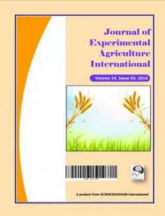

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Proteomic Analysis of Natural and Demucilaged Coffee Beans from Plantations at Different Altitudes in the Mantiqueira Mountains

Kalynka G. do Livramento[1*], Flávio M. Borém[1,2], Luana F. Torres[1], Fabrício L. da Silva[1], Dárlan Einstein do Livramento[3] and Luciano V. Paiva[1,4]

[1]Federal University of Lavras (UFLA), Lavras, Minas Gerais, Brazil.
[2]Department of Engineering, Federal University of Lavras (UFLA), Brazil.
[3]State University of Passos (UEMG), Passos, Minas Gerais, Brazil.
[4]Department of Chemistry, Federal University of Lavras (UFLA), Brazil.

Authors' contributions

This work was carried out in collaboration between all authors. Authors KGL, LFT and DEL designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors FMB and LVP managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Analysis of the effects of climatic conditions and processing methods on coffee beans is of great interest in research, chemical composition and consequent beverage quality are strongly influenced by growing conditions and/or processing methods.
Aims: In this study, beans of *Coffea arabica* cv. Acaiá coffee trees grown at two altitudes (below 1000 m and above 1200 m) were assessed through a proteomic approach in an attempt to better understand the protein changes that occur during two types of processing (dry and wet processing).

**Corresponding author: E-mail: kalynkagabriella@yahoo.com.br;*

Methodology: Samples of proteins of natural and demucilaged coffee bean were analyzed by two-dimensional electrophoresis. The gels stained with Commassie Blue G250 were evaluated by the ImageMaster 2D Platinum 7.0 program. The differentially abundant proteins were treated with the enzyme Trypsin e aliquots of each hydrolysate sample were analyzed by mass spectrometry. Sample molecular mass profile obtained through MS e MS/MS was subjected to comparative analysis using the MASCOT program, Brazilian Coffee Genome database, NCBI and Coffee Genome Hub.
Place and Duration of Study: The experiments were conducted in the following laboratories: the *Laboratório Central de Biologia Molecular (LCBM)* and the *laboratório de Processamento de Produtos Agricolas (LPPA)* at Federal University of Lavras, (Brazil), between 2014 and 2017.
Results: Differentially accumulated proteins, such as 11S globulin, glyceraldehyde-3-phosphate dehydrogenase, dehydrin, and *Heat shock* proteins, have been found among treatments through mass spectrometry.
Conclusion: These results contribute to an understanding of the effects of altitude and processing methods on the chemical composition of coffee beans, and thus serve as a basis for future research related to coffee beverage quality.

Keywords: 2-DE; coffee protein content; coffee beverage quality; coffee processing; effects of altitude; protein analysis; proteome.

1. INTRODUCTION

Considered as one of the world's most popular beverages, coffee is the second most important commodity after oil. Two commercially important species of the *Coffea* genus belonging to the Rubiaceae family, namely, *C. canephora* and *C. arabica* P. L., represent about 30% and 70% of world production, respectively. In 2015/16, world agribusiness export values for coffee reached around 20 billion dollars, with production of approximately 145 million bags (of 60 kg) and creation of jobs in rural areas in all 48 producing countries [1].

Although *Coffea arabica* cultivars are highly vulnerable to climatic conditions [2], their beans usually provide superior taste and produce high quality beverages.

Regarding climate conditions, it is empirically known that high altitude has beneficial effects on coffee quality [3].

Low temperatures normally delay the ripening process, which leads to a higher accumulation of flavour precursors [4]. It is generally acknowledged that high altitudes improve coffee quality [5,6]. The ideal annual average temperature range for coffee production is 19-21℃ [7], although there are some places, such as the Northeast of Brazil, with higher temperatures (24-25℃) that produce beans with satisfactory quality [8].

Coffee plantations have been undergoing the effects of global warming [9,10]. According to recent studies, these effects will intensify as temperatures will rise 1.8-4℃ by the end of the twenty-first century [11]. An understanding of the relationship between tolerance mechanisms and abiotic stress (i.e., temperature and rainfall) is essential in the search for biotechnological strategies that aim to elucidate the effect of these factors on "cup" quality.

Furthermore, post-harvest management can also affect beverage quality as the beans are subjected to many physical, biochemical, and physiological changes during drying and processing [12]. Although these changes have not been well explained, they result in important differences in flavour and aroma precursors that may impart particular characteristics to each beverage [13].

Better understanding of coffee chemical composition and its relationship to bean metabolic conditions should be taken into account in analyzing the importance of altitude and post-harvest treatments on rendering flavour.

As for chemical composition, little information is found in the literature on protein content in coffee beans. The proteins Globulin 11S, glyceraldehyde-3-phosphate dehydrogenase, LEA Dehydrin, UTP-glucose-1-phosphate uridyltransferase, Heat Shock Protein, Trichome birefringence like protein, alpha-galactosidase and a homologous protein to putative rMLC Termites like superfamily protein were identified in dry grain in the yard and dryer at 60℃, with the significant difference in the abundance [14].

Therefore, studying protein composition would help elucidate metabolic processes associated with the different phenomena that occur in coffee beans during their development and from post-harvest mechanisms. This may lead to identification of biomarkers, which might be useful in evaluating the quality of *Coffea arabica* beans and, consequently, beverage quality.

Thus, the main objective of this study was to analyze the proteome of coffee beans by using a combination of two-dimensional electrophoresis (2-DE) and mass spectrometry (MS) technologies to analyze proteins which are differentially accumulated in beans collected from growing areas in two different altitude ranges and subjected to the dry or wet processing method.

2. MATERIALS AND METHODS

2.1 Sources of Coffee Fruits

Acaiá (*Coffea arabica* L.) samples were collected during the 2009/10 coffee harvest from commercial plantations located in the municipality of Carmo de Minas (-22°6', 45°8'), Minas Gerais, in southeastern Brazil. This region is in the Mantiqueira Mountain Range and is known for the production of specialty coffees (Fig. 1).

The experimental design was based on the investigation of the interaction between environmental and processing variables.

The coffee field environment was stratified into two altitude categories (more less or equal to 1,000 m and more than or equal to 1,200 m), resulting in two environmental variable combinations. Red fruits of Acaiá were collected from each environment. Three repetitions were collected and processed using either the dry or the wet method, for all combinations involving environment. For each repetition, 132Ibs of coffee fruits were harvested.

Harvesting was carried out manually and selectively. After that, the fruits were separated according to differences in density and only denser beans were used. Approximately 22Ibs of selected fruits were laid on screens for drying in the sun, thus obtaining natural coffee samples through the dry method. The demucilaged coffee samples were obtained through the wet method: the pulp was eliminated by a pulper, followed by mechanic removal of mucilage from the parchment. Finally, about 22Ibs of demucilaged coffee beans were set on screens to be dried in the sun. Drying started immediately after the processing. Harvesting, processing and drying procedures were performed according to [12].

Fig. 1. Altitude, climate and soil favorable benefit the production of specialty coffee in the Serra da Mantiqueira in Minas Gerais state, which have quality recognized in international competitions

Subsequently, all defective beans were removed in order to establish uniformity and mainly to minimize interferences not related to genetic material, processing, or the cultivation environment.

In previous studies [15], some of these samples underwent sensory analysis using the methodology proposed by the Specialty Coffee Association of America - SCAA [16]. Samples originating from higher elevations (>1200 m) had higher beverage preference scores, with higher sensory complexity, than samples from lower elevations (<1000 m) [15].

However, all samples used in this study were "specialty coffee" and their beverages received a final grade equal to or greater than 80 points according to the rating scale suggested by the SCAA protocol.

Comparative analyses were set up as follows:

- Analysis 01 - below 1000 m altitude - natural beans X demucilaged beans.
- Analysis 02 - above 1200 m altitude - natural beans X demucilaged beans.
- Analysis 03 - demucilaged beans - below 1000 m altitude X above 1200 m altitude.

2.2 Total Protein Extraction

In order to perform analyses, coffee beans were frozen in liquid N_2 and pooled before storage at -80℃. Proteins were then extracted according to [17], only with a few modifications. A portion (250-300 mg) of the powered samples was homogenized in 660 µl lysis buffer [7 M urea (GE-Healthcare), 2 M thiourea (GE-Healthcare), 14 mM Tris base (GE-Healthcare), 100 µl proteases inhibitor cocktail (GE-Healthcare), 12 DNase I (1 mg/ml) units, 20 µl RNase A (1 mg/ml), 0.6 µl Triton X-100, 60 mM 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) (GE-Healthcare) and 17.5 mM Dithiothreitol (DTT) (GE-Healthcare)]. The mixture was homogenized in a vortex and then kept in ice for 15 minutes. Then the tubes were centrifuged at 14,000 rpm for 10 minutes at 4℃. The supernatant containing total proteins was centrifuged once more under the aforementioned conditions and then stored in 100 µl aliquots at -20℃.

The protein concentration of various extracts was quantified by means of optical density according to the Bradford method [18] by applying bovine serum albumin (BSA) as a standard, through a Gene Quant spectrophotometer (Amersham Healthcare).

2.3 Two-dimensional Electrophoresis

After checking their integrity, proteins were separated through isoelectric focusing (IEF). This was performed through 18 cm dehydrated polyacrylamide strips with an immobilized pH gradient from 3 to 10 (Immobiline Drystrips – GE-Healthcare) to which 250 µg of total protein was applied. After IEF, each strip was equilibrated with DTT and iodoacetamide. Two dimensional sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS-PAGE) was performed in 12.5% polyacrylamide gels at 600 V, 20 mA, 30 W for 35 minutes and then at 600 V, 50 mA, 30 W for 1.5 h. At least three replicate gels were run for each sample [19].

2.4 Protein Staining and Image Analysis

The two-dimensional gels were stained with Coomassie blue G250 for 48 h and stored in a 250 ml solution containing 50 g of ammonium sulfate. The colored two-dimensional gels were scanned at high resolution (ImageScanner, GE-Healthcare) with the aid of the software Ulmax MagicScan 4.6. Image analysis was performed with the program ImageMaster 2D Platinum 7.0 (GE-Healthcare). In order to evaluate differences in protein abundance across distinct 2-DE gels, the normalized protein spot volumes were used as a parameter (spot area multiplied by its intensity, measured by optical density). The relative spot volume (%Vol) represents the ratio of a given spot volume (Vol_s) to the sum of all spot volumes detected in the gel with “n” spots ($\%Vol = \frac{Vol}{\sum_{S=1}^{n}(Vol_S)} x\ 100$). Experimental molecular mass was calculated from digitized 2-D electrophoresis images using standard molecular mass marker protein.

2.5 Trypsin Digestion and Mass Spectrometry Analysis

Following electrophoresis, protein spots were excised individually from the gels, dehydrated with 200 µl acetonitrile, dried in a SpeedVac, and finally treated with the enzyme Trypsin Gold (Promega®) at 58℃ for 30 minutes. Aliquots of each hydrolysate sample were analyzed by mass spectrometry.

40 µL of the samples were injected (SIL-20A HT) in an HPLC system composed of two pumps

(LC-20AD), a column oven (CTO-20A), and a degasser (DGU-20A_3) (Shimadzu, Japan). The column was a C-18 150×2.1 mm, 2.7 µm, with pre-column (Supelco, Pennsylvania, USA). The mobile phases were 0.1% formic acid solutions in water (A) and in acetonitrile (LC-MS grade - Fluka, Buchs, Switzerland), and the elution was carried out at a 200 $\mu L.min^{-1}$ flow, as follows: 0–5 min, 5% B isocratic, 5–60 min, linear gradient to 40% B, 60–70 min, linear gradient to 50% B, 70–75 min, linear gradient to 90% B, 75–80 min, isocratic to 90% B, 80–80.1 min, alteration for 5% B, 80.1–90 min, isocratic 5% B. Data obtained through LC–MS/MS were treated with Bruker Data Analysis software, version 4.0 (Build 253). The MS/MS spectrum obtained during the 5-30 min interval was used for a search in the database by Biotools Version 3.2 software (Bruker Daltonik).

Sample molecular mass profile obtained through MS e MS/MS was subjected to comparative analysis using the MASCOT program (http://www.matrixscience.com) so as to conduct mass comparison of those peptides observed through the database, as well as the most probable protein identification. The databases used in the analysis were from the Brazilian Coffee Genome database [20], NCBI (http://www.ncbi.nlm.nih.gov/) and Coffee Genome Hub [21].

3. RESULTS

Theses results were the first to show a significant influence of processing method and altitude on the proteomic profile of cv. Acaiá coffee beans.

The protein profile of coffee beans under different aspects was evaluated in this study. The first analysis consisted of a comparative evaluation regarding the proteome of natural and demucilaged coffee beans from plants cultivated in regions with an altitude below 1000 m. During this analysis, two-dimensional gels were evaluated through the program ImageMaster 2D Platinum 7.0 (GE-Healthcare). As a result, approximately 240 protein points were detected from which 10 spots were selected (no. 96, 106, 177, 221, 250, 251, 254, 262, 264, 268) with more than 1.5-fold differential accumulation in three independent gels.

All spots were treated with the enzyme trypsin and injected in the mass spectrometer. Then the resulting peptides were compared with the databank through the MASCOT program. Only four spots were successfully identified based on the tryptic peptide sequence: three 11S globulin isoforms, glyceraldehyde-3-phosphatedehydrogenase, and DHb1dehydrin (Table 1, Fig. 2).

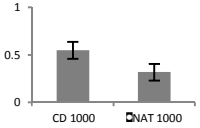
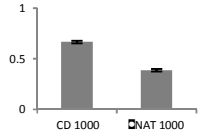
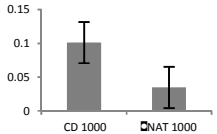
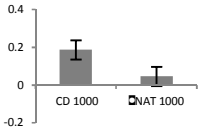
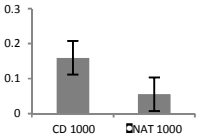
The second comparative analysis concerning proteomic profiles involved natural and demucilaged coffee beans from plants cultivated in the highlands of the Serra da Mantiqueira at an altitude above 1200 m. After evaluating two-dimensional gels dyed with Comassie Blue G-250, it was possible to visualize approximately 240 protein points among gels. These protein points enabled a comparative assessment made by the ImageMaster program, revealing the presence of six accumulated proteins (no. 36, 42, 73, 77, 81, 82), which were excised from gel and analyzed.

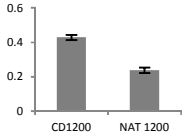
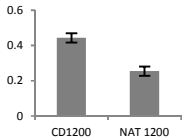
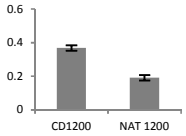
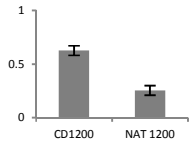
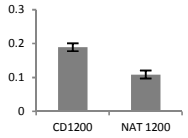
Peptides that were analyzed by spectrometry with ionization by electrospray (ESI-MS/MS) produced enough spectra to be compared to the databases. Some of the protein points exhibited homology to the following proteins: two 11S globulin isoforms, two DHb1 dehydrin protein isoforms, and the molecular chaperone Heat Shock Protein (Table 1, Fig. 3).

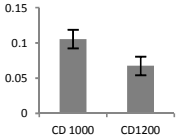
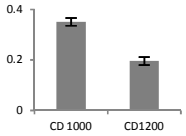
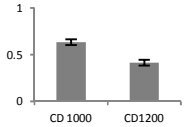
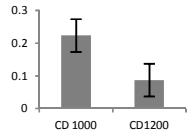
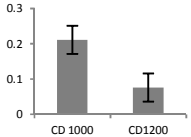
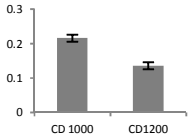
A third comparative analysis was performed relating coffee bean content to the growing site. In this analysis, total proteins were extracted from demucilaged beans of growing areas located in regions below 1000 m and above 1200 m. Two-dimensional gels were dyed with G250 Comassie Blue and their image was digitalized through high resolution scanner.

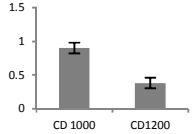
After image analysis, about 250 protein points were clearly visualized, which were then statistically evaluated by the T-test ($P = .05$) for relative volume differential detection. Under these conditions, 18 protein points (no. 13, 29, 33, 38, 55, 57, 67, 84, 93, 107, 109, 110, 116, 117, 121, 130, 135, and 136) identified with a 1.5-fold differential expression. All protein points were excised and digested with trypsin for peptide production that was ionized in a mass spectrometer. The spectra generated were compared with the databases, seeking homology to previously described proteins. Various proteins were found, including seven isoforms of storage 11S globulin and two non-characterized proteins (Table 1 and Fig. 4).

Table 1. Differentially accumulated proteins identified by mass spectrometry analysis of *Coffea arabica* L. cv. Acaiá beans

Spot number	Relative volume [c]	Correspondent protein	Species	Score[a]	Acess number	Sequence ID Peptide	Exp/ThMr[b]
Analysis 01 - Comparative Analysis regarding proteomic profile of desmucilated and natural coffee beans from regions below 1000 m.							
96		DH1b Dehydrin	*Coffea canephora*	70	Q1A523	K.SQYDEYGNPVR.Q	1326.58/1326.58
177		glyceraldehyde-3-phosphate dehydrogenase	*Coffea arabica*	50	J7LF18	R.SSIFDAK.A K.VLPALNGK.L R.AASFNIIPSSTGAAK.A	766.39/766.38 810.50/810.49 1433.75/1433.75
221		11S Globulin	*Coffea arábica*	29	Q9ZNY2	K.TNDNAMINPLVGR.L	1429.69/1429.69
251		11S Globulin	*Coffea arábica*	145	O82437	R.AIPEEVLR.S K.AGNQGFEYVAFK.T K.TNDNAMINPLVGR.L R.QEALLLSEQSQQGK.R	925.53/925.52 1329.63/1329.63 1413.70/1413.70 1557.80/1557.79
254		11S Globulin	*Coffea arabica*	72	P93079	R.AIPEEVLR.S K.AGNEGFEYVAFK.T K.TNDNAMINPLVGR.L	925.527/925.523 1330.61/1330.61 1429.69/1429.69

Spot number	Relative volume [c]	Correspondent protein	Species	Score[a]	Acess number	Sequence ID Peptide	Exp/ThMr[b]
Analysis 02	Comparative analysis regarding proteomic profile of desmucilated and natural coffee beans from regions above 1200 m.						
36		DH1b Dehydrin	*Coffea canephora*	70	Q1A523	K.SQYDEYGNPVR.Q	1326.58/1326.58
42		11S Globulin	*Coffea arabica*	51	Q9SAN3	K.LNAQEPSFR.F	1060.52/1060.53
73		DH1b Dehydrin	*Coffea canephora*	50	Q1A523	K.SQYDEYGNPVR.Q	1326.58/1326.58
81		11S Globulin	*Coffea arabica*	184	P93079	R.AIPEEVLR.S K.AGNEGFEYVAFK.T K.TNDNAMINPLVGR.L	925.527/925.523 1330.61/1330.61 1429.69/1429.69
82		*Heat shock* protein fragment	*Coffea arabica*	19	Q9AT71	R.FRLPENAK.M	973.56/973.53

Spot number	Relative volume [c]	Correspondent protein	Species	Score[a]	Acess number	Sequence ID Peptide	Exp/ThMr[b]
Analysis 03 - Comparative analysis regarding proteomic profile of desmucilated coffee beans from regions below 1000m and above 1200 m.							
29		11S Globulin	*Coffea arabica*	17	Q9SAN3	K.FFLAGNPQQGGGK.R	1060.52/1060.53
38		11S Globulin	*Coffea arabica*	90	O82437	R.AIPEEVLR.S K.AGNQGFEYVAFK.T K.TNDNAMINPLVGR.L R.QEALLLSEQSQQGK.R	925.53/925.52 1329.63/1329.63 1413.70/1413.70 1557.80/1557.79
107		11S Globulin	*Coffea arabica*	116	P93079	R.AIPEEVLR.S K.LNAQEPSFR.F R.SNGNEGFEYVAFK.T K.AGNEGFEYVAFK.T K.TNDNAMINPLVGR.L	925.527/925.523 1060.53/106053 1349.64/1349.64 1330.61/1330.61 1429.69/1429.69
110		11S Globulin	*Coffea arabica*	204	P93079	R.AIPEEVLR.S K.AGNEGFEYVAFK.T K.TNDNAMINPLVGR.L R.QEALLLSEQSQQGK.R R.SSFQISSEEAEELK.R K.LSENIGLPQEADVFNPR.A	925.53/925.52 1330.62/1330.61 1429.70/1429.69 1557.80/1557.79 1557.80/1557.79 1897.95/1897.95
116		11S Globulin	*Coffea arabica*	107	P93079	R.AIPEEVLR.S K.AGNEGFEYVAFK.T K.TNDNAMINPLVGR.L	925.53/925.52 1330.62/1330.61 1429.70/1429.69
130		Hypothetic Protein	*Vitis vinifera*	50	gi|225424256|	K.SIEVLEGDGK.S	1045.53/1045.52

Spot number	Relative volume [c]	Correspondent protein	Species	Score[a]	Acess number	Sequence ID Peptide	Exp/ThMr[b]
135		11S Globulin	*Coffea arabica*	669	O82437	K.VFDDEVK.Q R.AIPEEVLR.S K.AGNQGFEYVAFK.T K.TNDNAMINPLVGR.L R.QEALLLSEQSQQGK.R	850.38/850.40 925.53/925.52 1329.63/1329.63 1413.70/1413.70 1557.80/1557.79

a Mascot score for the most significant hits.
b Theoretic (Th) and experimental (Exp) molecular weight (Mr) of identified peptides
c Natural (NAT) and Desmucilated (CD) coffee beans

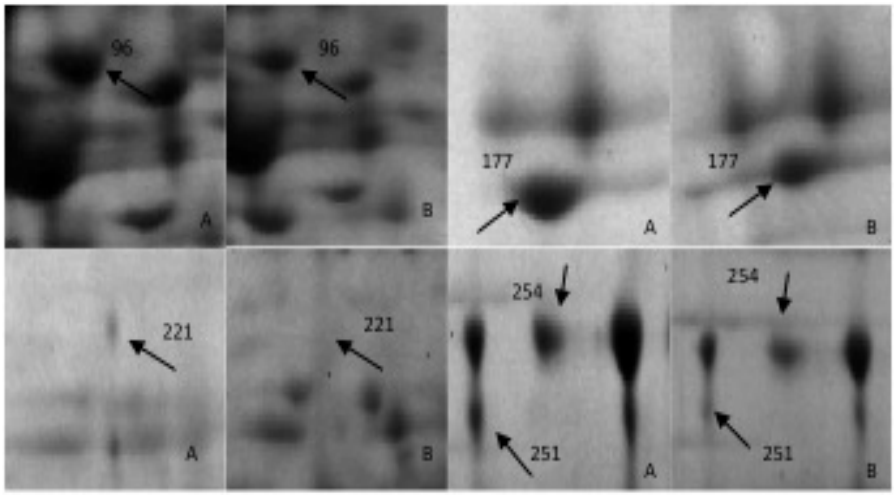


Fig. 2. Detail of differentially accumulated protein in desmucilated (A) and natural (B) coffee beans cultivated in the region below 1000 m

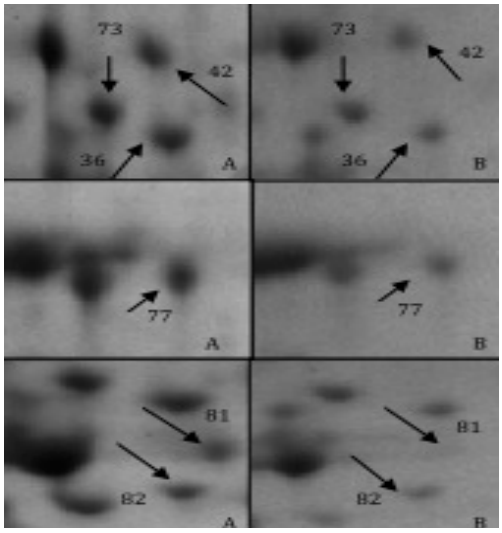


Fig. 3. Detail of protein points differentially accumulated in desmucilated (A) and natural coffee (B) beans from regions above 1200 m

The remaining protein points did not show homology with database peptides nor did they produce enough spectra to make comparisons.

4. DISCUSSION

In this study, the first comparative proteome evaluation was performed on both natural and demucilaged coffee beans from plantations located below 1000 m. The protein glyceraldehyde 3-phosphate dehydrogenase (GAPDH) displayed greater concentrations under the proteomic profile of demucilaged beans.

Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is involved in carbon and energy metabolism and, according to current proteomic literature, the presence of GAPDH has been observed in both greater and lesser abundance.

The protein profile of soil beans subjected to saline stress exhibited reduction of many glycolytic enzymes, including GAPDH, compared to the profile of untreated beans [22].

The increase in GAPDH expression was reported under different conditions when plants were

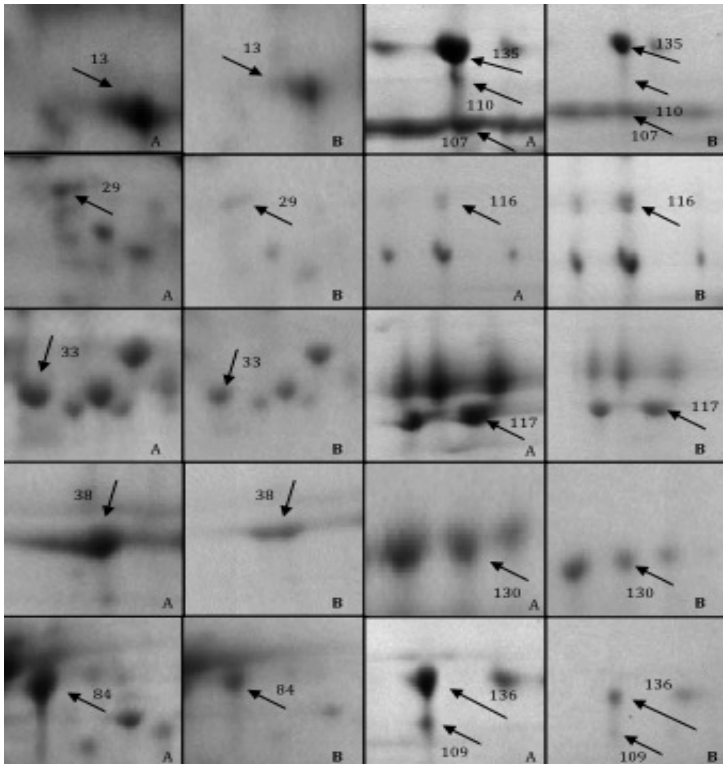


Fig. 4. Detail of differentially accumulated protein points in desmucilated coffee beans from cultivations located below 1000 m (A) and above 1200 m (B)

subjected to stress, and it was speculated that GAPDHs play roles in plant tolerance to abiotic stress [23].

In the proteome maps of seedling leaves from plants under PEG6000-mediated drought stress, the glyceraldehyde 3-phosphate dehydrogenase isoenzyme (GAPDH) was dramatically upregulated in drought-tolerant wheat (NC47) and drought-sensitive 'Chinese Spring' (CS) wheat (*Triticum aestivum* L.) cultivars [24]. Vine treatment with flumioxazin herbicide directly affected the proteomic profile through oxygen impairment, and thus drastically decreased photosynthesis proteins and increased GAPDH – known as anaerobic protein [25].

The higher GAPDH concentration in demucilaged beans might be linked to different stress during wet processing, which stimulates cells to produce more energy for the maintenance of cellular homeostasis.

The endosperm is a tissue characterized by high accumulation of storage compounds. Actually, in this study, various 11S globulin protein isoforms could be identified by the three comparative assessments of the proteomic profile.

Isoforms of the storage globulin 11S, highly present in the coffee bean endosperm, constitute about 45% of the total number of proteins [26]. Two-dimensional electrophoresis was employed to elucidate the presence of this protein, which was about 55kDa, as verified by the subunits (β arms – 20kDa, and α arms – 32kDa). Peptides were also found in numbers of 12 to 16 kDa probably as degradation products produced by protease activity. Furthermore, 23 variations were observed after sequence alignment of storage proteins was conducted with other proteins as codified by two 11S globulin cDNA in *Coffea arabica*. Differences such as gap and nucleotide addition, as well as amino acid changes, may help explain the presence of isoforms [27].

The protein 11S globulin is the main source of the peptides and amino acids in the coffee bean endosperm. This protein build-up occurs during seed development, with the peak of abundance being reached at the half-way point of fruit

maturation, which corresponds to maximum expansion of the bean endosperm [27].

Studies have shown that sucrose accumulation occurs in coffee beans at the end of the maturation period [28], thus, in a group with a large number of globulin proteins and free amino acids, it can be inferred that such a condition may be an important factor in generating beverage flavour and aroma. Throughout the roasting process of coffee beans, many of the compounds generated favor the aroma and flavour of the beverage, and these compounds are derived from the Maillard reaction, which takes place between reducing sugars and free amino acids.

Dehydrin proteins were selected from two-dimensional gels of demucilaged beans either in the first or the second comparative evaluation.

Dehydrins consist of LEA type II proteins that are highly induced under specific stress conditions, such as hydric deficit and salinity, which provides relative tolerance to cellular dehydration, thus minimizing negative oxidative processes. Their build-up under these conditions has frequently been reported [29-31].

Dehydrins usually act synergically with glucose in formation of the cytoplasmic matrix state, thus guaranteeing cell stability in desiccation tolerant organisms [32]. Taking the action of both sucrose and dehydrins into account, it can be inferred that the high level of dehydrin and higher sucrose concentration in coffee beans may contribute to the cell membrane and consequent preservation of chemical compounds that are precursors of the unique beverage flavour and aroma of demucilaged beans.

When performing proteome analysis of natural and demucilaged coffee beans from growing areas located above 1200 m, a significant increase in the low molecular weight fragment of Heat Shock proteins (HSPs) in respect to demucilaged beans was observed.

HSPs are proteins generally involved in cell protection and repair, as well as degradation of harmful components, especially during abiotic stress [33]. HSPs are synthesized and accumulated in highly dehydrated tissues and assist in stabilization of the protein tertiary structure [34]. HSP synthesis increases when the proteins are not properly formed (incorrect protein folding) and the amount of ATP is limited [35].

An increasing number of studies suggest that HSPs interact with other stress response mechanisms, hence acting synergistically to prevent cell injuries and to re-establish cellular homeostasis [36].

Recent studies have reported the presence of HSPs in protein profiles of different species when subjected to different types of stress. For example, two-dimensional gels have revealed five distinct HSP sub-families (classified according to their molecular weight: HSP70, HSP60, HSP90, Hsp100, and small HSPs) in a larger number of citrus fruits subjected to storage at low temperature in relation to those stored at room temperature [37]. In contrast, an increase in HSPs was systematically observed in different studies on rice leaf, *Carissa spinarum*, and *Raphanus sativus L.* when these crops were subjected to high temperature conditions or drought stress [38-40]. Moreover, HSPs were observed in larger quantities in ripe banana fruits after comparison of proteomes in two physiological phases [41].

In this study, the increase in HSPs and dehydrin protein in demucilaged beans may be related to wet processing itself, which involves various phases and, consequently, some kind of coffee bean cell stress, thus generating different physiological responses compared to natural beans. Future studies must be carried out in order to determine how much this protein activity may physiologically affect the beans and alter coffee beverage flavour and aroma.

5. CONCLUSIONS

For the first time, results have shown that environmental conditions formed by different altitudes had a significant impact on different proteins during development and processing of coffee beans, thus substantially altering them.

This study prepares the way for overall analysis of the general system of coffee beverage quality. Integration of data generated from transcriptomics, metabolomics and other studies related to large scale "-omics" may open new routes in understanding the changes occurring inside coffee beans before, during, and after harvest and thus avoid inadequate management practices that might jeopardize coffee bean yield and quality.

Future studies must be carried out in order to determine how much this protein activity may

physiologically affect the beans and alter coffee beverage flavour and aroma.

Moreover, these findings will be applied as a background for future studies related to coffee metabolism in the face of climatic changes since the functional genomic study may provide a basis for relating organisms and their response to stress. Finally, the proteome approach has proven to be a very powerful tool to study the pattern of proteins that result from differential gene expression.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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