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Quality profile of Peruvian dark chocolate: A preliminary approach

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Peru is considered to be the centre of cocoa genetic diversity, since it harbours around 60% of the worldwide cocoa cultivars. Peruvian fine flavour cocoa stands out at international level due to its recognized diversity and richness of aroma and taste, which allow the production of high quality chocolate, especially with high cocoa content due to its potential health benefits. In the last years, Peruvian cocoa production has shown a remarkable growth. Likewise, an increasing number of local chocolate producers have promoted the exhibition of Peruvian chocolate in international markets and worldwide cocoa competitions with great success. Peru has a great opportunity to be recognized worldwide as a producer of high quality chocolates made from native fine flavour cocoa. To promote this process, studies are necessary to demonstrate the chemical-organoleptic quality of these chocolates. In this sense, the present study focused on the evaluation of the chemical (total phenolics, flavonoids, theobromine and caffeine), nutritional (proteins, carbohydrates, fat, fatty acids and fibre), antioxidant activities and sensory profiles of 5 Peruvian dark chocolates and a control sample. These single origin samples (Piura, San Martín, Amazonas and Cusco) contained between 60% and 70% cocoa. An international semi-trained panel of cocoa and by-products carried out the sensory evaluation. For the quantitative descriptive analysis (QDA), 5 descriptors for odour (fruity, floral, cocoa/chocolate, sweet/caramel/malt and off-odour), 8 for taste (fruity, floral, bitter, sweet/caramel/malt, astringent, cocoa/chocolate, nutty and off-taste) and 1 for mouthfeel (melting degree) were defined by the panellists. A 10 point-scale was used to score attribute intensity and global quality of the samples. Based on ANOVA ($p>0.05$), only 8 descriptors (fruity, floral, cocoa/chocolate and sweet/caramel/malt odour; fruity, bitter and cocoa/chocolate taste; and melting degree) explained the chocolate differences. In addition, Principal Components Analysis of the sensory results showed that the first two principal components explained 89.1% of the data variance, in which two clusters of chocolate samples were identified. A first cluster of 2 products was characterized by its fruity flavour, while a second cluster of 3 products was associated to floral notes. According to the panellists, the overall quality of the five chocolates ranged from 5 to 6 points. The outcomes of this study provide a supporting base for further research towards the improvement of Peruvian chocolate quality.

Keywords: Peruvian chocolates, sensory profile, proximate analysis, methylxanthines, antioxidant

I. INTRODUCTION

Peru is one of the South American countries that contribute to the cocoa supply worldwide. Based on Motamayor *et al.* (2008) studies, the centre of origin of *Theobroma cocoa* L. is located in the Upper Amazon region where the highest cocoa genetic diversity is encountered.

Fourteen out of 24 Regions are cocoa producing zones in Peru. San Martín represents the largest cocoa producing zone in Peru with approximately 46% of the total local cocoa production (92592 ton) and 38% of the total cocoa cultivated hectares (120 374 ha) (MINAGRI, 2015). Piura, Cusco and Amazonas are other cocoa producing regions which have also been recognized as birth place of fine flavour native species denominated "cacao blanco", "cacao chuncho" and "cacao Amazonas Perú" respectively (Sierra y Selva Exportadora, 2015; Arévalo, 2017, INDECOPI, 2016). These locations have shown constant increases per year due to the global demand. Therefore there is a boost for cultivating this crop since it becomes a sustainable alternative for farmer's economy.

Little is known about chemical and organoleptic quality of Peruvian dark chocolates. Therefore this study attempts to enlighten the diversity of chemical and organoleptic profile found in local dark chocolate samples with different cocoa content, cocoa origin and manufacturing company.

II. MATERIALS AND METHODS

a) Proximate analysis

Moisture content was determined by weighing approximately 2 g of sample into a pre-weighed dish. Moisture analyzer equipment (Sartorius Moisture Analyzer, Model MA35) was programmed at 135 ° C for 6 minutes. The final weight and the percentage of humidity provided by the equipment were recorded. The Kjeldahl method was used for protein determination, consisting of 3 stages: digestion, distillation and titration. For the calculation of the percentage of proteins in the sample, the value of 6.25 was used as a protein factor. The percentage of fat in the sample was determined by the Soxhlet method. The amount of fat extracted from 2 g of sample by an organic solvent (petroleum ether) was calculated and reported as a percentage. A fiber extractor was used in which 0.5 g of defatted sample was subjected to acid hydrolysis with 1.25% sulfuric acid, followed by a basic hydrolysis with 1.25% sodium hydroxide. The obtained residue was weighed and reported as percentage of total fiber in the sample. 0.5 g of sample was weighed into a dry crucible and subjected to total calcination in the muffle furnace (Thermo Scientific ™ - Model FB1310M) at 525 °C for 3 hours for ash content determination. The weight of the residue obtained was calculated and reported as a percentage of ash in the sample. The percentage of carbohydrates in the sample was calculated by difference, starting from a value of 100% and subtracting the other components (moisture, fat, fiber, protein and ash) as percentages.

b) Fatty acids profiles

About 100 mg of previously extracted fat sample was weighed into a 14 mL Falcon tube; 10 mL of n-pentane was added to dissolve the sample and then 100 µL of 2N potassium hydroxide in methanol. The Falcon tube was vortexed for 1 min and centrifuged for 6 min at 5000 rpm at 10 °C. A volume of 1.5 mL of the supernatant was transferred to a vial through a Phenomenex 0.45 µm filter.

Subsequently, the vial was injected into the GC-MS (Agilent Technologies 7890 A-5975C) with helium as a carrier gas and a DB-5MS column (60m x 250µm x 0.25µm), injection temperature of 250 °C, detector temperature MS of 230 °C, Split ratio 200: 1 and a total running time of 36 min. The column was maintained at 100 °C for 1 min, then the temperature was increased 20 °C/min to 190 °C, maintained for 1 min, the temperature was further increased 3 °C/min to 210 °C, kept for 1 min and then increased to 1 °C/min to the final temperature of 230 °C. The injection volume of the sample was 5 µL. The identification of the compounds was carried out by comparing the mass spectra of the fatty acids with the mass spectra provided by the NIST 08 library and with standards of fatty acid methyl esters.

c) Theobromine and caffeine

0.2 g of defatted sample was weighed and placed in a round-bottom flask, and then 40 mL of ultrapure type I water was added and refluxed for 30 min. The extract was centrifuged for 5 minutes at 5000 rpm and brought to a final volume of 50 mL in a volumetric flask. 2 mL of the aqueous solution was placed into the previously conditioned Sep-pak C18 filter and the sample was eluted with 10 mL of chloroform. The solvent was evaporated and the residue obtained was dissolved with 5 mL of ultrapure water type I and transferred

to a vial for injection in the HPLC-DAD. The elution system was acetonitrile-water (20:80), isocratic, with a running time of 8 min, injection volume of 20 µL and a flow rate of 1.2 mL/min.
For the preparation of the calibration curve, stock solutions of theobromine (0.15 mg/mL) and caffeine (0.1 mg/mL) were prepared, 5 dilutions from these stock solutions were evaluated in the same way as the samples.

d) Total phenolics

0.5 g of defatted sample was weighed into a 15 mL Falcon tube, 5 mL of 80% ethanol was added, the mixture was stirred for 5 minutes on ultrasound equipment and centrifuged at 10 °C, 5000 rpm, for 10 min. The supernatant was transferred to a 25 mL volumetric flask (the previous procedure was repeated 3 times) and brought to volume with 80% ethanol. Then 50 µL of this solution was taken and mixed with 1000 µL of 10% Folin Ciocalteu plus 1000 µL of 7.5% Na_2CO_3 and 970 µL of ultrapure type I water.

The mixture was allowed to stand for 15 min at room temperature and in a dark place. Finally, the absorbance of the solution was read at 750 nm in a spectrophotometer (Spectroquant® Pharo 300). For the preparation of the calibration curve of gallic acid, solutions of this standard were prepared at different concentrations, which were analyzed in the same way as the sample. The total phenol content was expressed as milligram-equivalents of gallic acid per gram of sample (mg AG/g).

e) Total flavonoids

One gram of sample was extracted with 20 mL of 80% methanol for 3 h at room temperature, then filtered and brought to volume in a 25 mL volumetric flask. Two mL of this solution was mixed with 1.5 mL of distilled water and 0.15 mL of 0.05% $NaNO_2$. After 5 min, 150 µL of 0.1% $AlCl_3$ was added and the mixture was allowed to stand for 6 min. At this time, 1 mL of NaOH 1 M was added and the volume of the solution was completed to 5 mL with distilled water. After 30 min, the absorbance of the solution was measured at 510 nm in a spectrophotometer. For the preparation of the catechin calibration curve, dilutions of this standard were prepared at different concentrations (6 to 14 µg/mL) which were analyzed in the same way as the sample. The total flavonoid content was expressed as milligram-equivalents of catechin per gram of sample (mg CAT/g).

f) Antioxidant activity (DPPH test)

0.5 g of the defatted sample was weighed into a Falcon tube and 5 mL of 80% ethanol was added, sonicated for 5 min and centrifuged for 10 min at 5000 rpm, 10 °C. The supernatant obtained was placed in a 25 mL volumetric flask (this procedure was repeated 3 times). All supernatants were pooled and brought to volume with 80% ethanol. From this solution dilutions were prepared with concentrations of 0.063 to 0.625 mg/mL, each of them were mixed with 3950 µL of DPPH (2,2-diphenyl-1-picryl hydrazyl) and enough 80% ethanol to complete a final volume of 4000 µL. The test tubes were then placed in the darkness for 30 min. To prepare a DPPH control solution, 1.97 mg of DPPH was dissolved in 5 mL and then brought to volume with 80% ethanol in a 50 mL volumetric flask. The reduction of DPPH was determined at a wavelength of 517 nm in a spectrophotometer. The percentage of antioxidant activity was calculated with the following formula:

$$\% AA = 100 \times (1 - (\text{Absorbance sample} / \text{Absorbance DPPH control}))$$

The median effective concentration (EC_{50}) of the antioxidant activity was obtained from the curve of the percentage of antioxidant activity versus sample concentration (mg/mL).

g) Sample preparation and sensory evaluation

Sensory training and organoleptic evaluation of dark chocolate samples by a semi trained panel were carried out at Hamburg University of Applied Sciences in Germany.

Single origin chocolate samples with different percentage of cocoa content (60-85%) were used during panel training. For profiling, five single origin chocolates (between 60 and 70% cocoa content from San Martín, Amazonas, Cusco and Piura) were chosen as shown in Table 1. Besides a control sample (commercial dark chocolate with 75% cocoa content and composed by cocoa mass, cocoa butter, sugar and soy lecithin) was also included during panel evaluation. Approximately 2 g ± 0.10 g of sample was portioned out in similar size and shape into plastic containers. To avoid code similitude and positioning effect, random 3 digit numbers were generated using FIZZ software by Biosystemes to code the samples. Sealed samples were served at room temperature in a free-odour, spacious environment with adequate

lightning. Warm water and water crackers were used as neutralizing agents of desaturation while red light in the booths was used to mask the colour differences of the samples.

Six panellists from different origin country formed the semi trained panel. The panel followed a 2 month training based on series of sensory tests (basic tastes recognition, odour recognition, threshold, ranking, discriminative and descriptive tests) following the ISO DIN standards 3972, 5495, 8586, 8587, 8589, 10399 and 13299. Quantitative descriptive analysis (QDA) was used to obtain the organoleptic profile of each sample. During panel session, 5 odour attributes (fruity, floral, cocoa powder/chocolate, sweet/caramel/malt, off-odour), 8 flavour attributes (fruity, floral, bitter, sweet/caramel/malt, astringent, cocoa powder/chocolate, nutty, off-taste), melting degree and overall quality were chosen for the evaluation using a 10 points scale to score the attribute intensity. In case of melting degree, this descriptor refers to the ease of melting during tasting. Panellists were firstly asked to perform the odour testing so they needed to remove the lid of the container, inhale and assess the intensity of the defined odour attributes. Then taste testing was followed scoring the intensity of the taste attributes. The sensory evaluation was conducted one more time (in duplicate) using different sample codes and session time.

The results from the QDA forms were analysed using the Panel Check V1.4.2 software. Analysis of variance (ANOVA, $p>0.05$), Principal Component Analysis (PCA) and spider web diagrams were used to identify the attributes that discriminate the samples and their organoleptic profile (Naes *et al.*, 2010).

Table 1. Detailed list of dark chocolate samples

Code	Brand	Cocoa content (%)	Origin	Composition
1	A	70	San Martín	Cocoa mass, cocoa butter, sugar, soy lecithin
2	A	60	Cusco	Cocoa mass, cocoa butter, sugar
3	A	70	Piura	Cocoa mass, cocoa butter, sugar, soy lecithin
4	B	70	Amazonas	Cocoa mass, sugar
5	C	70	San Martín	Cocoa mass, cocoa butter, sugar cane

III. RESULTS AND DISCUSSION

Nutritional and chemical profile

The nutritional composition of the chocolates with 60-70% cocoa solids is reported in Table 2. Similar protein content was characteristic among the samples. Chocolate 5 had the highest fat concentration (44.1%) and caloric value (594.5 kcal/100 g) while chocolate 3 contained the highest fibre content (11.1%) and the lowest energy value (546.8 kcal/100 g). According to De Clercq *et al.* (2012), variable fat content levels could be attributed to the cocoa origin, variety, agronomy handling, pod ripeness and cocoa butter extraction. The narrow range of fat content among samples might be attributed to the addition of cocoa butter as part of the chocolate formulation. However, chocolate 4 is the only sample made with cocoa mass and sugar so its fat content mainly comes from the typical beans grown in the Amazon region.

Table 2. Proximate analysis of chocolate samples

Code	Fat (%)	Ash (%)	Protein (%)	Fibre (%)	Carbohydrates (%)	Energy (kcal/100 g)
1	38.9 ± 0.4	1.7 ± 0.0	7.9 ± 0.2	2.9 ± 0.1	48.6 ± 0.3	576.1
2	38.6 ± 0.2	1.5 ± 0.0	7.1 ± 0.3	8.4 ± 0.2	44.4 ± 0.3	553.4
3	40.0 ± 0.2	2.1 ± 0.0	7.9 ± 0.1	11.1 ± 0.1	38.8 ± 0.2	546.8
4	41.4 ± 0.3	1.9 ± 0.1	8.2 ± 0.1	3.0 ± 0.1	45.4 ± 0.3	587.0
5	44.1 ± 0.3	2.1 ± 0.0	8.0 ± 0.1	4.3 ± 0.2	41.4 ± 0.4	594.5

The fatty acid profile of all chocolate samples showed mainly the presence of palmitic (25.62 – 28.75%), oleic (35.15 – 39.81%) and stearic (29.28 – 35.58%) acids (see Table 3.), which are the main fatty acids found in cocoa beans. Similar fatty acid profile has also been reported for dark chocolates from Ecuador and Ghana with 51% cocoa content (Torres-Moreno *et al.*, 2015).

Table 3. Fatty acids profile of chocolate samples

Fatty acids	Relative concentration (%)				
	1	2	3	4	5
Myristic (C14:0)	0.03	0.04	0.03	0.02	0.03
Palmitoleic (C16:1)	0.09	0.14	0.11	0.13	0.11
Palmitic (C16:0)	27.14	26.07	28.75	25.62	28.29
Margaric (C17:0)	0.12	0.10	0.08	0.08	0.10
Stearic (C18:0)	33.81	34.60	29.28	35.58	34.19
Oleic (C18:1)	36.60	36.90	39.81	36.34	35.15
11-methyl-octadecenoic (C18:1)	0.28	0.33	0.31	0.30	0.22
Linoleic(C18:2)	1.49	1.33	1.25	1.40	1.38
Araquidic (C20:0)	0.44	0.50	0.38	0.52	0.53

Table 4 summarizes the results for content of total phenolic compounds, flavonoids, antioxidant capacity and methylxanthines of Peruvian dark chocolate samples. Total phenolic compounds were in the range of 1630 – 2139 mg/100 g, lower than the concentrations found in some commercial chocolates from Malaysia and Ghana (2059-6158 mg/100 g) (Ramli *et al.*, 2001). Antioxidant activities were higher (lower EC_{50}) in the chocolates with greater amounts of total phenolic and flavonoid compounds.

Methylxanthines (theobromine and caffeine) stimulate central nervous system and give a bitter taste to cocoa and chocolates (Franco *et al.*, 2013). The methylxanthines content is generally determined by the genotype and fruit ripeness. Thus the theobromine/caffeine ratio could be used as a parameter for genotype differentiation (Amores *et al.*, 2006). Concentrations of theobromine (36-104 mg/g) and caffeine (3-9 mg/100 g) in commercial samples from Malaysia and Ghana were much lower than the ones found in Peruvian chocolates (187-421 and 17-48 mg/100 g, respectively) (Ramli *et al.*, 2001). Chocolate 1 and 5 from San Martín had similar theobromine/caffeine ratio while the samples from different origins (Cusco, Piura and Amazonas) evidenced different ratio values. Based on the theobromine/caffeine ratio used by Trognitz *et al.* (2013) for cacao classification, cocoa mass of chocolate 2 may correspond to Trinitario-Nacional cacao while the cocoa masses of the other chocolate samples could come from Forastero cacao type.

Table 4. Total flavonoids, phenolics and antioxidant activities of Peruvian dark chocolates

Code	Total flavonoids (mg CAT/100 g)	Total Phenolics (mg AG/100 g)	EC_{50} (µg extract/mL)	Methylxanthines (mg/100 g)		
				Theobromine	Caffeine	Theobromine/caffeine ratio
1	484.6 ± 5.4	2024.3 ± 25.8	229.3 ± 1.8	248.6 ± 3.3	18.6 ± 0.5	13.4
2	534.1 ± 9.2	2139.1 ± 25.4	201.9 ± 3.7	187.1 ± 1.2	47.9 ± 1.4	3.9
3	587.6 ± 14.7	2072.6 ± 12.1	200.7 ± 7.1	421.2 ± 1.2	42.3 ± 0.4	10.0
4	493.3 ± 11.9	2032.4 ± 22.2	223.2 ± 7.2	238.4 ± 4.3	37.9 ± 0.9	6.3
5	215.3 ± 9.9	1630.7 ± 22.2	377.3 ± 2.1	240.1 ± 1.5	17.5 ± 0.8	13.7

Sensory evaluation

According to the ANOVA ($p>0.05$), eight out of 14 sensory attributes allowed the samples discrimination. These attributes were fruity, floral, cocoa/chocolate, sweet/caramel/malt odour; fruity, bitter, cocoa/chocolate taste and melting degree. On the other hand, attributes such as off-odours, off-flavours, floral, sweet/caramel/ malt, astringent and nutty tastes were not significant for the panel to differentiate the samples. Based on the PanelCheck methods, the panel evidenced a good performance in terms of variance explanation and results reproducibility.

A standardized PCA plot (Figure 1) showed the main attributes that characterized each chocolate sample. Approximately 90% of the total data variance is represented by component 1 and 2. Odour notes of cocoa/chocolate and sweet/caramel/malt, taste notes of bitterness and cocoa/chocolate as well as the highest melting degree characterized the control sample. Chocolate samples 1, 3 and 4 with 70% cocoa from San Martín, Piura and Amazonas, respectively owned floral notes of odour. These floral notes were described

by the panellists as herbal, grassy and hay for sample 1 and 3 while tobacco, earthy and wood notes were additionally used to describe the odour of sample 4. Fruity taste and odour (described as yellow, brown and red fruit) were associated to sample 2 with 60% cocoa from Cuzco and sample 5 with 70% cocoa from San Martín.

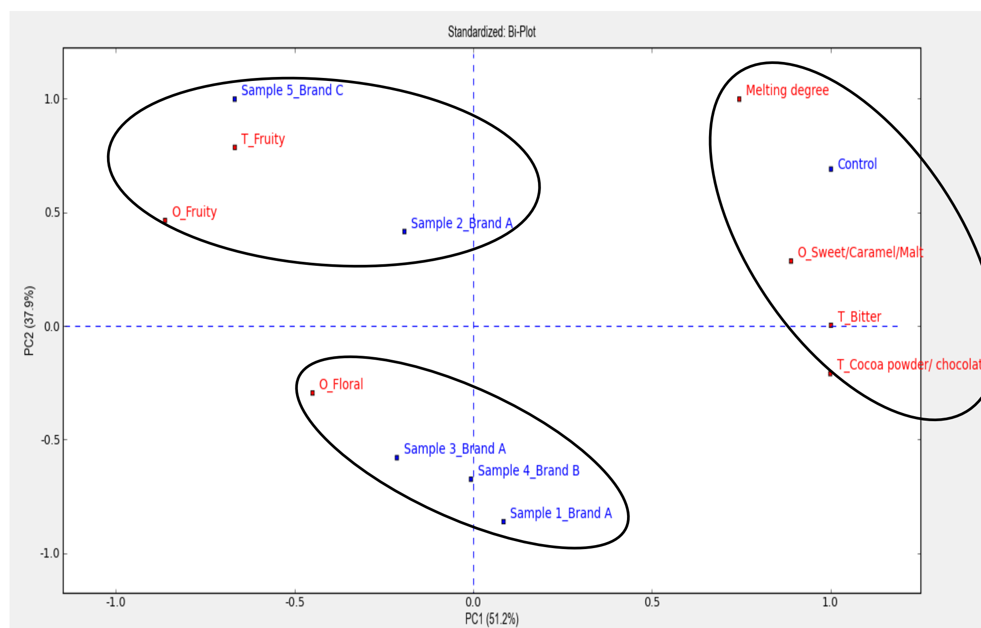

Figure 1. Bi-Plot loadings of principal component analysis (PCA) with descriptors and Peruvian dark chocolate samples

Figure 2 represents the sensory profile (14 attributes and overall quality) of the five dark chocolates and the control sample. Samples 1 and 3 with 70% cocoa from San Martín and Piura showed a similar organoleptic profile. Sample 1 slightly differs from sample 3 in terms of presence of off-taste and less perceptible fruity notes. Sample 2 with 60% cocoa from Cuzco has higher melting point and more intense fruity notes than sample 1 and 3 (See Figure 2a).

Samples from same cocoa origin (Peruvian Amazon) and same cocoa content exhibited particular profiles as shown in Figure 2b. Moderate intensity of fruity notes and higher melting point were found in sample 5 compared to sample 1 and 4. Besides, off-flavour and off-odour traces were perceived for these samples. Profile differences may be related to chocolate recipe and processing protocols which highlight different taste/odour notes even though the cocoa origin and content is the same among the evaluated samples.

Figure 2c and 2d show the sensory profile of the control sample compared to the other chocolate samples. It is evident that cocoa/chocolate notes of odour and taste predominant over the rest of the samples while fruity and floral notes are less perceptible.

In terms of overall quality, all samples were qualified with 5-6 points.

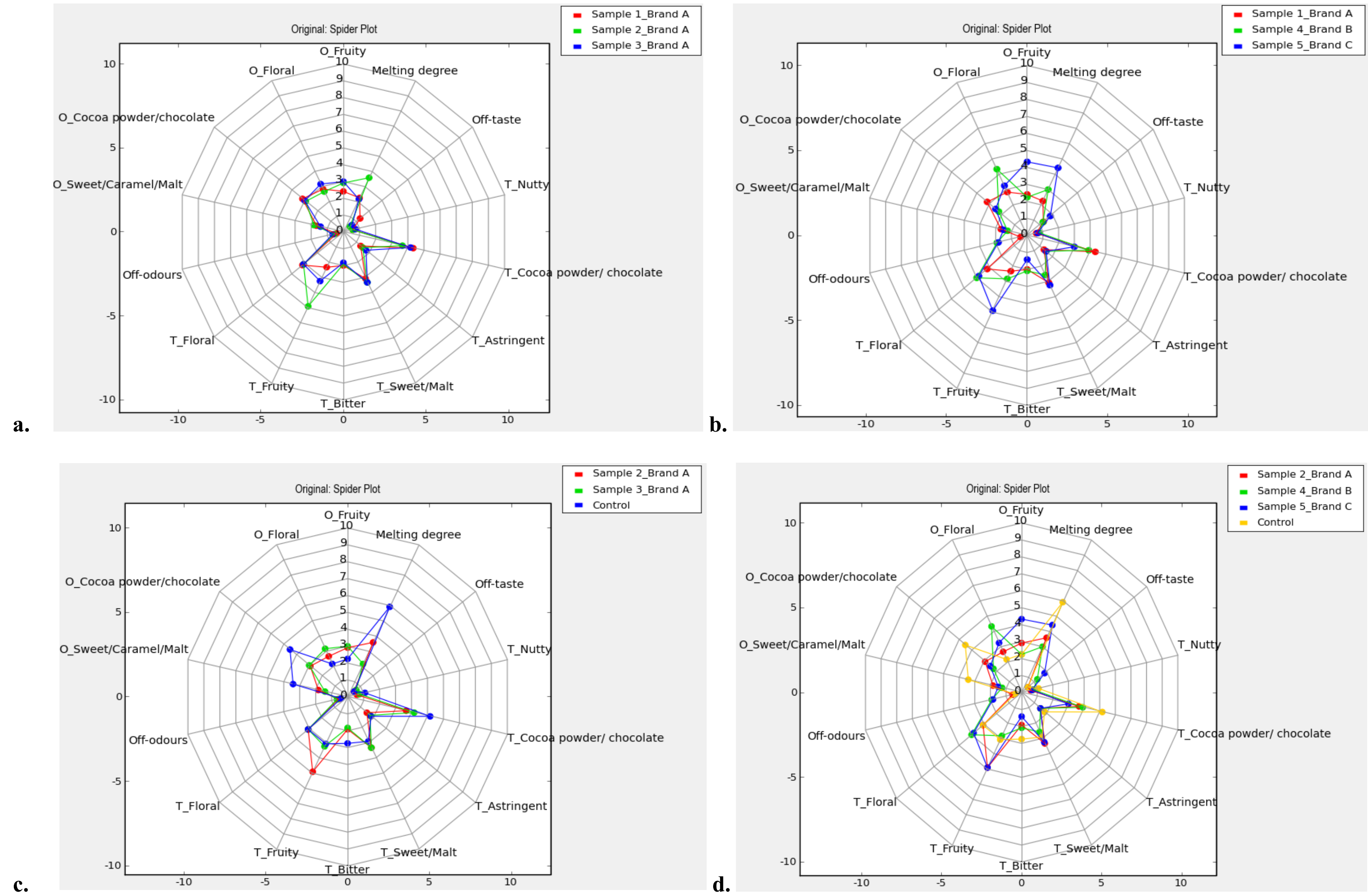

Figure 2. Spider web plots of Peruvian dark chocolates

IV. CONCLUSION

Concentrations of fat were higher than 38% in the Peruvian samples evaluated. The main fatty acids were palmitic, oleic and stearic acids. Antioxidant activities were correlated to the total amount of flavonoids and phenolic compounds. Theobromine and caffeine concentrations in Peruvian samples were higher than commercial samples from other countries. Sensory evaluation performed by an international semi trained panel evidenced the broad diversity of organoleptic attributes perceived in fine flavour dark chocolates made from single origin regions and local manufacturers from Peru.

V. ACKNOWLEDGMENT

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