

Original Research Article

Proteomic analysis of natural and demucilaged coffee beans from plantations at different altitudes in the Mantiqueira mountains.

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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).

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

27 *laboratório de Processamento de Produtos Agrícolas (LPPA)* at Federal University of
28 Lavras, (Brazil), between 2014 and 2017.

29 **RESULTS:** Differentially accumulated proteins, such as 11S globulin, glyceraldehyde-
30 3-phosphate dehydrogenase, dehydrin, and *Heat shock* proteins, have been found
31 among treatments through mass spectrometry.

32 **CONCLUSION:** These results contribute to an understanding of the effects of altitude
33 and processing methods on the chemical composition of coffee beans, and thus serve
34 as a basis for future research related to coffee beverage quality.

35 **Keywords:** 2-DE, coffee protein content, coffee beverage quality, coffee processing ,effects of
36 altitude, protein analysis, proteome.

37

38 1. INTRODUCTION

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

46 Although *Coffea arabica* cultivars are highly vulnerable to climatic conditions [2],
47 their beans usually provide superior taste and produce high quality beverages.
48 Regarding climate conditions, it is empirically known that high altitude has beneficial
49 effects on coffee quality [3].

50 Low temperatures normally delay the ripening process, which leads to a higher
51 accumulation of flavour precursors [4]. It is generally acknowledged that high altitudes
52 improve coffee quality [5, 6]. The ideal annual average temperature range for coffee
53 production is 19-21°C [7], although there are some places, such as the Northeast of

54 Brazil, with higher temperatures (24-25°C) that produce beans with satisfactory quality
55 [8].

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

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

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

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

77 Therefore, studying protein composition would help elucidate metabolic
78 processes associated with the different phenomena that occur in coffee beans during
79 their development and from post-harvest mechanisms. This may lead to identification

80 of biomarkers, which might be useful in evaluating the quality of *Coffea arabica* beans
81 and, consequently, beverage quality.

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

87

88 **2. MATERIALS AND METHODS**

89 **2.1 Sources of coffee fruits**

90 Acaiá (*Coffea arabica* L.) samples were collected during the 2009/10 coffee
91 harvest from commercial plantations located in the municipality of Carmo de Minas (-
92 22°6', 45°8'), Minas Gerais, in southeastern Brazil. This region is in the Mantiqueira
93 Mountain Range and is known for the production of specialty coffees (Figure 1).



Figure 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.

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 or 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, 132lbs 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 22lbs 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 22lbs 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].

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

120 preference scores, with higher sensory complexity, than samples from lower elevations
121 (<1000 m) [15].

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

125 Comparative analyses were set up as follows:

- 126 • Analysis 01 - below 1000 m altitude - natural beans X demucilaged beans
- 127 • Analysis 02 - above 1200 m altitude - natural beans X demucilaged beans
- 128 • Analysis 03 - demucilaged beans - below 1000 m altitude X above 1200 m
129 altitude

130

131 **2.2 Total protein extraction**

132 In order to perform analyses, coffee beans were frozen in liquid N₂ and pooled
133 before storage at -80°C. Proteins were then extracted according to [17], only with a few
134 modifications. A portion (250-300 mg) of the powdered samples was homogenized in
135 660 µl lysis buffer [7 M urea (GE-Healthcare), 2 M thiourea (GE-Healthcare), 14 mM
136 Tris base (GE-Healthcare), 100 µl proteases inhibitor cocktail (GE-Healthcare), 12
137 DNase I (1 mg/ml) units, 20 µl RNase A (1 mg/ml), 0.6 µl Triton X-100, 60 mM 3-[(3-
138 Cholanidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) (GE-Healthcare)
139 and 17.5 mM Dithiothreitol (DTT) (GE-Healthcare)]. The mixture was homogenized in a
140 vortex and then kept in ice for 15 minutes. Then the tubes were centrifuged at 14,000
141 rpm for 10 minutes at 4°C. The supernatant containing total proteins was centrifuged
142 once more under the aforementioned conditions and then stored in 100 µl aliquots at -
143 20°C.

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 = $\frac{\text{Vol}}{\sum_{s=1}^n (\text{Vol}_s)} \times 100$). Experimental molecular mass was calculated from digitized 2-D electrophoresis images using standard molecular mass marker protein.

170 2.5 Trypsin Digestion and Mass Spectrometry Analysis

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

175 40 μ L of the samples were injected (SIL-20A HT) in an HPLC system composed
176 of two pumps (LC-20AD), a column oven (CTO-20A), and a degasser (DGU-20A₃)
177 (Shimadzu, Japan). The column was a C-18 150×2.1 mm, 2.7 μ m, with pre-column
178 (Supelco, Pennsylvania, USA). The mobile phases were 0.1% formic acid solutions in
179 water (A) and in acetonitrile (LC-MS grade - Fluka, Buchs, Switzerland), and the elution
180 was carried out at a 200 μ L.min⁻¹ flow, as follows: 0–5 min, 5% B isocratic, 5–60 min,
181 linear gradient to 40% B, 60–70 min, linear gradient to 50% B, 70–75 min, linear
182 gradient to 90% B, 75–80 min, isocratic to 90% B, 80–80.1 min, alteration for 5% B,
183 80.1–90 min, isocratic 5% B. Data obtained through LC–MS/MS were treated with
184 Bruker Data Analysis software, version 4.0 (Build 253). The MS/MS spectrum obtained
185 during the 5-30 min interval was used for a search in the database by Biotoools Version
186 3.2 software (Bruker Daltonik).

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

193

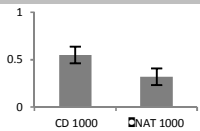
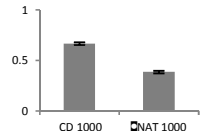
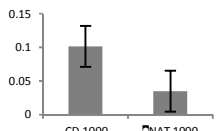
194 3. RESULTS

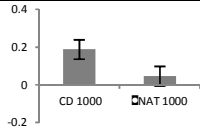
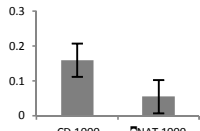
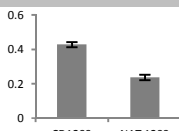
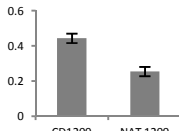
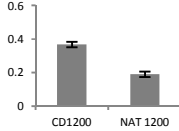
195 **Theses results** were the first to show a significant influence of processing
196 method and altitude on the proteomic profile of cv. Acaiá coffee beans.

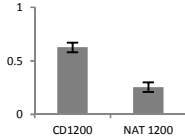
197 The protein profile of coffee beans under different aspects was evaluated in this
198 study. The first analysis consisted of a comparative evaluation regarding the proteome
199 of natural and demucilaged coffee beans from plants cultivated in regions with an
200 altitude below 1000 m. During this analysis, two-dimensional gels were evaluated
201 through the program ImageMaster 2D Platinum 7.0 (GE-Healthcare). As a result,
202 approximately 240 protein points were detected from which 10 spots were selected (no.
203 96, 106, 177, 221, 250, 251, 254, 262, 264, 268) with more than 1.5-fold differential
204 accumulation in three independent gels.

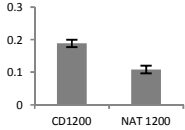
205 All spots were treated with the enzyme trypsin and injected in the mass
206 spectrometer. Then the resulting peptides were compared with the databank through
207 the MASCOT program. Only four spots were successfully identified based on the tryptic
208 peptide sequence: three 11S globulin isoforms,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

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

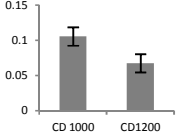
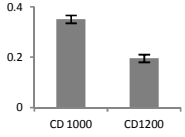
Spot number	Relative Volume ^c	Correspondent Protein	Species	Score ^a	Acess number	Sequence ID Peptide	Exp/ThMr ^b
Analysis 01 - Comparative Analysis regarding proteomic profile of desmucilated and natural coffee beans from regions below 1000 m.							
96		DH1b Dehydrin	<i>Coffea canephora</i>	70	Q1A523	K.SQYDEYGNPVR.Q	1326.58/1326.58
177		glyceraldehyde-3-phosphate dehydrogenase	<i>Coffea arabica</i>	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	<i>Coffea arabica</i>	29	Q9ZNY2	K.TNDNAMINPLVGR.L	1429.69/1429.69
251		11S Globulin	<i>Coffea arabica</i>	145	O82437	R.AIPEEVLR.S K.AGNQGFYVAFK.T	925.53/925.52 1329.63/1329.63

254						K.TNDNAMINPLVGR.L	1413.70/1413.70
						R.QEALLLSEQSQGK.R	1557.80/1557.79
		11S Globulin	Coffea arabica	72	P93079	R.AIPEEVL.R.S	925.527/925.523
						K.AGNEGFEYVAFK.T	1330.61/1330.61
						K.TNDNAMINPLVGR.L	1429.69/1429.69
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

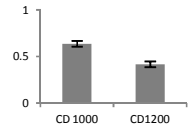
81		11S Globulin	<i>Coffea arabica</i>	184	P93079	R.AIPEEVLR.S	925.527/925.523
						K.AGNEGFEYVAFK.T	1330.61/1330.61
						K.TNDNAMINPLVGR.L	1429.69/1429.69

82		Heat shock protein fragment	<i>Coffea arabica</i>	19	Q9AT71	R.FRLPENAK.M	973.56/973.53
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Analysis 03 - Comparative analysis regarding proteomic profile of desmucilated coffee beans from regions below 1000m and above 1200 m.

29		11S Globulin	<i>Coffea arabica</i>	17	Q9SAN3	K.FFLAGNPQQGGGK.R	1060.52/1060.53
38		11S Globulin	<i>Coffea arabica</i>	90	O82437	R.AIPEEVLR.S	925.53/925.52
						K.AGNQGFYVAFK.T	1329.63/1329.63
						K.TNDNAMINPLVGR.L	1413.70/1413.70
						R.QEALLLSEQSQQGK.R	1557.80/1557.79

107



11S Globulin

Coffea arabica

116

P93079

R.AIPEEVLR.S

925.527/925.523

K.LNAQEPSFR.F

1060.53/1060.53

R.SNGNEGFYVAFK.T

1349.64/1349.64

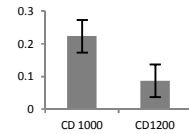
K.AGNEGFYVAFK.T

1330.61/1330.61

K.TNDNAMINPLVGR.L

1429.69/1429.69

110



11S Globulin

Coffea arabica

204

P93079

R.AIPEEVLR.S

925.53/925.52

K.AGNEGFYVAFK.T

1330.62/1330.61

K.TNDNAMINPLVGR.L

1429.70/1429.69

R.QEALLLSEQSQQGK.R

1557.80/1557.79

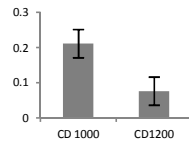
R.SSFQISSEEAELK.R

1557.80/1557.79

K.LSENIGLPQEADVFNPR.A

1897.95/1897.95

116



11S Globulin

Coffea arabica

107

P93079

R.AIPEEVLR.S

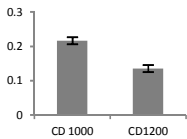
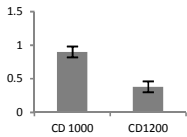
925.53/925.52

K.AGNEGFYVAFK.T

1330.62/1330.61

K.TNDNAMINPLVGR.L

1429.70/1429.69

130		Hypothetic Protein	<i>Vitis vinifera</i>	50	gi 225424256	K.SIEVLEGDGK.S	1045.53/1045.52	210
								211
								212
135		11S Globulin	<i>Coffea arabica</i>	669	O82437	K.VFDDEVK.Q	850.38/850.40	213
						R.AIPEEVLR.S	925.53/925.52	214
						K.AGNQGFYVAFK.T	1329.63/1329.63	215
						K.TNDNAMINPLVGR.L	1413.70/1413.70	216
						R.QEALLLSEQSQQGK.R	1557.80/1557.79	217

a Mascot score for the most significant hits.

b Theoretic (Th) and experimental (Exp) molecular weight (Mr) of identified peptides

218 c Natural (NAT) and Desmucilated (CD) coffee beans

219

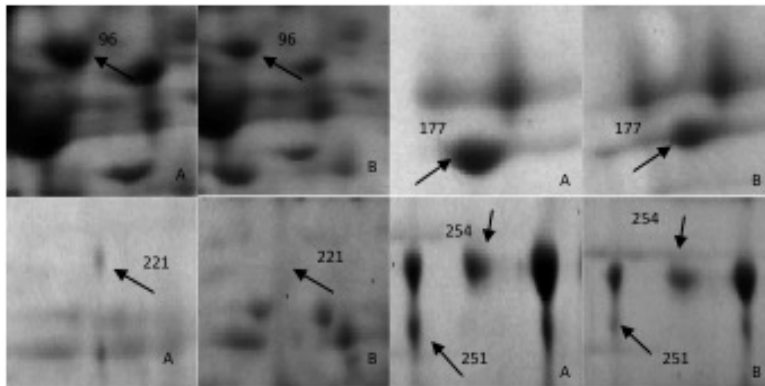


Figure 2 Detail of differentially accumulated protein in desmucilated (A) and natural (B) coffee beans cultivated in the region below 1000 m.

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

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

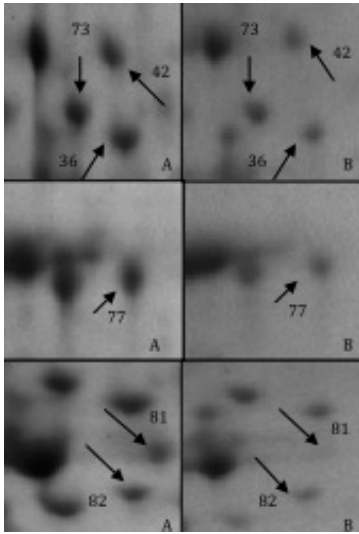


Figure 3 Detail of protein points differentially accumulated in desmucilated (A) and natural coffee (B) beans from regions above 1200 m.

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 Figure 4).

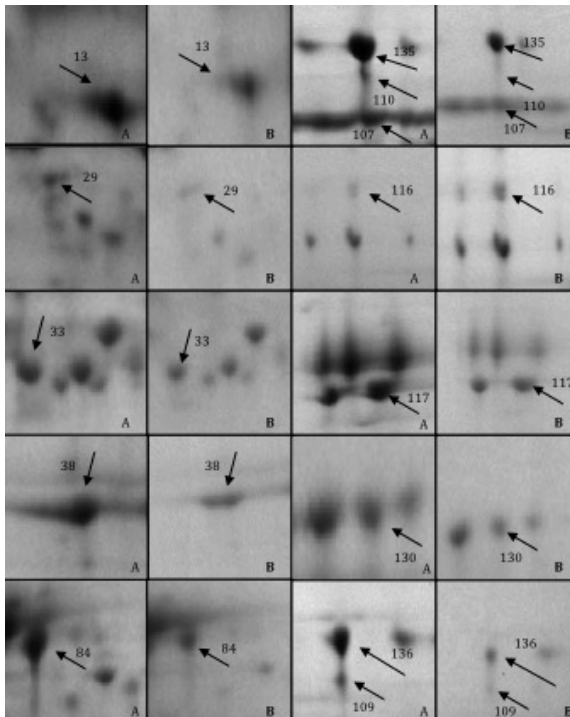


Figure 4 Detail of differentially accumulated protein points in desmucilated coffee beans from cultivations located below 1000m (A) and above 1200m (B).

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].

265 The increase in GAPDH expression was reported under different conditions
266 when plants were subjected to stress, and it was speculated that GAPDHs play roles in
267 plant tolerance to abiotic stress [23].

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

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

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

281 Isoforms of the storage globulin 11S, highly present in the coffee bean
282 endosperm, constitute about 45% of the total number of proteins [26]. Two-dimensional
283 electrophoresis was employed to elucidate the presence of this protein, which was
284 about 55kDa, as verified by the subunits (β arms – 20kDa, and α arms – 32kDa).
285 Peptides were also found in numbers of 12 to 16 kDa probably as degradation products
286 produced by protease activity. Furthermore, 23 variations were observed after
287 sequence alignment of storage proteins was conducted with other proteins as codified
288 by two 11S globulin cDNA in *Coffea arabica*. Differences such as gap and nucleotide
289 addition, as well as amino acid changes, may help explain the presence of isoforms
290 [27].

291 The protein 11S globulin is the main source of the peptides and amino acids in
292 the coffee bean endosperm. This protein build-up occurs during seed development,

293 with the peak of abundance being reached at the half-way point of fruit maturation,
294 which corresponds to maximum expansion of the bean endosperm [27].

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

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

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

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

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

318 HSPs are proteins generally involved in cell protection and repair, as well as
319 degradation of harmful components, especially during abiotic stress [33]. HSPs are
320 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.

342

343 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

349 and other studies related to large scale "-omics" may open new routes in
 350 understanding the changes occurring inside coffee beans before, during, and after
 351 harvest and thus avoid inadequate management practices that might jeopardize coffee
 352 bean yield and quality.

353 Future studies must be carried out in order to determine how much this protein
 354 activity may physiologically affect the beans and alter coffee beverage flavour and
 355 aroma.

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

361

362 6. REFERENCES

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