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Full Paper

Determination of Smuggled Cigarette Characteristics in Brazil and Their Potential Risk to the Human Health

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Abstract: The objective of this study was to determine the different characteristics of tobacco found in thirty brands of smuggled cigarettes in Brazil. Determination of arsenic through atomic absorption spectrometry in graphite oven was carried out and classical methodologies were employed to determine dirtiness, total ash, insoluble ash, humidity, tobacco pH and sidestream smoke pH. The methodology used to quantify arsenic presented quantification limit of 15.0 ng g^{-1} and detection limit of 4.0 ng g^{-1} in dry tobacco mass. The recovery of arsenic for the method purpose was 98.2% and relative standard deviation 6.0%. About 56% of the brands were observed to have arsenic concentrations above 20.0 ng g^{-1}, which means nearly twice as much as the arsenic found in cigarettes sold legally in Brazil. Levels above the recommended value for humidity were found in 53% of brands. About 96% of the brands presented total ash content above that indicated by the Brazilian Pharmacopoeia. About 53% of the samples contained levels of insoluble ash above the limit. In 90% of the samples, the smoke was alkaline. In dirtiness tests, 81.2% of the brands presented some kind of contaminant, such as fungi, insect fragments, grass or mites. The characteristics revealed that the consumption of this kind of cigarette can increase risks to consumer health.

Keywords: Contamination; Smuggling; Cigarettes; Arsenic.

1. INTRODUCTION

Smoking is considered a global epidemic by the World Health Organization, killing around 6 million people every year [1]. In order to slow the advance of problems caused by smoking, some measures have been implemented aiming to reduce cigarette consumption such as the prohibition to advertise it and increase in cigarette taxation [2]. However, these measures might stimulate smuggling and falsification. Recent data shows that around 11% of all cigarettes consumed worldwide are obtained for smuggling [3]. Also, because they are easily transported and generate high profits, the illegal cigarette market has become an extremely appealing and profitable activity all over Latin America [4]. It is believed that over 30% of the cigarettes consumed in Brazil is smuggled, reaching 60% in some border regions with Paraguay [5-6]. Over 1.7 million smuggled cigarettes were seized in Brazil only in the first semester of 2014 [6].

About 4700 compounds are found in tobacco, many are toxic such as phenols, cresols, acrolein, non-metals, and metals [7-8]. Inhalation of these substances is related to multiple diseases such as various cancers (esophagus, larynx, trachea, kidneys, lungs, stomach, pancreas, ureters, bladder, cervix), and chronic diseases (blindness, cataracts, periodontitis, cerebral infarction, aortic aneurysm, pneumonia, coronary heart disease, asthma, atherosclerosis) [9]. Between various toxic components, metals and non-metals are the most important due to their catalytic activity in the human body cells. Arsenic is one of these substances, which was identified in legal cigarettes in China, Canada and in fake brands of cigarettes in the United States [9-11]. The Food and Drug Administration report on the

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cigarettes potentially harmful constituents, classifies arsenic as carcinogenic and toxic to the cardiovascular and reproductive systems [12]. The al tobacco physical, chemical and biological characteristics can change volatilization conditions and absorption speed of highly carcinogenic substances, these characteristics can be also used to measure the quality of the product. Cigarettes are produced from the *Nicotiana tabacum* plant, and their behavior depends on the humidity content. This factor is important to determine the ideal conditions of production and sale. High humidity content can decompose the tobacco during storage and transport, favoring the occurrence of fungi and other microorganisms [13].

Any undesirable material found in processed products is called dirtiness. These materials are classified in lightweight dirtiness such as insect fragments, mites and fungi, or heavy dirtiness like metal pieces and silica sand, which can be visually identified through microscopic or gravimetric techniques [14-15]. Several additives are mixed to the cigarette tobacco including sugars and ammonia forming agents and the Sanitary National Agency (ANVISA) has been considering the prohibition of these additions due to their toxic potential, in order to make the cigarette less appealing to young people and adolescents. The Brazilian National Congress (2013) has been studying a bill that bans the commercialization of cigarettes whose smoke has pH above 7.0 [16]. Too much of these additives in adulterated cigarettes contribute to the alkalizing of tobacco smoke. These cigarettes are known to release great amounts of free nicotine, which when absorbed by the organism tend to result in chemical dependency [17].

This study aims at characterizing smuggled cigarettes in Brazil. Therefore, thirty cigarette brands seized by the Brazilian Federal Revenue were characterized through determination of arsenic, gravimetric analyses (humidity, total and insoluble ash), tobacco pH and sidestream smoke and dirtiness.

2. MATERIAL AND METHODS

Sample collection and treatment

Thirty brands of smuggled cigarettes shown in Table 1 were made available to the study through a partnership between the Federal Revenue Station – 9th Region in Ponta Grossa and the State University of Ponta Grossa (UEPG). The samples available to this study had been seized in routine inspections carried out by the Brazilian Federal Police. The storage site is a well ventilated, protected from weathering warehouse which belongs to the Federal Revenue, and the packets are stored on shelves until they are legally sent to incineration. Simple random probability sampling was used. Ten packets out of the five hundred contained in a sealed box were collected and numbered. Next, the cigarettes contained in the previously picked packets were numbered and a hundred were randomly collected, and this process was repeated with all brands. For each determination, the corresponding number of cigarettes was randomly collected. For the sampling and gravimetric analyses, tobacco pH and dirtiness, the tobacco from the smuggled cigarettes was separated from the filter and the wrapping paper.

Table 1. Seizure volume in millions of cigarette packets seized in Brazil between 2010-2013 by manufacturer (M) [6, 18].

Brands	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15	A16	A17	A18	A19	A20	A21	A22	A23	A24	A25	A26	A27	A28	A29	A30
Packets	0.07	60.6	3.25	3.48	23.6	5.44	0.77	5.1	23.7	5.6	0.03	8.41	0.03	0.26	2.46	0.09	0.03	3.56	6.58	3.6	5.44	7.78	0.67	8.41	8.05	6.33	3.4	0.03	0.57	0.03
M	6	1	5	5	2	4	2	4	2	2	6	3	6	3	1	6	1	4	1	3	4	1	7	3	1	4	5	6	7	5

Determination of arsenic in the samples

For the sampling and measurement of arsenic, a cigarette of each portfolio was sampled. An (Ohaus®) analytical scale was used to measure 1.0 ± 0.01g of each sample, then each sample was subjected to the acid digestion process. The sample was acidified with concentrated HNO_3 (Biotec®) followed by heating at 90±5°C for 6h in digester block (Tecnal® model TE-040/25) and the addition of H_2O_2 30% v/v at 90°C for 4h. Due to the presence of precipitates in the sample, the solutions obtained were filtered with filter paper Whatman nº 540, 0.05 L volume with ultrapure water reverse osmosis and water filter (Gehaka®) and kept under refrigeration at 4°C until the determination was carried out.

All arsenic determinations were carried out in triplicate in an atomic absorption spectrometer (Varian®, AA 240Z), with electrothermal atomization in a graphite oven (model GTA 120), equipped with Zeeman background corrector and sampler system for automated dilution (model PSD 120). A hollow-cathode arsenic lamp was used and argon High Purity (99.9999%) as the inert gas in a 0.3 L min^{-1} flow. The heating program used for arsenic determination in graphite furnace atomic absorption spectrometry (GFAAS) following Agilent instructions is shown in Table 2. A $Pd(NO_3)_2$ and $Mg(NO_3)_2$ chemical modifier was employed at the 5/3 ratio, acidified with 1% nitric acid [19]. The arsenic recovery was tested with fortification with the stock solution 1000 mg L^{-1} in 5 replicates of 3 known concentrations of arsenic (4.0; 8.0 and 16.0 ng g^{-1}) fortified in a period of 24 hours before the start of digestions. The linear regression showed correlation coefficient of 0.997 in a linear range of 2.0 to 16.0 µg L^{-1}[20].

Table 2. GFAAS heating program used to determine arsenic in tobacco samples.

	Temperature (°C)	Ramp (s)	Landing (s)	Gas Flow (L min^{-1})	Reading
Drying 1	90	40.0	5.0	0.3	No
Drying 2	120	10.0	5.0	0.3	No
Calcination	1400	5.0	2.0	0.3	No
Atomization	2600	1.0	2.0	0	Yes
Cleaning	2800	2.0	1.0	0.3	No

Gravimetric analyses

In order to determine humidity, the method AOAC 966.02 (1990) was employed, and three cigarettes from each packet were used. The samples were successively heated at around 80°C and cooled in a dissecator until a mass with difference lower than 1mg was obtained. The AOAC 930.05 (1990) method was employed to determine total ash content. Previously dried samples were collected in the humidity determination process and placed in porcelain crucibles. Next, the samples were calcined in muffle at approximately 550 °C for 5 hours. After reaching room temperature, the samples were weighed.

The insoluble ash determination was carried out in order to determine dirtiness according to the AOAC 920.08 (1990) method. Total ash was solubilized in HCl 1 mol L^{-1} and heated for 5 minutes at 80°C. The samples were filtered in quantitative filter paper Whatman nº 540 and transferred to previously dried and weighed porcelain crucibles, followed by heating in muffle at 550 °C for 5h and cooling in dissecator up to room temperature and weighing.

pH determination in the tobacco and sidestream smoke.

The tobacco pH was determined through the US-EPA 9045D method (2004) [21]. The tobacco was homogenized with ultrapure water, 18.2 MΩ^{-cm} resistivity at 1 to 2m/v ratio for 15 minutes on a shaker and later on the pH was measured from the liquid extracted. In order to determine the sidestream smoke pH the Brunnemann and Hoffmann method (1974) was used [22]. A glass electrode was covered with a thin layer of buffer adjusted at pH around 6.0, then a flow of smoke was released to recover the electrode and the pH was measured.

Light dirtiness determinations

In order to determine lightweight dirtiness, the AOAC 970.66 method (1990) was employed. The material was identified using a stereo binocular microscope with 20 and 50 times enlargement in two different scanning procedures and mechanical separation of elements found which were different from the product under analysis (tobacco).

Statistical analyses

The statistical analyses were carried out by employing the software Assistat 7.7 and PAST 3.08.

The variance analysis (ANOVA) with a completely randomized design using the Scott-Knott test at 5% probability was carried out to test significant differences between the smuggled cigarette arsenic concentration, humidity content, ash, insoluble ash, tobacco pH and tobacco smoke pH averages. Values below 0.05 were considered statistically significant, and represented by ($p<0.05$). Principal Component Analysis (PCA) was employed to correlate the characteristics of the thirty different brands of smuggled cigarettes. All data was pre-processed through self-scaling.

3. RESULTS AND DISCUSSION

Arsenic determination in smuggled cigarettes

The method used for arsenic recovery presented 15.0 ng g^{-1} Limit of Quantification (LQ) in mass of dry tobacco of cigarettes, while the Limit of Detection (LD) was 4.0 ng g^{-1} and Characteristic Mass (Mo) 19.4 pg. The result of the arsenic recovery test in the tobacco of smuggled cigarettes was 98.2% and Relative Standard Deviation 6.0% on average, as shown in Table 3. The arsenic could be quantitatively determined and recovered from the cigarette tobacco samples by using the method described. The recovery of an analyte as a function of its concentration should ensure the reliability of the results, presenting recoveries around 75 to 125% and a 32% maximum relative standard deviation [20].

Table 3 presents the results of 3 fortifications (Fort.) in 5 replications evaluated to recover the arsenic in the cigarette tobacco.

Table 4 presents the results of arsenic determinations and Standard Deviation (SD) for the 30 brands of smuggled cigarettes with the brands identified as A1 - A30.

Table 3. Result of the arsenic recovery test in the tobacco of smuggled cigarettes.

	Fort. 1	Fort. 2	Fort. 3
Fortified value ng g^{-1}	4.0	8.0	16.0
Measured value ng g^{-1}	4.1	7.6	15.5
Recovery %	103.2	94.9	96.5
Relative Standard Deviation %	10.1	4.3	3.6

Table 4. Result of the arsenic concentrations (ng g^{-1}) in mass of dry tobacco of smuggled cigarettes. Mean ± SD, n= 3.

	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15
Mean ± SD	38.5 ± 0.020	20.6 ± 0.034	25.4 ± 0.038	25.3 ± 0.040	20.9 ± 0.013	22.7 ± 0.037	22.8 ± 0.045	28.2 ± 0.034	19.0 ± 0.022	17.8 ± 0.034	23.2 ± 0.030	23.8 ± 0.024	17.5 ± 0.032	28.3 ± 0.026	0.61 ± 0.002

	A16	A17	A18	A19	A20	A21	A22	A23	A24	A25	A26	A27	A28	A29	A30
Mean ± SD	22.4 ± 0.043	36.4 ± 0.071	20.1 ± 0.008	16.2 ± 0.002	25.2 ± 0.048	13.3 ± 0.015	15.9 ± 0.023	2.9 ± 0.001	21.3 ± 0.014	21.5 ± 0.027	10.9 ± 0.018	11.1 ± 0.017	10.7 ± 0.011	13.5 ± 0.021	11.1 ± 0.005

About 56% of the samples, including the brands A2, A5 and A9, which were the most seized of all brands (Table 1), presented concentrations above 20.0 ng g^{-1}, which means twice as much as the concentration found in legal cigarettes in Brazil (9.0 ± 0.02 ng g^{-1}) [24]. The ANOVA test was employed, which presented $p<0.05$ indicating the significantly higher difference of arsenic in the brands A1 and A17 when statistically compared with the remaining ones. The brands A1 and A17 presented, respectively, the highest arsenic concentrations 38.5 ± 0.02 and 36.4 ± 0.071 ng g^{-1}. Since about 20% of the arsenic is transferred from the tobacco to the smoke [23] and considering that each cigarette is about 0.68 g tobacco mass, one can say that for each cigarette consumed from brands A1 and A17, approximately 63.8 ng arsenic can be absorbed by the human body.

Comparing the average arsenic determined in smuggled cigarettes 18.9 ng $g^{-1}$with the average observed in legalized cigarettes in Brazil, the potential health risk for the users can be observed. In the human body, arsenic is readily absorbed through the ingestion or inhalation [10]. Studies have reported arsenic levels in the urine of children (4.2 ug g^{-1}) for non-smoking parents, and children with two smoking parents (13.0 ug g^{-1}) [11]. The male smokers exposure to arsenic is mainly related to reduced lung capacity when compared to women and non-smoker individuals. Another important factor is the significant incidence of squamous cell carcinoma of the lungs, vasoconstriction and cardiovascular diseases, skin cancers and dermal sensitization [10-11]. Cell necrosis might occur after intense absorption of arsenic compounds [25]. This occurs because arsenic induces DNA suppression causing chromosome anomalies, acting as a secondary mutagenic, genotoxic and carcinogenic agent, becoming toxic through the inactivation of several enzymes [26]. The arsenics are also cardiotoxic agents and present high affinity with proteins of the sulfhydryl group, which are directly involved in the cell metabolism. The arteriosclerosis as well as the blackfoot disease, which are peripheral vascular disturbances and result in gangrene of the body extremes are both associated to arsenic poisoning [26-27].

Gravimetric determinations

Table 5 presents mean humidity, total ash and insoluble ash content with standard deviation (SD) for the 30 brands of smuggled cigarettes.

Table 5. Humidity, total ash and insoluble ash content in percentage. Mean ± SD, n= 3.

	Humidity	Total ash	Insoluble ash		Humidity	Total ash	Insoluble ash
A1	17.28 ± 0.21	23.65 ± 1.3	8.96 ± 0.85	**A16**	12.5 ± 0.46	21.98±0.32	16.94±0.42
A2	18.34 ± 0.09	17.57 ± 0.31	1.84 ± 0.19	**A17**	16.67 ± 0.26	12.22±1.04	4.98±0.31
A3	13.17 ± 0.68	18.28 ± 0.34	2.36 ± 0.33	**A18**	16.39 ± 0.37	17.74±0.53	1.24±0.73
A4	22.28 ± 0.15	19.49 ± 1.59	4.17 ± 0.41	**A19**	18.94 ± 0.53	16.42±0.25	2.27±0.56
A5	20.26 ± 0.33	19.66 ± 0.38	3.53 ± 0.8	**A20**	14.17 ± 0.41	18.77±0.07	10.86±1.37
A6	15.41 ± 0.49	19.78 ± 0.29	4.19 ± 0.97	**A21**	13.37 ± 0.04	18.29±0.51	2.65±0.91
A7	14.73 ± 0.31	19.57 ± 0.88	3.01 ± 0.54	**A22**	15.07 ± 0.14	16.55±0.27	1.73±0.97
A8	11.91 ± 0.54	18.3 ± 0.84	2.69 ± 0.35	**A23**	16.8 ± 0.36	16.68±0.57	2.09±1.14
A9	13.48 ± 0.19	17.8 ± 0.05	2.3 ± 0.3	**A24**	14.66 ± 0.43	18.94±0.23	5.43±0.9
A10	14.86 ± 0.53	17.7 ± 0.43	1.82 ± 0.35	**A25**	14.04 ± 0.09	16.48±0.34	9.27±0.66
A11	18.46 ± 0.12	20.81 ± 0.36	2.52 ± 0.35	**A26**	21.10 ± 0.63	18.69±0.77	5.84±0.53
A12	15.45 ± 0.56	20.92 ± 0.69	5.45 ± 0.1	**A27**	20.35 ± 0.43	18.24±0.46	5.31±0.54
A13	14.26 ± 0.45	19.38 ± 0.46	8.45 ± 0.2	**A28**	20.04 ± 0.66	18.67±0.36	11.11±0.38
A14	17.67 ± 0.68	18.98 ± 0.15	9.84 ± 2.0	**A29**	10.67 ± 0.38	18.81±0.67	10.39±1.33
A15	14.66 ± 0.71	17.03 ± 0.19	8.46 ± 1.0	**A30**	12.17 ± 0.25	18.97±0.32	3.28±0.48

For the humidity analysis, the ANOVA test presented $p<0.05$, indicating the existence of a significant variation in humidity content when the average between brands was compared. It was observed that 53% of the brands presented humidity above 14%, indicated as ideal for tobacco conservation [13, 29]. Brands A4 22.28 ± 0.14% and A26 21.1 ± 0.62% outstood with the highest humidity content which was above that described as ideal (12 to 14%) for industrialized cigarettes [28-30].

Humidity is an important parameter to determine the industrialized tobacco quality, since excess humidity favors the proliferation of fungi and accelerates the cigarette degradation, producing undesirable substances which alter the product flavor and odor [13, 28]. Nicotine is a water-soluble alkaloid, high humidity contributes to the increase in the smoke particle size, which influences the conditions of absorption of chemical compounds which are quickly transferred to the tissues. Thus, particles larger than 0.3 µm can be absorbed directly by the mouth and throat [31-32].

Ajab et al (2014) determined the humidity content in 20 brands of cigarettes legally sold in Pakistan [33]. Their results revealed that 10 Pakistani brands presented 10.3% average value and 10 imported brands, which were legally sold in the same country, presented 8.8% on average. In legal cigarettes in the United States, the humidity content average found was 13 to 15% [34-35]. In Brazil, the humidity content found in legally sold cigarettes was 13.5% [36].

Therefore, it was possible to observe that brands A4 and A26 presented humidity content on average 7.7% above the cigarettes in the United States and Brazil and 12.1% above those sold in Pakistan. Thus, the characteristic under analysis might accelerate the tobacco decomposition process altering its composition, producing undesirable substances and possibly increasing the nicotine and other components release in the cigarette smoke, through the increase in particle size.

Regarding total ash, the ANOVA test presented $p<0.05$ indicating a significantly high variation when compared to the averages between brands. In Table 5 it is possible to see that 96% of the samples presented ash content above that recommended by the Brazilian regulation, whose maximum limit is 12% [37]. Brands A1 (23.6 ± 1.1%), A11 (20.8 ± 0.3%), A12 (20.9 ± 0.6%) and A16 (21.9 ± 0.3%) outstood presenting the highest total ash content percentage. Some metals were found in significantly higher concentrations in the smuggled cigarettes in Brazil when compared to the legal cigarettes [8, 38]. Ash content can be used as an indicator of the mineral salt amount and possible adulteration through inorganic compounds. High ash content suggests the presence of inorganic substances such as metallic oxides and silicates [15].

For insoluble ash determination, the ANOVA test was employed, which presented $p<0.05$ indicating the significantly higher presence of insoluble solids in the brands A1, A13, A14 A15, A16, A20, A25, A28 and A29 when statistically compared to the others. Table 5 shows that 53% of the samples presented over 4% insoluble ash content, which is the maximum limit recommended as the product hygiene ideal practice [37]. The highest insoluble ash content was found in brands A16 16.9 ± 0.4%, A28 11.1 ± 0.3% and A20 10.8 ± 1.3%. The insoluble ash determination indicated the presence of heavy dirtiness such as silicate present in the soil, resulting from unsuitable hygiene practices during the industrialization and storage processes [15].

pH determination

Table 6 presents the tobacco mean pH and sidestream smoke with standard deviation (SD).

Table 6. Smuggled cigarettes tobacco mean pH and sidestream smoke. Mean ± SD, n= 20.

	tobacco pH	smoke pH		tobacco pH	smoke pH
A1	8.06 ± 0.44	9.33 ± 0.18	**A16**	7.18 ± 0.06	7.83 ± 0.25
A2	8.05 ± 0.33	9.13 ± 0.19	**A17**	4.62 ± 0.46	6.69 ± 0.33
A3	4.96 ± 0.05	8.14 ± 0.36	**A18**	5.28 ± 0.11	8.46 ± 0.34
A4	5.04 ± 0.09	8.65 ± 0.31	**A19**	5.33 ± 0.05	8.29 ± 0.31
A5	5.01 ± 0.65	8.22 ± 0.34	**A20**	5.17 ± 0.28	8.04 ± 0.33
A6	7.43 ± 0.21	8.62 ± 0.28	**A21**	5.22 ± 0.15	8.32 ± 0.32
A7	7.31 ± 0.50	9.01 ± 0.23	**A22**	5.34 ± 0.25	6.67 ± 0.34
A8	5.35 ± 0.23	8.54 ± 0.25	**A23**	4.91 ± 0.05	7.86 ± 0.21
A9	7.72 ± 0.03	8.56 ± 0.33	**A24**	5.15 ± 0.52	8.58 ± 0.29
A10	5.1 ± 0.07	8.21 ± 0.26	**A25**	5.04 ± 0.02	7.73 ± 0.34
A11	5.66 ± 0.11	7.74 ± 0.26	**A26**	5.18 ± 0.03	8.31 ± 0.2
A12	5.21 ± 0.09	8.81 ± 0.33	**A27**	4.77 ± 0.05	8.15 ± 0.32
A13	5.76 ± 0.11	8.34 ± 0.36	**A28**	5.59 ± 0.04	8.47 ± 0.23
A14	5.54 ± 0.34	8.36 ± 0.19	**A29**	5.13 ± 0.15	7.52 ± 0.35
A15	5.21± 0.03	6.97 ± 0.24	**A30**	5.34 ± 0.04	8.41 ± 0.31

For the analysis of pH, the ANOVA test presented $p<0.05$ indicating significantly high variation in the tobacco pH values and in the cigarette smoke. When the brand averages were compared, statistically different groups were found in both cases, from which the group formed by the brands A1, A2, A7 and A12 outstood for presenting the highest pH averages in the smoke released when the tobacco burned. Table 6 reveals that 90% of the brands presented sidestream smoke pH above 7.0. Studies show that alkaline sidestream smoke is able to release nicotine in free form, which is a way that is more absorbed by the human body [17, 22] and considering the bill that intends to ban cigarettes which produce alkaline smoke, these brands are the most harmful to the human health [16]. Therefore, brands A1 8.06 ± 0.44 and A2 8.05 ± 0.33 were shown to have the highest tobacco pH values, in Brazil the cigarette components pH are around (5.3) [38]. Therefore, brands A1 and A2 were considerably above the levels suggested as quality standard by some of the regulating agencies [30]. Brands A1 9.33 ± 0.18 and A2 9.13 ± 0.19 were the most alkaline regarding the smoke they released. Regarding Brazilian legal cigarettes, the pH value for the smoke released was approximately 5.9, and therefore, the brands A1 and A2 were significantly above the levels previously found in legal cigarettes [38].

The tobacco pH is important because depending on its acidity or alkalinity, the nicotine can be found in different protonation ways. Several substances such as sugar and scents are added to the cigarettes aiming to disguise their unpleasant flavor and odor and make them more appealing [17]. For this reason ANVISA has been studying the possibility of eliminating or reducing the addition of such products to cigarette tobacco [16]. Besides, some additives such as ammonium salts might alter the tobacco pH and, consequently, the cigarette smoke [17, 39]. The diprotonated nicotine is found in the pH band (0 to 5) as a bound non extractable salt, and the intermediary monoprotonated form is in the pH band (3 to 9) and the deprotonated free nicotine is in the band (7 to 12) [39].

Therefore, it was possible to observe that the smuggled cigarette tobacco and sidestream smoke pH of brands (A1 and A2) were potential sources of deprotonated nicotine, because they had more alkaline sidestream smoke. This nicotine conformation at pH 7 to 12 is the one that is most available to the human organism, and easily absorbed by lungs and gastrointestinal tract [17, 31, 39]. Nicotine stimulates autonomic ganglia and the central nervous system [40]. Therefore, users of these brands are likely to become more and more addicted to them due to the higher nicotine offer.

Dirtiness determination

Figure 1 represents the main kinds of lightweight dirtiness found in the thirty smuggled cigarette brands through microscopic analysis.

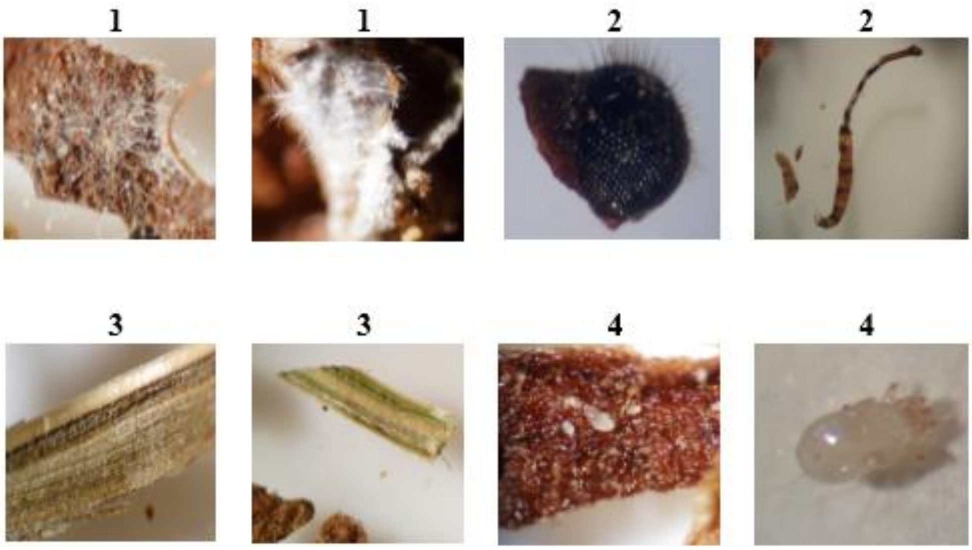

Figure 1. Kinds of contaminants detected in smuggled cigarettes (1) fungi; (2) insect fragments; (3) Grass; (4) mites.

Table 7 presents the amount of lightweight dirtiness observed in twenty cigarette units, which represent 13.6 ± 2.0 g of the tobacco analyzed from each brand. Where: F = Fungi; I= Insect fragments; G= Grass and M= Mites.

Table 7. Number of cigarettes contaminated in relation to the most significant amount of dirtiness found.

	A1	A2	A3	A4	A5	A6	A9	A10	A11	A12	A13	A14	A15	A16	A17	A19	A20	A23	A24	A26	A27	A28	A29	A30
F	2 - U	-	4 - U	8 - U	*	*	*	10 - U	5 - U	*	*	*	*	19 - U	*	*	*	*	2 - U	*	9 - U	2 - U	*	*
I	*	*	2 - 2	4 - 4	4 - 4	4 - 4	*	2 - 2	2 - 2	2 - 2	2 - 2	3 - 4	*	*	*	2 - 3	*	*	*	*	2 - 2	2 - 2	2 - 2	2 - 2
G	*	2 - U	2 - U	*	*	*	*	*	4 - U	*	9 - U	*	4 - U	*	7- U	-	4 - U	2 - U	*	*	*	10 - U	*	*
M	*	*	*	*	*	4-8	19 - U	4 -10	*	*	*	15 - U	*	*	*	6 - 21	*	*	6 - 16	19 - U	8 – 20	*	9 - 24	*

U= uncountable amount of dirtiness. *= not found

According to the results presented in Table 7 and Figure1, 30% of the brands presented fungi contamination. Brands A16 and A10 presented, respectively, 95% and 50% cigarettes contaminated by fungi colonies. A study carried out in the United States with 14 different cigarette brands revealed that 37% of the samples were contaminated by fungi. The exposure to fungal spores in the air is considered an important risk factor to develop infection mainly in patients with a weakened immunologic system [41].

Insect fragments were found in 46.6% of the brands. Brands A4, A5 and A6 outstood for presenting 20% contaminated samples. According to ANVISA (2014) [42], the presence of insect fragments reveal unsuitable handling practices of these materials and the maximum limit is 1 fragment for each 10 g of the material under analysis. In this sense, it was possible to see that brands A4, A5 and A6 excess was twice as high as the value indicated as good hygiene practice.

Plant leaves, different from tobacco were also found, representing grass contamination in 46.5% of the samples under analysis. Brands A28 and A13 presented the highest levels of contamination with this kind of dirtiness totaling 50 and 45%, respectively. Grass is not harmful to the human health when consumed in the form of cigarette; however, it is an indicative that the product was not produced in ideal hygiene conditions. In such case, during the cigarette production, different plants could have been introduced as a volume agent [43].

Mites were found in 30% of the brands under analysis, and brands A9 and A26 presented mite contamination in 95% of the samples. Mites do not represent direct risk to the smoker. However, dead mites or their feces might contain substances that when inhaled, together with the smoke while smoking, might trigger allergic reactions [44].

The dirtiness tests revealed that 81.2% of the brands presented some kind of contamination such as: fungi, insect fragments, grass or mites above the levels indicated as good hygiene practices by ANVISA.

Chemometric analysis

The PCA was calculated by using arsenic, total ash, insoluble ash, humidity, tobacco pH and tobacco smoke pH as variables. The concentrations obtained from the samples of thirty smuggled cigarette brands were used as observation. The original data was used to generate the multivariate regression mode and the analysis was carried out through the correlation matrix. Both principal components explained 65.3% of the data total variance, the first component was responsible for the explanation for the 38.1% when variables were related. The explanation for 65.3% variance between data for both components is satisfactory, due to the fact that the variables under study depend on several factors, such as the production conditions, manufacturer, etc. Figure 2 shows the Biplot graph (scores and loadings) relating the two principal components. The scores are represented by the number of brands A1 to A30 and represent the spatial distribution and the relationship between samples and manufacturers. The loadings are represented by the variables arsenic, total ash, insoluble ash, humidity, tobacco pH and tobacco smoke pH, which allow the visualization and understanding of relations between variables.

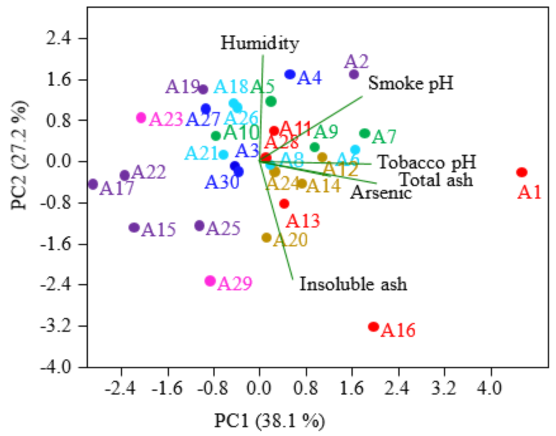

Figure 2: Biplot for PC1 and PC2 obtained from the analysis of multivariate data of the 30 samples of smuggled cigarette different brands. The colors refer to the different manufacturers. Manufacturers: (1) purple; (2) green, (3) brown, (4) light blue, (5) blue, (6) red and (7) pink.

Figure 3 presents the correlation matrix coefficients (loadings) for each variable. In figure 3, it is possible to see that the PC1 is positively correlated to the variables arsenic, total ash, tobacco pH and sidestream smoke pH. While PC2 is positively correlated to the variable humidity and inversely correlated to the variable insoluble ash.

Figure 2 shows that manufacturer 6's brands had the points grouped in the PC1 positive axis, and high content of total ash was verified while some brands were correlated to the PC2 negative axis and presented high insoluble ash content. There was some agglomeration of the points representing manufacturer 3's brands in the PC1 positive axis and PC2 negative axis, which indicates that these manufacturer's brands tend to present high content of insoluble ash, total ash, arsenic, tobacco and sidestream smoke more alkaline pH. However, it was also seen that for these brands, the humidity average content was relatively lower, 15.49%. High ash content suggests the presence of inorganic substances, such as the oxides and silicates containing heavy metals, another indication of contamination is the high arsenic content, >20.0 ng g^{-1} and insoluble ash, >5%, found in this manufacturer's brands[5, 18]. Regarding manufacturer 1's samples, except for brand A2, the points were grouped in the PC1 negative axis, and the cigarettes tended to produce sidestream smoke presenting more alkaline pH, however, this manufacturer's most sold brand presented the most alkaline pH when compared to all the other brands under study. Alkaline cigarettes are known to release free nicotine, which might result in increase in nicotine chemical dependency [17]. Therefore, brand A2 is one of the brands with the most alkaline smoke, and the most seized among the manufacturer 1's brands [6, 18]. The remaining samples did not present significant correlation between the brand characteristics and manufacturers.

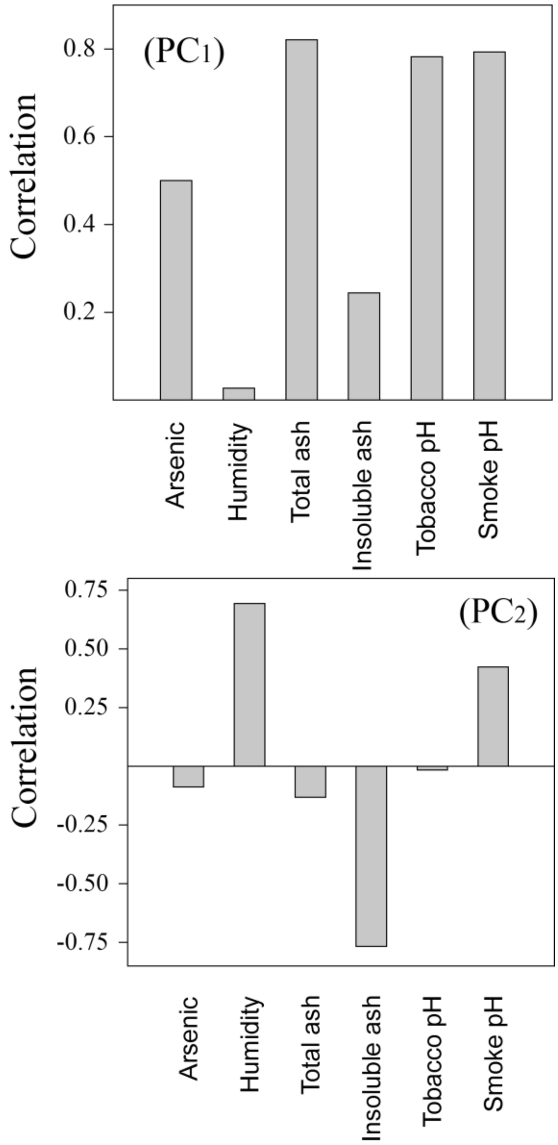

Figure 3. Histogram of loadings obtained through PCA

Since the 30 brands of smuggled cigarettes evaluated in this study were representative samples of the main cigarette brands which enter irregularly in Brazil, one can say that the smuggled cigarettes can increase the damage to the smokers' health, when compared to legally sold cigarettes.

4. CONCLUSION

The methodology used to quantify arsenic was satisfactory. It presented quantification limit of 15.0 ng g^{-1} and detection limit of 4.0 ng g^{-1} in dry tobacco mass. The arsenic recovery for the method purpose was 98.2% and relative standard deviation 6.0%. The results obtained pointed out that 56% of the cigarette brands presented arsenic contamination above 20.0 ng g^{-1} on average, which means twice as much as the concentration found in legal cigarettes in Brazil.

Levels above of the recommended value for humidity (14%) were found in 53% of the brands and thus the smoke particle distribution might have been altered during the act of smoking. About 96% of the brands presented total ash content above 12% which is the maximum limit indicated by the Brazilian Pharmacopoeia. About 53% of the samples presented over 4% insoluble ash, being above the tolerable limit. About 90% of the samples were verified to have smoke pH above 7.0, and two cigarette brands presented tobacco with pH above 8.0; and two brands presented sidestream smoke pH above 9.0. The most consumed brand in Brazil (A2) was the second most alkaline cigarette regarding both conditions, and this is a potential factor for the increase in addiction. In dirtiness contamination analysis, 81.2% of the brands presented some kind of contamination, among which were fungi, insect fragments, grass and mites and, therefore, did not meet the minimum requirements for suitable hygiene practices.

The characteristics observed revealed the low quality of smuggled cigarettes and consequently, their consumption might represent additional risk to the consumers' health.

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