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ARTICLE

Metabolic Fingerprint of Brazilian Maize Landraces Silk (Stigma/Styles) Using NMR Spectroscopy and Chemometric Methods

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Aqueous extract from maize silks is used by traditional medicine for the treatment of several ailments, mainly related to the urinary system. This work focuses on the application of NMR spectroscopy and chemometric analysis for the determination of metabolic fingerprint and pattern recognition of silk extracts from seven maize landraces cultivated in southern Brazil. Principal component analysis (PCA) of the ^{1}H NMR data set showed clear discrimination among the maize varieties by PC1 and PC2, pointing out three distinct metabolic profiles. Target compounds analysis showed significant differences ($p < 0.05$) in the contents of protocatechuic acid, gallic acid, *t*-cinnamic acid, and anthocyanins, corroborating the discrimination of the genotypes in this study as revealed by PCA analysis. Thus the combination of ^{1}H NMR and PCA is a useful tool for the discrimination of maize silks in respect to their chemical composition, including rapid authentication of the raw material of current pharmacological interest.

KEYWORDS: Maize silks; landraces; anthocyanins; polyphenols; ^{1}H NMR spectroscopy; principal component analysis; metabolomics

INTRODUCTION

Many ethnopharmacological studies, from various regions of the world, on the use of maize (*Zea mays* L.) silks based on popular knowledge, have been published (*1*). The silk refers to the styles and stigmas from the maize's female flower and has been used mainly as tea for the treatment of various diseases (*2*, *3*) also recognized by official medicine (*4*). The biological actions of silk aqueous extracts are well cited in the literature and include mild diuretic, tonic, and urinary demulcent (soothing or softening agent), besides antispasmodic, anti-inflammatory, and antioxidant properties. It has also been used to pass kidney stones, to cure bladder ailments, gout, benign prostatic hyperplasia, edema (water retention), lowering blood pressure, and even helping rheumatism symptoms or hypertension (*2*, *4*, *5*).

The extensive genetic and chemical diversity of maize results from a long domestication process carried out since Pre-Colombian civilizations (*6*). More than 250 maize varieties are known worldwide, and their origin is linked directly or indirectly to the maize domestication and breeding processes performed by ancient civilizations (*7*). One of the properties of maize is the spectacular diversity in morphology among its races, which is seemingly paralleled by an extensive allelic variation as detected by molecular methods (*6*). On the other hand, despite the fact modern farming techniques have greatly increased the yield of maize, the genetic breeding programs adopted have led to a greater genetic uniformity as well as meaningful loss of diversity. In fact, nowadays, very few of the world's maize germplasm consist of local varieties (landraces), showing the genetic vulnerability of that species.

In this context, small farmers in some regions of the world still cultivate maize landraces that are populations with high genetic variability and represent a valuable source of potentially useful traits such as resistance or tolerance to (a)biotic stress factors (*8*). In fact, one can easily observe the genetic diversity in maize landraces by observing the wide range of colors in their leaves, flowers (silks), grains, stems, and roots, for example.

In some counties at the far-west region of Santa Catarina State (Southern Brazil), the occurrence of more than 30 maize landraces with distinct agronomic and nutritional traits have been registered so far (*9*, *10*). In that county, characterized by small rural establishments, around 40.6% of the farmers cultivate

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Table 1. Grain Types, Grain Colors, Plant Height, Cycle Duration, and Productivity of Maize Landraces Developed and Cultivated in Anchieta County (Southern Brazil, Santa Catarina State)

varieties	code	grain color	grain type	cultivation cycle (days)	height (m)	no. of cobs per plant	productivity of grains (kg/ha)
Cateto Vermelho[c]	*CV*	yellow and red	semident		2.8	1.36	7348
Língua de Papagaio[c]	*LP*	yellow and purple	dent	111	3.1	1.00	4944
Mato Grosso Palha Roxa[c]	*MGPR*	yellow and purple	semident	104	3.1	1.02	4828
Rajado 8 Carreiras[c]	*R8C*	variegated	dent and flint		2.7	1.25	5454
Rosado[c]	*RS*	variegated	semident	109	3.3	0.97	5225
Roxo 29[c]	*R29*	purple	dent		3.0	1.16	5823
Roxo 41[a]	*R41*	purple					

[a] Not analyzed. [b] Trials carried out in 2002/2003. [c] Trials carried out in 2003/2004 (adapted from Canci et al., 2004).

maize landraces (*11*). Anchieta county (26°31'11"S, 53°20'26"W; 229.53 km^2) is a strategic field for studying the genetic diversity and chemical exploitation of that species as source of compounds of nutritional and phytomedicinal interest. The chemical diversity of maize landraces can be analyzed by a metabolomic fingerprint approach, which employs diverse analytical tools such as nuclear magnetic resonance spectroscopy (NMR) and chromatographic techniques. ^{1}H NMR spectroscopy, for instance, has been used as a fingerprint tool for authentication and assessment of the quality control of natural products (*12*−*14*). Also, NMR has been combined with multivariate statistical techniques such as the well-known principal component analysis (PCA) to metabolomically profile several types of plants (*15*−*17*).

In this work, we assess the metabolic fingerprint of maize silk from seven landraces cultured in southern Brazil by using 1D and 2D NMR spectroscopy followed by chemometric analysis. The quick screening of the extracts with distinct chemical composition was possible using that metabolomic approach. In addition, the analysis of the major components in the silk extracts using UV−vis and HPLC explained the discrimination obtained by PCA analysis.

MATERIALS AND METHODS

Plant Materials and Ethics. The seeds of seven maize landraces originating from Anchieta county (latitude 26° 30' S and longitude 50° 30' W) were donated by a small local farmer association (SINTRAF/SC) to the Federal University of Santa Catarina in accordance with the current Brazilian legislation on biodiversity usage and assay (Genetic Heritage Management Council, Provisional Act 2.186-16, August 23, 2001). **Table 1** shows the principal agronomic characteristics of those genotypes such as grain type, grain color, plant height, cycle duration, and productivity as previously reported (*11*). According to their local names, the maize landraces were encoded as *LP* (Língua de Papagaio), *R29* (Roxo 29), *R41* (Roxo 41), *MGPR* (Mato Grosso Palha Roxa), *RS* (Rosado), *R8C* (Rajado 8 Carreiras), and *CV* (Cateto Vermelho).

The maize landrace populations were agro-ecologically cultured (Florianopolis, Santa Catarina State, southern Brazil, September 2005), at a population density of 20000 plants/ha. The silk samples (0.5−5 g, fresh weight) were harvested between fourth day and seventh day after they emerged from the husk, from individual plants, and immediately frozen in liquid N_2. The samples were subsequently lyophilized and stored at −18 °C until further analysis.

Extraction Procedure. The lyophilized plant material (30 mg/individual; n = 8 individuals/variety) was reduced to a fine powder with liquid N_2 and extracted with 6 mL of methanol-HCl (1%, v/v), for 30 min, at 0 °C. The extracts were rapidly vacuum-filtered through a sintered glass funnel on a cellulose membrane (0.45 μm). The end volume was adjusted to 5 mL and used immediately as described below. All the experiments were performed in triplicate.

NMR Sample Preparation and Data Collection. The concentrated extracts were dissolved in MeOH-d_4 (700 μL). All spectra were recorded at room temperature on a Bruker Avance DRX 400 spectrometer (Bruker GmbH, Rheinstetten, Germany), operating at 400.13 MHz for ^{1}H and equipped with a 5 mm inverse detection probe. For each sample, 16 scans (FIDs) were recorded with the following parameters: 64k data points; pulse width 8.5 μs (90°), spectral width of 4401 Hz, acquisition time of 7.4 s, and relaxation delay of 1.0 s. For spectrum processing, 64k points were used and an exponential multiplication associated to a line broadening of 0.3 Hz was applied. The spectra were referenced to tetramethylsilane (TMS) at 0.0 ppm. For obtaining metabolic profiles of the samples in study, a second set of NMR experiments was performed through a series of 1D and 2D experiments. For that, the dried methanolic extract was dissolved in 700 μL of 0.4 mM of sodium 3-(trimethylsilyl)-propionate-2,2,3,3-d_4 (TSP) solution in D_2O PBS buffer (pH = 7.2) in order to avoid chemical-shift changes due to pH variation. The dissolved extracts were transferred to a 5 mm NMR tube, followed by replacement of the internal atmosphere of the tubes by N_2 injection.

High-resolution 1D and 2D NMR spectra were recorded on a Bruker Avance DRX-500 spectrometer, equipped with an autosampler device (NMRcase, Bruker Biospin), operating at 500.13 MHz for proton and 125.77 MHz for carbon, at 298 K and nonspinning. One-dimensional ^{1}H NMR spectra were acquired using an modulate shape pulse (NOESYPR1DSP pulse sequence, Bruker library) with triple offset and amplitude scaling applied during the relaxation delay (15 s) and the mixing time (100 ms) for saturation of water (4.87 ppm) resonance. A total of 256 scans were acquired, with data collected into 64k data points over a 5482.45 Hz bandwidth in the absolute intensity mode, with an acquisition time of 2.88 s. Previous to Fourier transformation, the signal-to-noise ratio of the spectra was improved by multiplying each free induction decay with an additional exponential factor corresponding to 0.08 Hz, allowing an optimum balance between the noise reduction and the line-broadening effects considering a digital resolution of ±0.083655 Hz. Spectra were referenced to TSP (0.00 ppm) for chemical shift. Total correlation spectroscopy (TOCSY) spectra were acquired in phase sensitive mode using time proportional phase incrementation (TPPi) with a MLEV17 pulse sequence for excitation and spin-lock (*18*). Twenty-four scans were acquired for each of the 512 increments with 1024 data points over a spectral width of 5482.45 Hz in both dimensions, a relaxation delay of 1.7 s, and a mixing time of 100 ms. The 2D heteronuclear single quantum correlation (HSQC) spectra, acquired with inverse detection and ^{13}C decoupling during acquisition, consisted of 2048 data points over a 5482.45−25153.81 Hz bandwidth in the ^{1}H and ^{13}C dimensions, respectively. Forty-eight scans were acquired for each of the 2048−300 increments. HSQC spectra were processed by applying a sine squared function and sine squared constant both F1 and F2 dimension. The 1D and 2D ^{1}H/^{13}C NMR spectra were phased and baseline corrected using the softwares XWin-NMR (version 3.1, Bruker-BioSpin, Rheinstetten, Germany) and TopSpin (version 1.3, Bruker-BioSpin, Rheinstetten, Germany).

Multivariate Analysis: PCA, SIMCA, and HCA. The ^{1}H NMR spectra were reduced to ASCII files using XWin-NMR software, and the resulting data matrix was imported into Origin statistical software (version 5.0, Microcal, USA). Further, the regions of the spectra were imported into Pirouette statistical software (version 3.11, Infometrix, USA). After that, the signals corresponding to solvent, TMS, and noises from water suppression were removed from the data set prior to statistical analysis. The exploratory analyses (PCA, HCA, and soft independent modeling of class analogy (SIMCA)) were carried out autoscaling the variables and employing normalization and calculations of the first derivative as transformation. In the hierarchical cluster analysis (HCA) analysis, the

Euclidian distance was used as the SIMCA analysis was performed in same conditions of PCA.

Anthocyanin Quantification. Total anthocyanin content was determined in methanol-HCl (1%) extracts by reading the absorbance at 460 and 525 nm. The concentrations were expressed as equivalent of cyanidin-3-glucoside (molar extinction coefficient of 34300 $M^{-1} \cdot cm^{-1}$ and molecular weight of 449.2 $g \cdot mol^{-1}$) (*19*). All measurements were done in triplicate, and the results were expressed as mean ± standard error of mean (SEM).

High Performance Liquid Chromatography Analysis of Polyphenols. The methanolic extract was concentrated, and ethanol (100 μL) and ethyl acetate (400 μL) were added in each tube and then centrifuged at 5000 rpm for 10 min. The supernatants were collected, filtered (0.22 μm), and used for HPLC analysis. An aliquot (10 μL, triplicate analyses) was injected into a liquid chromatograph (Shimadzu LC-10A) equipped with a C_{18} (Shim-Pack CLC-ODS, 250 mm × 4.6 mm ∅, 40 °C) reverse phase column, fitted with a 5 μm C_{18} reversed phase guard column (Shim-Pack CLC-ODS, 4.6 mm ∅), maintained at 40 °C, and a UV−visible detector (280 nm). Elution consisted in H_2O:AcOH:*n*-BuOH (350:1:10, v/v/v), with a flow rate at 0.8 mL/min. Identification and quantification of phenolic acids were performed using the retention times of standard compounds [protocatechuic acid (Sigma P-5630), gallic acid (Sigma G-7384), *t*-cinnamic acid (Fluka 96340), syringic acid (Fluka 86230), caffeic acid (Sigma C-0625) 100 μg/mL], and external standard curves [gallic acid (2.5 μg/mL to 100 μg/mL, $r^2 = 0.98$, $y = 1589.9\times$) and caffeic acid (1 μg/mL to 100 μg/mL, $r^2 = 0.99$, $y = 532.83\times$)] obtained under the same experimental conditions. Besides, the identification of the compounds of interest was also confirmed by cochromatography of reference compounds, i.e., chromatographic standards. All measurements were done in triplicate and the results were expressed as mean ± SEM.

RESULTS AND DISCUSSION

^{1}H NMR and PCA. Metabolomic strategies based on nuclear magnetic resonance (NMR) coupled with multivariate statistical analysis have been developed to obtain the metabolite profile in a given complex biological sample. This approach defines the biochemical phenotype of a tissue or cell, providing the quick discrimination of other samples (*20*).

The ^{1}H NMR spectra of the methanol-HCl (1%) extract for the *LP*, *RS*, and *R29* landraces are shown in **Figure 1**. By visual inspection, the ^{1}H NMR spectra showed a predominance of signals in the carbohydrate region (2.5−4.5 ppm) followed by aliphatic/organic acids (0.0−3.0 ppm) and aromatic (6.0−8.0 ppm) regions. Further assignment of the peaks, performed by comparison of the chemical shifts from the 1D and 2D ^{1}H/^{13}C NMR spectra with data previously reported (*21*, *22*) and with a NMR data bank of our research group, revealed a number of assigned organic acids, sugars, amino acids, and phenolic acids resonances as shown in **Table 2**. The NMR spectra provided similar metabolic profiles for the maize landraces' silk, as discrepancies seem to be related to the concentration of the metabolites among the genotypes. Thirty-seven compounds were identified in all the samples and confirmed previous findings for this species, arginine, gallic acid, citric acid, β-fructose, and niacin that seem to have been identified for the first time. Despite the fact that the NMR spectra of the silk extracts were acquired using experimental parameters, allowing quantitative analysis of the metabolites, in the aromatic region, e.g., the resonances were relatively higher in the *RS* and *R41* genotypes and smaller in the *CV* and *MGPR* samples. However, we were not able in to identify by visual inspection of the spectra a clear picture regarding a discriminating effect of aromatic compound among the maize landraces. Further chemometric analysis (PCAs) revealed that signals of carbohydrate and aliphatic/organic acids regions were the most important for the discrimination of samples as described. Taking into consideration that the antioxidant effect of silk aqueous extract used as phytomedicine is regarded to be its phenolic content (*1*, *2*), we further analyzed the extracts by HPLC in order to gain insights as to a more detailed composition of these metabolites.

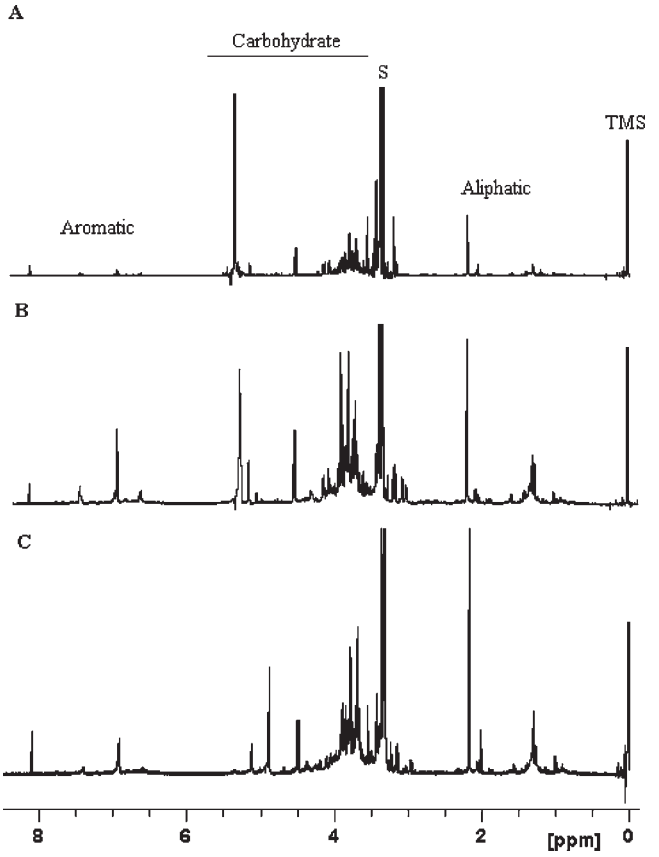

Figure 1. ^{1}H NMR spectra (0.0 to 8.0 ppm) of methanolic-HCl (1%) extract of silks from *LP* (A), *RS* (B), and *R29* (C) maize landraces cultivated in southern Brazil. TMS as internal standard.

An NMR spectrum is a physical characteristic of a compound and is thus highly reproducible. That is a major advantage, as it means that NMR-metabolomics data stand forever as long as the same extraction procedures and the same NMR solvents are used. Data mining from the raw data from previous experiments is thus always possible. In fact, an NMR spectrum contains many variables per sample, making detailed visual analysis very difficult. Thus, in this work, PCA was used to objectively interpret and compare the ^{1}H NMR data. PCA is among the most versatile of all chemometric methods by reducing the dimensionality of data and preserving most of the variance without requiring any knowledge of the data set (*23*). Our application of PCA for metabolomic profile allowed the large ^{1}H NMR data set obtained for various metabolites to be reduced to PC1 and PC2 that showed clear separation between the silks of maize landraces (**Figure 2**). PC1 (33.3%) and PC2 (28.1%) express 61.4% of the total variance of the ^{1}H NMR data set. In the PC1 vs PC2 scores scatter plot, the group formed by *MGPR*, *R29*, and *R41* genotypes (negative PC1) was clearly distinguished from *R8C*, *LP*, and *CV* landraces positioned at positive values of PC1. *RS* sample was discriminated from both groups, positioned at negative values of PC2, suggesting a distinct chemical composition for this variety. Interestingly, these chemometric results point to three distinct metabolic profiles of the silk extracts of maize landraces, which were cultivated in the same geographical area (Southern Brazil) and under similar agronomic traits (agro-ecological ones).

The discrimination between the group formed by *MGPR*, *R29*, and *R41* with respect to the genotypes *R8C*, *LP*, and *CV* occurred

Table 2. ^{1}H and ^{13}C Chemical Shifts and Proton Multiplicity for Assigned Compounds Found in Methanol-HCl 1% Extracts of Silks from Maize Landraces Cultivated in Southern Brazil

compd	δ_{ppm} ^{1}H (multiplicity,[a] assignment)−δ_{ppm} ^{13}C
acetate	**1.92** (s, βCH_3)−23.55
alanine	**1.42** (d, βCH3)−17.52; **3.78** (q, αCH)−52.66
arginine	**2.00** (m, βCH2)−31.72; **3.32** (δCH_2); **4.11** (αCH)−52.87
asparagine	**2.85** (dd, βCH); **4.01** (dd, αCH)−52.46
caffeic acid (3,4-dihydroxycinnamic acid)	**6.43** (d, C8′H); **7.15** (C5′H-ring); **7.17** (C2′H-ring)−113.01; 7.69 (d, C7′H)
citrate	**2.74** (d, α,γCH)−43.55; **2.94** (d, α',γ'CH)
formate	**8.43** (s, HCOOH)
β-fructose	**3.57** (C1H)−62.65; **3.85** (C5H); **4.08** (C4H)−74.55; **3.79** (C6H)
fumaric acid	**6.65** (s, CH)
β-Gal	**4.58** (d, CH1); **3.93** (dd, CH4)
GABA (γ-aminobutyric acid)	**1.82** (q, βCH2); 2.27 (t, αCH2)−33.77; **3.04** (t, γCH2)−41.75
gallic acid (3,4,5-trihydroxybenzoic acid)	**7.07** (s, C2H, C6H-ring)−117.36
glutamine	**2,45** (m, γ, CH2); **3.78** (t, αCH)
glutamic acid	**2.08** (m, βCH, β'CH)−26.74; **2.33** (m, γCH2)−31.50
α-Glc	**3.51** (C2H); 3.86 (C5H)−69.17; **5.22** (d, C1H)−95.20
β-Glc	**3.26** (t, C2H)−77.25; **3.40** (dd, C4H)−71.37; **4.62** (d, C1H)−99.10
glycine	**3.55** (s, αCH2)−44.29
histidine	**7.09** (s, C4H-ring)−118.84; **7.83** (s, C2H-ring)
isoleucine	**0.93** (t, CH3); 1.22 (m, γCH3)−27.21; **1.44** (m, γ'CH); **1.94** (m, βCH)
leucine	**0.97** (d, δ, δ'CH3); 1.73 (m, βCH2); **3.76** (t, αCH)
lysine	**3.76** (t, αCH); 1.85 (m, βCH2); **1.64** (m, δCH2)
malate	**4.29** (dd, αCH)
niacin	**8.81** (dd, C4H, C6H); **9.11** (s, C2H)
ornithine	**1.73** (m, γCH); **1.92** (m, βCH2); **3.03** (t, δCH2)
phenylalanine	**3.06** (βCH); 7.38 (m, C2,C4H-ring; C3, C5H-ring)−131.77; **7.43** (m)
proline	**2.05** (γCH2)−26.74; **2.15**, (m, β'CH), **2.32** (m, βCH2)−26.91; **3.45** (t, δCH)−44.29; **4.13** (t, αCH)−63.69
protocatechuic acid (3,4-dihydroxybenzoic acid)	**6.81** (s, CH4-ring); **7.37** (s, CH6-ring)
pyruvate	**2.38** (s, βCH3)−31.49
serine [(t)]	**3.86** (d, αCH); **3.98** (m, βCH)
succinate	**2.39** (s, α, βCH2)−31.57
sucrose[#]	**3.48** (t, G3H); **3.84** (c, F6H); **3.90** (m, F5H); **4.22** (d, F3H); **5.42** (G1H, d)
syringic acid (4-hydroxy-3,5-dimethoxybenzoic acid)	**7.31** (s, CH5-ring)
t-cinnamic acid	**6.46** (d, CH7); **7.40** (s, CH5-ring)
threonine	**1.33** (γCH3)−22.52; **4.03** (d, αCH); **4.43** (m, βCH)−70.04
tryptophan	**7.11** (t, C5H-ring; **7.28** (t, C6H-ring); **7.52** (C7H-ring)
tyrosine	**6.89** (d, C3, C5H-ring)−118.34; **7.21** (d, C2, C6H-ring)−133.81
uridine[b]	**5.88** (C5H-ring, C1′H); 7.90 (C6H-ring)

[a] s, singlet; d, doublet; dd, doublet of doublets; t, triplet; q, quartet; m, multiplet; c, complex. [b] Tentatively assigned.

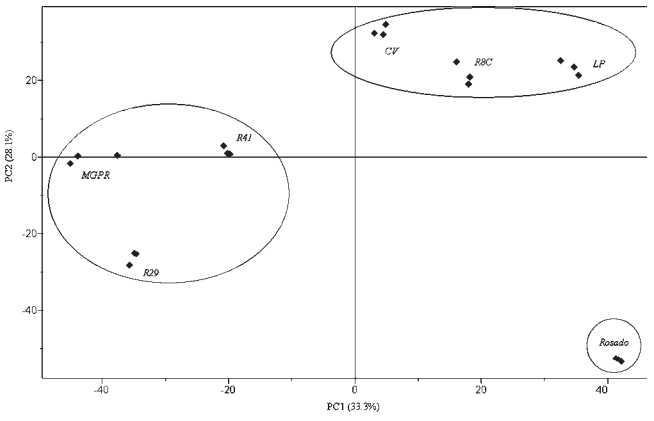

Figure 2. PCA scores scatter plot of the ^{1}H NMR spectra of silk methanolic-HCl (1%) extracts of seven maize landraces from southern Brazil.

predominantly in PC1 while for the *RSI* landrace in PC2. Nevertheless, the loadings plot analysis of PC1 and PC2 revealed that it is not possible to determine the signals responsible for the discrimination of samples once the autoscaling of data attributes the same weight to all variables, making the loading plot interpretation difficult. For this reason, soft independent modeling of class analogy (SIMCA) was performed. In this analysis, the variables with low discriminatory power were determined and deleted from the data set because they contribute only to the noise in the principal component models. Hence, a new PCA analysis was performed only with the variables of high discriminatory power (**Figure 3**). As can be seen in the scores scatter plot of PC1 vs PC2 (65.9% of the total variance), the samples were discriminated again, with the same pattern of classification obtained in the previous PCA analysis (**Figure 4**). The loadings of this second set of PC1 and PC2 values (**Figure 5**) indicated that the signals of carbohydrate and aliphatic/organic acids regions were the most important for the discrimination of samples. Though genetic and environmental effects create significant variation in the amount and quality of metabolites (*24*), probably the variations on the metabolic profile of maize landrace silks herein found reflect genotypic differences because the plants were cultivated using similar agronomic traits in the same geographic area under homogeneous climatic and soil conditions. Further, the dendogram obtained from HCA analysis (**Figure 6**) using a similarity index of 0.595 clearly revealed the existence of three groups of maize landraces. This result is similar to that obtained in PCA and confirms the major similarity between the silk extract of *RS* variety with group formed by *R8C*, *LP*, and *CV* landraces.

UV−Vis Spectrophotometry and HPLC. The determination of total anthocyanin and polyphenol contents confirmed the results

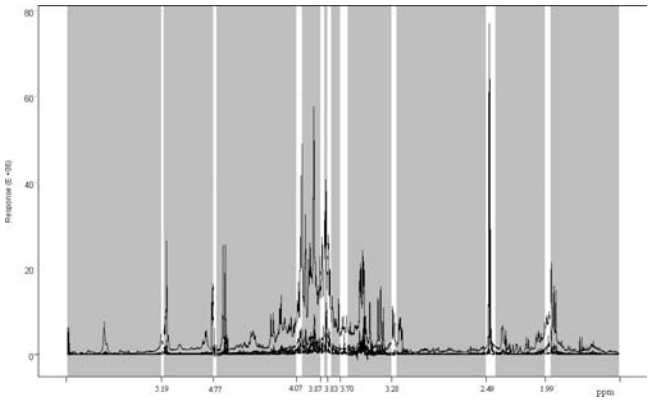

Figure 3. SIMCA analysis showing the ^{1}H chemical shifts with high discriminatory power (in white).

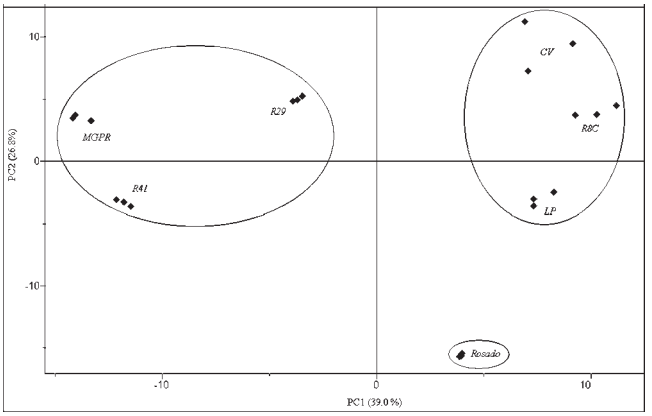

Figure 4. Classification plot of maize landraces silks for ^{1}H NMR after SIMCA analysis.

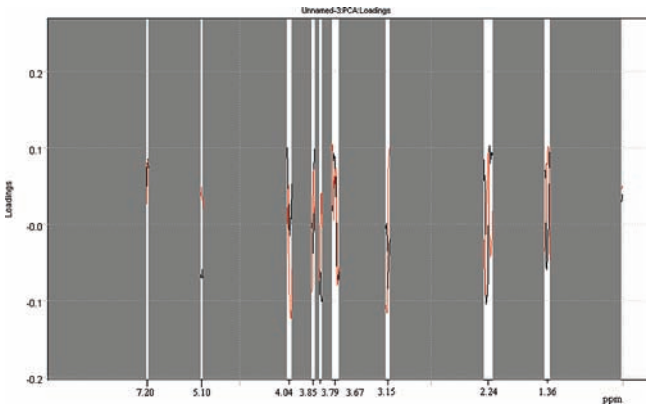

Figure 5. Loading plot after SIMCA analysis. A PC1 loading is represented by black line and PC2 loadings by red one.

of ^{1}H NMR spectroscopy and PCA. The silk extracts of maize landraces cultivated in the same geographical area and with similar agro-ecological traits are not chemically homogeneous. Anthocyanins are molecules with biological actions well established in the literature (*25*, *26*). Anthocyanins show peaks in the visible spectral range between 475 and 550 nm as anthocyanidins have maximum absorbances between 520 and 542 nm in MeOH-HCl solution (*27*). Spectral properties are especially useful for identifying the anthocyanidin type. Ring B functional groups may have some influence on the wavelength of maximum absorption in the visible so that the anthocyanins that possess two functional

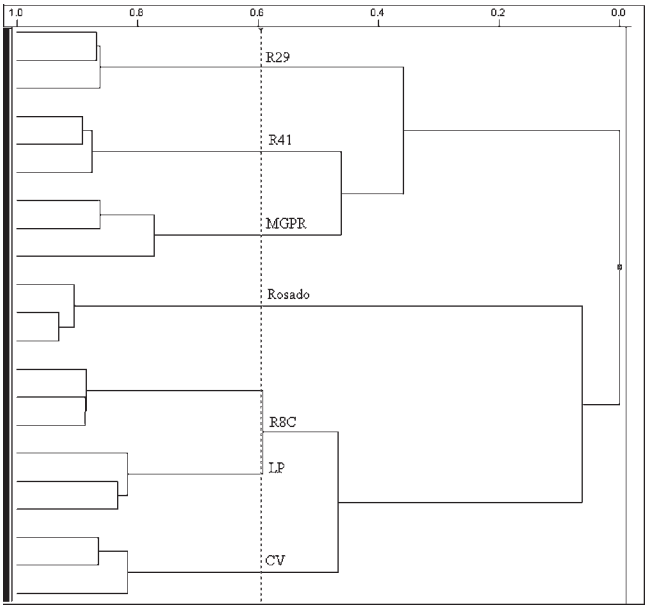

Figure 6. Hierarchical cluster analysis (HCA) based on the ^{1}H NMR spectral data of methanolic extracts of maize silk from seven landraces cultured in southern Brazil.

groups in that cycle (cyanidin and peonidin) have absorption maxima 11 nm lower than those with three functional groups (delphinidin, petunidin, and malvidin) (*28*). The highest content of total anthocyanins determined at 460 nm and 525 nm were recorded in the silk of *R41* genotype, followed by *R8C*, and *RS*, while lower levels were detected in *CV*, *MGPR*, and *RV* landraces (**Table 3**), the latter genotype showing ca. of 3.0 times less anthocyanins (460 ηm). Glycosidic substitution pattern of the anthocyanins can be inferred by absorption in the 400−460 nm region since the 3-glycosides exhibit ratios of $E_{440}/E_{\lambda max}$ about 2 times greater than those for 3,5-diglycosides (*27*). Thus, the *RS* sample seems to have less content of 3-glycosides as compared to other varieties. These findings corroborate the NMR results showing higher aromatic resonances intensity for *R41* and *RS* genotypes and lower aromatic peak intensity for *CV* and *MGPR* ones.

Previous studies detected in maize tissues (seeds, leaves, silks, and roots) identified the common 3-hydroxyanthocyanins cyanidin 3-glucoside, cyanidin 3-galactoside, pelargonidin 3-glucoside, peonidin 3-glucoside, cyanidin 3-(6″-malonylglucoside), cyanidin 3-(3″,6″-dimalonylglucoside), peonidin 3-(6″-malonylglucoside), peonidin 3-(dimalonylglucoside), and the rare 3-deoxyanthocyanins apigeninidin and luteolinidin (*25*, *26*, *29*). Preliminary studies of our research group using MALDI-TOF mass spectrometry revealed distinct metabolic profiles confirming the presence of cyanidin 3-glucoside, pelargonidin 3-glucoside, peonidin 3-glucoside, peonidin 3-(6″-malonylglucoside), apigeninidin, and luteolinidin in the silk and leaf methanolic extracts of the maize landraces in this study (unpublished data). Thus, an anthocyanin composition distinct for the *RS* genotype could be explained in the discrimination found in PCA analysis. Such a finding has a straight implication related to the use of plant material as raw material by traditional medicine as well as on the composition of the tissues that cover the seeds, i.e., the pericarp, for example. Such a tissue has origin from the ovary wall so that it is a motherly tissue, not depending on the fecundation. The pericarp and aleurone tissues from maize seeds can present different colorations such as white, yellow, red, purple, and variegated, indicating their distinct chemical composition. Besides, this phenotypic trait is observed among the maize landraces from southern Brazil and

Table 3. Anthocyanin[a] and Polyphenol Contents (mg/100g) (mean ± SEM) Determined by UV−Vis (460 nm and 525 nm) and RP-HPLC−UV−Vis, Respectively, of the Organosolvent Extract (Methanol-HCl 1%) of Silks from Maize Landraces Cultivated in Anchieta County (Southern Brazil, Santa Catarina State)

varieties	anthocyanins[b] (460 nm)	anthocyanins[b] (525 nm)	protocatechuic acid	gallic acid	*t*-cinnamic acid	syringic acid	caffeic acid	total polyphenols
CV	10.95 ± 1.53	17.71 ± 2.95	151.11 ± 4.25	38.74 ± 0.53	227.23 ± 3.35	2.88 ± 0.11	10.18 ± 0.03	430.16
LP	16.11 ± 3.62	18.03 ± 4.49	183.62 ± 0.33	410.78 ± 1.16	71.34 ± 8.21	2.11 ± 0.11	12.01 ± 0.30	679.86
MGPR	14.16 ± 2.31	16.43 ± 2.69	99.70 ± 3.24	18.59 ± 2.46	136.30 ± 5.37	nd[c]	13.29 ± 2.90	268.03
R8C	19.91 ± 3.41	16.97 ± 3.57	185.32 ± 4.14	275.10 ± 70.99	138.82 ± 4.36	nd	7.43 ± 2.50	606.69
RS	8.29 ± 3.37	27.07 ± 5.53	104.49 ± 1.37	43.37 ± 0.68	55.82 ± 2.28	1.04 ± 0.02	22.34 ± 0.19	227.06
R29	14.69 ± 2.09	15.94 ± 2.52	203.09 ± 6.16	68.78 ± 6.80	126.06 ± 9.77	0.65 ± 0.01	16.29 ± 3.08	414.87
R41	18.63 ± 2.77	19.58 ± 3.02	255.88 ± 13.26	96.87 ± 0.44	137.21 ± 6.54	3.71 ± 0.43	7.20 ± 0.40	500.87

[a] Expressed as mg cyanidin-3-glucoside/100g dry plant material. [b] All values are presented as mean of three extractions and three consecutive injections/sample. [c] nd = not detected.

could be of commercial importance in the use of the maize as functional food.

Previous studies have attributed some of the therapeutic properties of the silk extracts to the presence of polyphenolic acids and their antioxidant ability (*1*, *30*). In this work, protocatechuic acid, gallic acid, and *t*-cinnamic acid were the major phenolic acids detected in the silk extracts using the HPLC method (**Table 3**). Small amounts of caffeic acid and syringic acid were also detected in some samples. The highest contents of total polyphenols were found in *LP* and *R8C* landraces, with minor amounts detected in the *RS* and *MGPR* genotypes (**Table 3**). However, the distribution pattern of polyphenolic acids in the silks differed among the genotypes analyzed. The *R8C* and *LP* varieties discriminated in PCA analysis showed higher levels of gallic acid as compared to protocatechuic acid. On the other hand, the protocatechuic acid contents were higher compared to gallic acid in the *R41*, *R29*, and *MGPR* genotypes in accordance with results of chemometric analysis. These discrepancies on the chemical composition of the silk extracts are thought to be related to the distinct pharmacological potential for the Brazilian landraces as shown by other authors (*1*). In fact, the antioxidant activity and contents of various polyphenolic acids in the maize silks originated from Serbia showed a positive linear correlation (*1*).

In conclusion, the ^{1}H NMR and PCA methods allowed the discrimination of silk extracts based on metabolomic profiles, suggesting their use in the quick screening of genotypes with special pharmacological properties. This approach also can be applied to verify the quality and consistency of raw material for production of herbal drugs. In addition, the maize silk is considered a byproduct in the process of maize grain production, being inexpensive and readily available. The use of maize silks as raw material for the pharmaceutical industry could add value and stimulates the small farmers of Anchieta County in the maize germplasm conservation.

ABBREVIATIONS USED

CEUA, care and ethical use of animals on research committee; PCA, principal component analysis; SIMCA, soft independent modeling of class analogy; HCA, hierarchical cluster analysis; Maize landraces are encoded as *LP* (Língua de Papagaio), *R29* (Roxo 29), *R41* (Roxo 41), *MGPR* (Mato Grosso Palha Roxa), *RS* (Rosado), *R8C* (Rajado 8 Carreiras), and *CV* (Cateto Vermelho).

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