the ten groups suggested a possible grouping and further a clustering between the three groups of *Brassica* treatment types (i.e. between the FRH, FM_SL and FM_SL_AC over all the vegetables). This was the reason that the PCA was done considering these three groups of *Brassica* treatment types. In order to have a comprehensive view of the results, the biplot in **Figure 2a** is the graphical representation of loadings and factor scores for the latter PCA case, but preserving the indexing samples of the ten groups. The first two principal components of the biplot (**Figure 2a**) describe 76.725 % cumulative variance. The samples from each of three treatment groups are interconnected with a convex hull line. In agreement with the very small standard deviations of GLS for all *Brassica* vegetables (**Table 1**) all the groups present very close points in the biplot. All the *Brassica* groups are non-overlapped, but some of them are very close each other in terms of Euclidian distance.

The fresh (non-fermented) broccoli, red cabbage and fermented with salt broccoli groups have positive factor scores for the first component (PC 1), meanwhile the other *Brassica* groups have negative factor scores. Thus, it can be noticed that the first PCA component cannot discriminate the GLS profiles of fermented and non-fermented *Brassica* groups. The white cabbage fermented with salted water (WHITE CBG_FM_SL) and all non-fermented groups have positive scores for the second principal component, meanwhile the other groups have negative scores for the second principal component. Therefore, one can notice that the second principal component cannot make the simultaneous discrimination between fermentation types and also the *Brassica* types, simultaneously. However the Euclidian distances between the fermentation groups are not large enough to fully separate them in such a manner that clustering these groups should not be an issue. This hypothesis was proven by HCA (Hierarchical Cluster Analysis) with Euclidian distance, paired group, two-way, where there was no similarity distance that could generate the three treatments clusters (data not shown). The graphical distribution of the variables correlations (i.e. loadings) with factors or

principal components presented in the biplot does not have suggestive variables associations. First association includes GIB (PC 2: 33.8%), GNA (PC 2: 31.6%) and SIN (PC 2: 15.9%) variables, which are correlated and have high positive loadings and high contributions (i.e. %) to the second principal component (PC 2) and very low contributions to the first principal component (PC 1). This variables association contributes to the non-fermented white cabbage and cauliflower contrast and all the other samples groups. Also their content for the mentioned samples groups is higher than the others.

The next variables association consists of 4MeGBS (PC1: 21.1%), NGBS (PC1: 17.4%), GBS (PC1: 17.4%), PRO (PC1: 16.4%) and GRA (PC 1: 13.4%), with positive loadings, high contributions (i.e. %) for the first principal components and very low contributions to the second principal component (PC 2). The latter two variables associations contribute to contrast the non-fermented *Brassica* vegetables and the fermented samples groups, simultaneously. The corresponding GLS contents of these variables are higher in the non-fermented samples than in the fermented ones. The 4OHGBS variable has positive and loadings moderate (PC1: 8.8%; PC 2: 8.2%) contribution for both principal components. This variable does not associate with others in the PCA analysis. In order to determine the existence of fermentation groups clustering the Canonical Variates Analysis (CVA) was performed with the variables values; CVA is a much developed PCA method [18, 26, 27]. CVA realized an optimization of the principal axes, denoted the canonical axes, in order to obtain maximal and second to maximal separation between all groups (i.e. multigroup discriminant analysis) [18, 26]. Despite the canonical transformation, in the CVA biplot (**Figure 2b**), the variables associations are similar with the PCA ones, but with different orientation of the factor loadings. The loadings for the two canonical axes represent the input for the hierarchical cluster analysis (Euclidian distance, paired group, two-way) [18, 27]. Clustering process was successful, with the 29.2 cut-off similarity distance value (data not shown). Currently, one can assert that GLS profiles can be considered as markers (discrimination factors) for the three treatment types (including the fresh one as non-fermentation type or the control) of the *Brassica* vegetables.

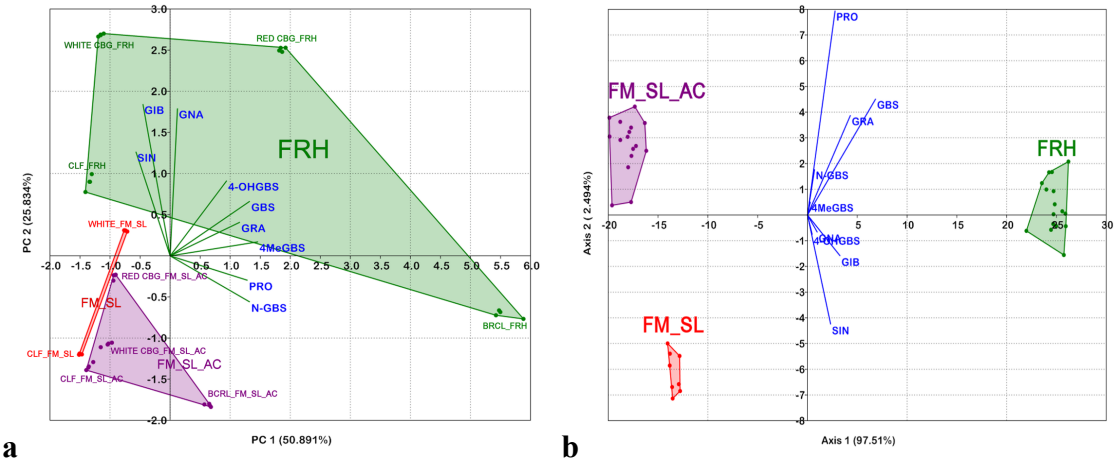


Fig. 2 a - PCA biplot with the factor scores (three fermentation samples groups) and factor loadings (GLS variables) for principal component 1 (PC 1: 50.834% explained variance) and component 2 (25.834% explained variance); b - CVA scatter biplot with the factor scores (three fermentation samples groups) and factor loadings (GLS variables) for principal canonical Axis 1 (97.51% explained variance) and canonical Axis 2 (2.494% variance explained)

4. Conclusions

Both home-made fermentations (FM_SL_AC and FM_SL) affect the content of both indole and aliphatic GLS from *Brassica* vegetables. The total GLS content was more affected in cauliflower and red cabbage under acidic fermentation (73% respectively, 70%). Instead, in broccoli samples the total GLS content decreased up to 49%.. The presence of salt in the fermentation process, increasing the osmotic pressure, may be one of the principal factors involved in the GLS losing into the brine solution. On the other hand, the increase of 4-OH GBS in the white cabbage, after fermentation, is interesting, and we can presume that it is due the stress conditions caused by lack of oxygen due to immersion of cabbage into brine. PCA and CVA analyses of GLS profile for fresh and fermented groups were able to discriminate them, thus these bioactive compounds could be considered markers for these fermentation treatments.

Based on the results, one can conclude that both fermentation types change the content of GLS in the end products. Hence, the fermented *Brassica* vegetables provide a low content of total GLS, they could have a beneficial effect and are more suitable to be consumed during the winter season in order to prevent the chronic diseases.

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