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Preliminary Findings on the Salivary Metabolome of Hookah and Cigarette Smokers

Ellen Greenfield, Mariana de Sá Alves, Fernanda Rodrigues, Júlia Oliveira Nogueira, Letícia Ferrari da Silva, Henrique Pereira de Jesus, Desiree Rosa Cavalcanti, Bruna Fernandes do Carmo Carvalho, Janete Dias Almeida, Maria Anita Mendes, and Mônica Ghislaine Oliveira Alves*

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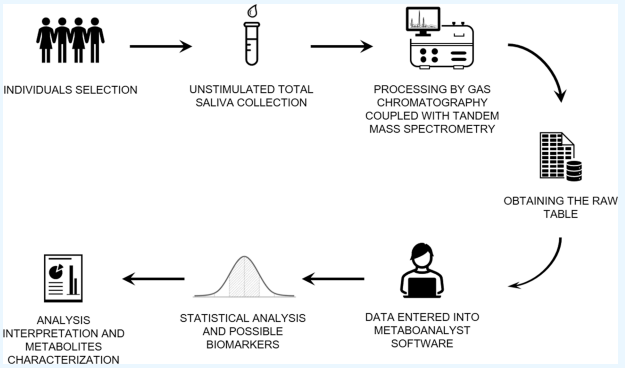
ABSTRACT: The aim of the study was to evaluate the salivary metabolomic profile of patients who habitually smoke hookah and cigarettes. The groups consisted of 33 regular and exclusive hookah smokers, 26 regular and exclusive cigarette smokers, and 30 nonsmokers. Unstimulated whole saliva was collected for the measurement of salivary metabolites by gas chromatography coupled with tandem mass spectrometry (GC−MS/MS). The MetaboAnalyst software was used for statistical analysis and evaluation of biomarkers. 11 smoking salivary biomarkers were identified using the area under receiving-operator curver criterion and threshold of 0.9. Xylitol and octadecanol were higher in cigarette smokers compared to controls; arabitol and maltose were higher in controls compared to cigarette smokers; octadecanol and tyramine were higher in hookah smokers compared to controls; phenylalanine was higher in controls compared to hookah smokers; and fructose, isocitric acid, glucuronic acid, tryptamine, maltose, tyramine, and 3-hydroxyisolvaleric acid were higher in hookah smokers compared to cigarettes smokers. Conclusions: The evaluation of the salivary metabolome of hookah smokers, showing separation between the groups, especially between the control versus hookah groups and cigarette versus hookah groups, and it seems to demonstrate that the use of hookah tobacco is more damaging to health.



INTRODUCTION

According to the World Health Organization (WHO), tobacco is a major global public health threat, killing about 8 million people every year, with 7 million of these deaths caused by the direct use of tobacco.[1] In addition, smoking is considered the greatest risk factor for the development of several malignant neoplasms and is also associated with the development and the complication of several chronic diseases.[2] In Brazil, an increase in the number of hookah consumption has been observed in recent years, especially in young people.[3,4]

Like the use of tobacco in conventional cigarettes, the use of hookah tobacco is also harmful to health, but users know little about these risks.[1] In Brazil, an increase in the number of hookah places throughout the country has been observed in recent years, which are a great attraction, especially for youngsters.[3,5] The rate of hookah experimentation (59.6%) is high among young people (average age of 23 years).[6]

About 300 chemical compounds have been identified in hookah smoke; 82 of them are toxic substances, and of these, 23 are carcinogens, including polycyclic aromatic hydrocarbons, heterocyclic compounds, primary aromatic amines, N-heterocyclic amines, tobacco-specific nitrosamines, and metals, as well as nicotine that causes chemical dependency.[7] Thus, hookah smoke contains large amounts of toxic substances that are known to cause diseases, such as cancer, in addition to chemical dependency. Moreover, tobacco consumption through hookah smoking is also associated with oral, esophageal, lung, gastric, and bladder cancers.[8]

Saliva is an aqueous solution present in the oral cavity. It is a complex fluid mix, consisting of several substances of different molecular weights and 99% of water, also containing bacteria, metabolites, epithelial desquamated cells, and food remains. It presents functions such as digestion, speech, mastication, swallowing, tissue protection, and also acting in the regulation of ionic balance in the remineralization of teeth. It can undergo changes due to hormonal issues, diet medication, and habits,

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such as smoking and alcoholism. Saliva can be used as an object of study and for diagnosis, since it is easy to collect and store in a noninvasive and low-cost way. In this sense, salivary diagnosis can be applied to detect various diseases through biomarkers.[9−12]

Perturbed biochemical functions associated with tobacco smoking can be investigated using omics platforms coupled with knowledge-based bioinformatics tools, and metabolic profile evaluation can be applied to next-generation tobacco and nicotine products for comparative risk assessment.[13] Metabolites are the end products of cellular regulatory processes, and their levels can be considered the final response of the biological system to genetic or environmental changes. The set of metabolites synthesized by a biological system constitutes its "metabolome."[14] The metabolites identified may serve as potential biomarkers to evaluate the status of smoking cessation and characterize smoking-related diseases.[15]

The area of studies of identification of metabolites related to the exposure to xenobiotics and the exposure time, exposome,[16] is increasing,[17] since it is important comparing the risk of novel tobacco products against conventional tobacco.[13]

The ability to monitor the health status and the onset and progression of diseases by noninvasive methods is an extremely desirable goal in terms of the promotion of health and the provision of healthcare. Saliva can be explored for health and disease surveillance, permitting valuable applications and providing new opportunities for the understanding not only of oral diseases but also of other systemic disorders.[18] Within this context, the aim of the present study was to compare the salivary metabolomic profile between hookah smokers, cigarette smokers, and nonsmokers.

EXPERIMENTAL SECTION

Selection, Preparation, and Collection of Samples. This study was approved by the Ethics Committee (Approval number 4.964.698). The samples were obtained by whole saliva collection from the following groups:

- Hookah group: exclusive smokers of hookah, with at least two sessions per week[19] for 2 years.[20]
- Cigarette group: exclusive smokers of manufactured cigarettes who have smoked at least 10 cigarettes per day for 2 years.
- Control group: nonsmokers who were not regular passive smokers according to the information provided by the patient.

The participants were submitted to intra- and extraoral clinical examinations. A questionnaire with questions about current tobacco use, age at onset, type, quantity and duration of use, and consumption of other substances were included. The Alcohol Use Disorders Identification Test (AUDIT) was used to assess the alcohol-related risk score.[21] In the cigarette group, smoking load was quantified by calculating pack-years, which is the number of cigarettes smoked per day/20 multiplied for the number of years the subject had smoked.[22]

Inclusion criteria were: individuals of both sexes, between 18 and 40 years, healthy. Exclusion criteria were: individuals with a personal history of oral malignancy, who had weekly ingestion of more than three doses of alcoholic beverages, users of daily medications, illicit drugs and who had any oral[23] or systemic diseases.[13]

All samples were collected between 10:00 and 12:00 h, and the participants were asked to rinse their mouth with distilled water for 1 min, 10 min before sampling. The samples were collected at least 60 min after food intake and at least 12 h after alcohol consumption. The participants were sitting upright in an airy and tranquil environment, and unstimulated saliva was collected by expectoration into a disposable plastic tube (Falcon, Rio de Janeiro, Brazil).[24] The saliva samples were stored at −80 °C until the time of analysis.

Analysis of Salivary Metabolites. Unstimulated whole saliva was collected for the measurement of salivary metabolites by gas chromatography coupled with tandem mass spectrometry (GC−MS, TQ 8050, Shimadzu, Brazil). The table with the raw data obtained was loaded into the software of the MetaboAnalyst 4.0 platform (available at: metaboanalyst.ca/docs/About.xhtml, updated and maintained by Xia Lab at McGill University, Canada), where the following statistical analyses were performed: Partial least squares − discriminant analysis (PLS-DA) and creation of volcano plots, dendrograms, and heatmaps. Classic univariate ROC curve analysis and multivariate ROC plot-based exploratory analysis (Explorer) were also performed. Metabolites with an area under the curve higher than 0.9[25] and a p-value = 0.05 were considered significant. The p values to assess differences in metabolite concentrations between groups were corrected by the false discovery rate (FDR) analysis of Benjamin−Hockberg to consider several independent tests at a value of $q < 0.05$.[26] The QC sample was the mixture of all samples from the analyzed subjects and was run on the same batch as the experimental samples.

For the study of metabolites, the online platform Human Metabolome Database was used (HMDB, 2023).

RESULTS

There were 26 subjects in the cigarette group, 33 in the hookah group, and 30 in the control group. Table 1 shows the age, sex, tobacco use, duration of tobacco use, and alcohol-related risk score of the participants in the three groups. In the cigarette group, smoking history was 9.57 pack-years.

Sixty-five metabolites were identified (Table S1). The analysis was made in pairs of groups (control versus cigarette; control versus hookah; cigarette versus hookah). The partial least squares-discriminant analysis (PLS-DA) is shown in Figure 1 and the VIP score is given in Figure 2. The PLS-DA analyses demonstrated separation between groups. In the analysis between the control versus hookah and cigarette versus hookah groups, a significant separation was observed, which may demonstrate the greater harmfulness for the health of individuals who use hookah tobacco. In the analysis between the control versus cigarette groups, despite the separation between the groups, some cases were close to the control group. This may come from the fact that the individuals participating in the smoker group, users of industrialized cigarettes, were young smokers.

Figures S1−S3 show dendrograms and the heatmaps. Through the three analyses, it is possible to visualize the separation between the groups. It was observed again that, in the comparison between the cigarette versus hookah groups, there was a complete separation of the groups, a good separation between the control versus hookah groups, while the separation was, again, smaller in the comparison between the control versus cigarette groups. The hypothesis is that this smaller separation is due to the more recent consumption of

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Table 1. Profile (Age, Sex, Tobacco Use, Duration of Tobacco Use, and Alcohol-Related Risk Score) of the Patients in the Three Groups Studied[a]

		cigarette group $n = 26$	hookah group $n = 33$	control group $n = 30$
age (years)	average	27.44	21.69	25.60
	standard deviation	5.15	3.89	7.29
	range	20−36	18−31	18−35
sex	male	13	9	7
	female	13	24	23
tobacco consumption	average[b]	15.42	5.24	NA
	standard deviation[b]	5.19	2.02	NA
tobacco consumption period (years)	average[b]	12.42	4.03	NA
	standard deviation[b]	4.92	1.63	NA
alcohol-related risk score	low-risk drinking	20	20	27
	at-risk drinking	6	9	2
	hazardous drinking	0	2	0
	probable dependence	0	2	0

[a]NA: not applicable. [b]Tobacco consumption for the Hookah Group = number of sessions per week and for the Cigarette Group = number of cigarettes per day.

tobacco by cigarettes and the greater harmfulness of hookah smokers.

Figures S4−S6 show volcano plots, which demonstrate important features selected by this analysis, using the bending violation limit and t-test limit in this evaluation. The software takes as a basis one of the two groups used in the comparison to determine the concentration and dispersion of each metabolite with a significance value in both groups, represented by circles, which in red are high values and blue ones are low. It is possible to observe the presence of metabolites with a significance value presented in the study, as well as their concentrations and distribution in each group.

The univariate ROC curves and box plots of metabolites considered to be significant in the comparison between control versus cigarette, control versus hookah, and cigarette versus hookah are shown in Figure 3.

DISCUSSION

We observed good separation between the control versus cigarette, control versus hookah, and cigarette versus hookah groups in all analyses. Our analysis of biomarkers identified the following 11 metabolites that were significant salivary candidate biomarkers for smoking: xylitol, octadecanol, arabitol, maltose, tyramine, phenylalanine, fructose, glucuronic acid, isocitric acid, tryptamine, and 3-hydroxyisovaleric acid.

There is evidence that hookah smoking presents health risks similar to those of cigarette smoking.[27] Amer et al. concluded that hookah smoking has the same harmful effect on the oral mucosa as cigarette smoking, imposing a subtle but constant risk of oral carcinogenesis.[28] However, to the best of our knowledge, this is the first study that evaluates salivary metabolome of hookah smokers. And we highlight, metabolomics research can lead to the identification of new biomarkers related to specific health disorders, and saliva can serve as an informative substance in the armamentarium of metabolomic profiles.[29]

Studies were conducted using different mass spectrometry platforms, evaluating biofluids from cigarette smokers and nonsmokers.[17] Garcia-Perez et al. highlight the importance of identifying common pathways that discriminate smokers and nonsmokers, with nontargeted metabolomics being a powerful tool that can aid in this process of discovering biomarkers, employing multivariate statistics analysis methods in order to identify combinations of metabolites to discriminate metabolic profiles, and in the future, to identify molecules that can provide insight into biochemical mechanisms.[17]

Few studies have evaluated the salivary metabolome of different forms of smoking, observing different profiles between tobacco users and nonusers. As an example, Mueller et al. evaluated conventional cigarette smokers, and Aghila Rani et al. evaluated smokers of medwakh.[30,31]

Studies on oral squamous cell carcinoma have investigated salivary biomarkers by different spectroscopy methods in order to identify and validate deregulated metabolites. The results showed altered metabolic pathways and differences in the spectral signatures of the saliva between the groups compared.[32−36]

The usefulness of salivary diagnosis has also been reported, aiding the clinical detection and improving the prognosis of lesions with potential for malignant transformation,[37,38] emerging as a promising tool for early diagnosis.[38] However, all of the cited studies did not discuss the influence of smoking on this process.[32−38]

The PLS-DA, dendrogram, and heatmap analyses demonstrate the separation between the groups. Good separation was observed between the control versus hookah and cigarette versus hookah groups, which may indicate greater damage caused by hookah use. On the other hand, in the analysis of the control versus cigarette groups, despite the separation between groups, some cases were similar to the control group. This finding might be explained by the young age of the participants in the smoker group (mean age of 27.44 years), who consumed a mean number of 15.42 cigarettes per day, for an average of 12.42 years. This level of smoking (9.57 pack-years) may not have been sufficient to cause major alterations in these cases.[39]

The volcano plots and VIP scores show all potential salivary biomarkers, as well as their concentrations in each group and their comparisons. We discuss each metabolite below.

Xylitol was higher in smokers of cigarettes compared to the control group. This metabolite is a dietary five-carbon sugar alcohol that is not produced endogenously. It is found naturally in fruits and vegetables and can also be produced industrially. Its consumption gives a strong and refreshing impression, and the compound is therefore widely used in the food industry and in mouthwashes, toothpastes, and cosmetics. This metabolite can be found in saliva, feces, urine, blood, and cerebrospinal fluid. Associations with colorectal cancer and hepatocellular carcinoma have been reported.[40]

Li et al. studied the metabolic profile of tobacco leaves cultivated in two different regions and found xylitol in both samples.[41] Rainey et al. chemically characterized four dissolvable tobacco products, and xylitol was also identified.[42] These findings thus explain the higher frequency of xylitol in the cigarette smoker group in our study when compared to the control group.

Additionally, Rainey et al. explain that xylitol is commonly used as a sucrose substitute, mainly based on the assumption

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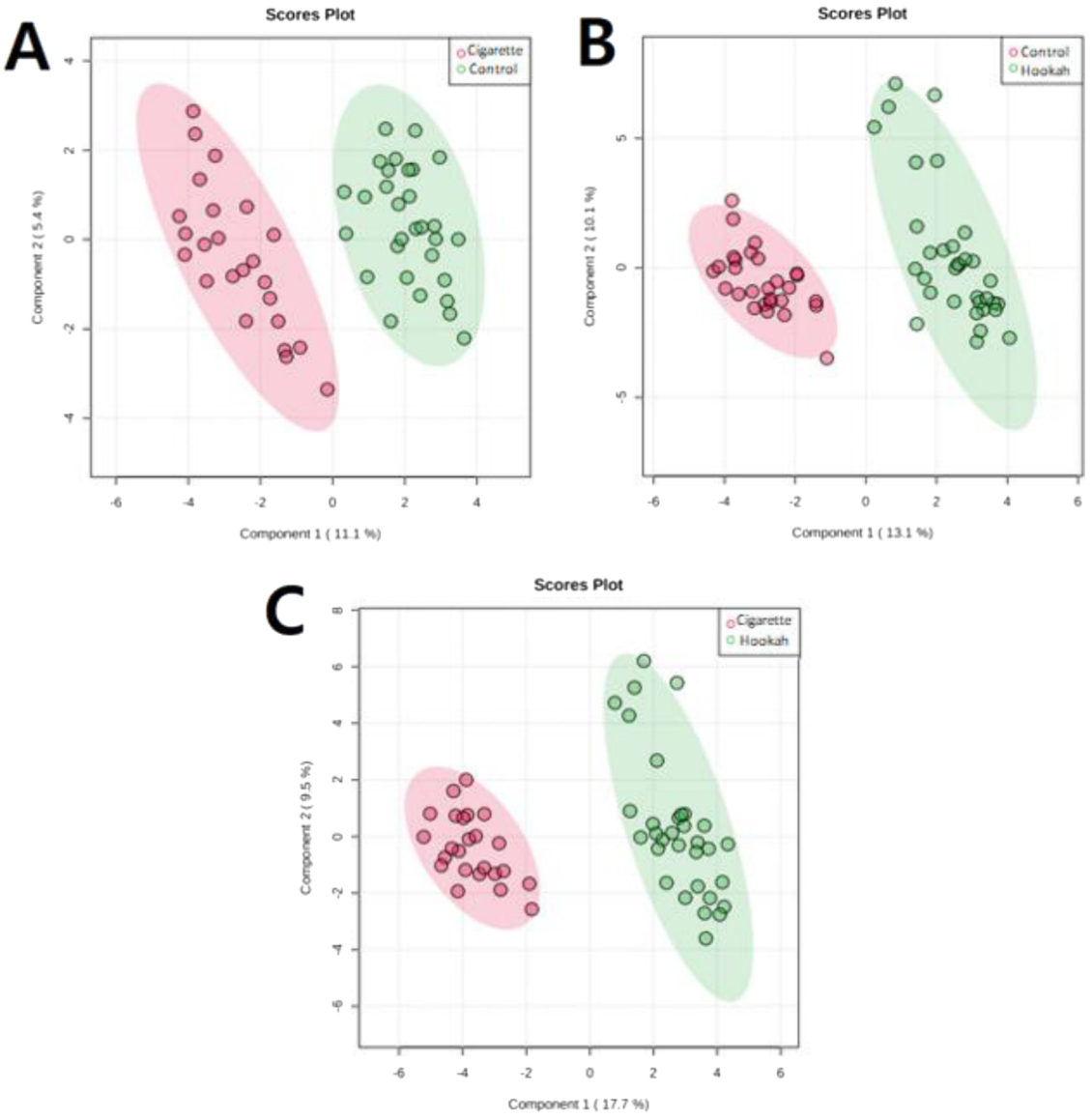

Figure 1. Partial least squares − discriminant analysis − PLS-DA. (A) Control versus cigarette. (B) Control versus hookah. (C) Cigarette versus hookah.

that it is supposedly less harmful to health. However, its consumption for long periods of time can result in the selection of *Streptococcus mutans* (a microorganism found in dental biofilms) resistant to xylitol, causing an increase in these oral bacteria.[42]

Octadecanol was found to be higher in both cigarette and hookah smokers when compared to the control group. Present in humans, this fatty alcohol is normally found in feces, urine, and saliva and has been associated with colorectal cancer.[43] Florin et al. identified octadecanol among the components of tobacco smoke and classified it as a nonmutagenic organic compound. However, the authors explain that, like other substances, octadecanol showed precipitation on the plates used in the mutagenicity test, a fact that makes interpretation of the results difficult.[44] This substance is also used in industry for its emollient characteristic, and emollient additives are also added as hookah essences.[45,46] In our study, higher concentrations of octadecanol were observed in both the hookah and the cigarette groups when compared to the control group, probably due to its presence in the tobacco consumed in this group, where, as mentioned above, it is introduced for its emollient characteristics.

Arabitol was found to be lower in cigarette smokers than in the control group. This sugar alcohol is present in all living species, from bacteria to humans. Several foods are sources of arabitol, including sweet potatoes, blackberries, moth beans, and European chestnuts, which may serve as a potential biomarker for the dietary intake of these foods. Arabitol can be found in saliva, feces, urine, blood, and cerebrospinal fluid. Associations with colorectal cancer and uremia have been reported.[47]

According to Kordowska-Wiater, arabitol can be obtained in two forms, as L-arabitol or D-arabitol, which both belong to the pentitol family along with xylitol and ribitol. Arabitol is widely used in the food industry as a low-calorie sweetener because of its similar sweetness to sucrose but much lower calorie value, as well as in the production of human therapeutics.[48] Furthermore, Yeo et al. found arabitol to be a marker for the early diagnosis and prognosis of severe fungal infections caused by *Candida*.[49] In our study, arabitol was

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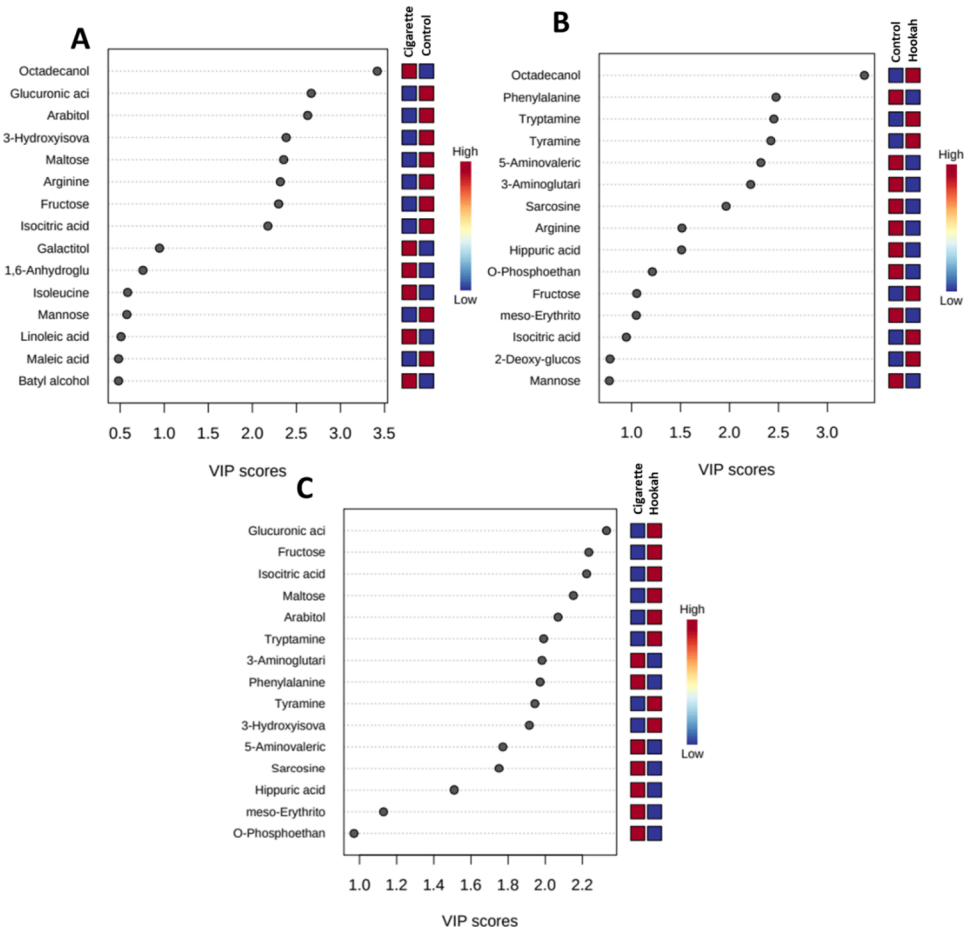

Figure 2. VIP scores (A) Control versus cigarette. (B) Control versus hookah. (C) Cigarette versus hookah.

increased in the control group when compared to smokers of manufactured cigarettes. We found no studies describing the relationship of arabitol with smoking; thus, this difference is possibly related to nutritional factors.

Maltose was lower in the group of cigarette smokers when compared to the control group and to the group of hookah smokers. Maltose, also known as alpha-malt sugar or cextromaltose, is an o-glycosyl compound found in all living species. In humans, this sugar participates in several enzymatic reactions. Outside the human body, maltose is found in foods such as fruit gums, marshmallows, oriental wheats, sweet potatoes, grape wine, spinach, cetaceans, and octopus and may therefore be a potential biomarker for the consumption of these foods. Maltose can be found in feces, urine, blood, and sweat and has been associated with colorectal cancer.[50]

Li et al. also detected maltose in both tobacco leaf samples.[41] Talhout et al.[51] analyzed the contribution of tobacco compounds to the aroma characteristics of Chinese cigarettes and, like Yin et al.[52] observed that, although sugars are natural components of tobacco, they are frequently added to tobacco during the manufacturing process to replace the sugars lost during curing in order to maintain a balanced flavor and neutralize the coarse sensation of smoke in the throat.[51,52] Another important factor is the pyrolysis of sugars, which break down into a mixture of organic acids and aldehydes. Qi and Tester concluded that maltose has health benefits by providing calories but is also associated with different health problems, including obesity, osmotic diarrhea, and dental caries. These findings suggest that the higher levels of maltose in the control group compared to the cigarette group are probably diet related, while the higher levels in the hookah group compared to the cigarette group are possibly related to high added sugars in hookah tobacco, which is famous for its variety of flavors and aromas.[53]

It is important to highlight that organic acids also act as precursors of a variety of inflammatory metabolites, as the eicosanoids, which are chemical messengers that act on the immune system. In inflammatory processes, one of the main fatty acids of interest is the arachidonic acid, a precursor of inflammatory eicosanoids.[54,55]

Higher levels of tyramine were found in the hookah group compared to the control group and the cigarette group. Tyramine belongs to the class of organic compounds known as phenethylamines and is a monoamine derived from the amino acid tyrosine, which is metabolized by the enzyme monoamine oxidase. In foods, tyramine is often produced by the decarboxylation of tyrosine during the process of fermentation or decomposition. Considerable amounts of this compound are found in certain foods such as fish, chocolate, alcoholic beverages, cheese, soy sauce, sauerkraut, and processed meat, which is considered a biomarker of cheese consumption. Tyramine can be found in feces, urine, and blood and has been associated with diseases such as colorectal cancer and periodontal diseases.[56]

In a metabolome profiling study of saliva from smokers, Mueller et al. demonstrated elevated levels of tyramine in saliva and concluded that its decomposition by monoamine oxidase is inhibited by smoking.[30] In our study, lower levels of

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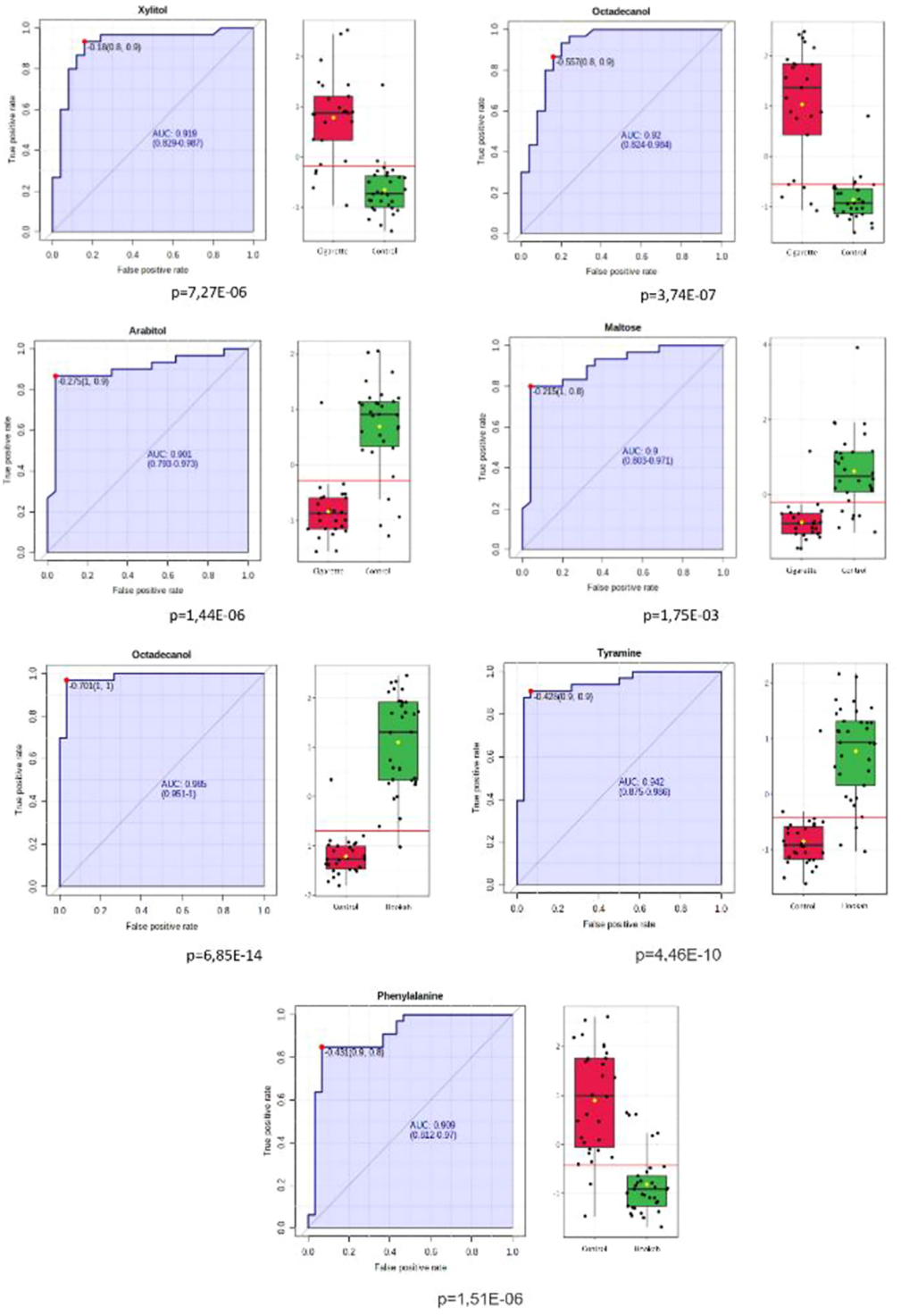

Figure 3. continued

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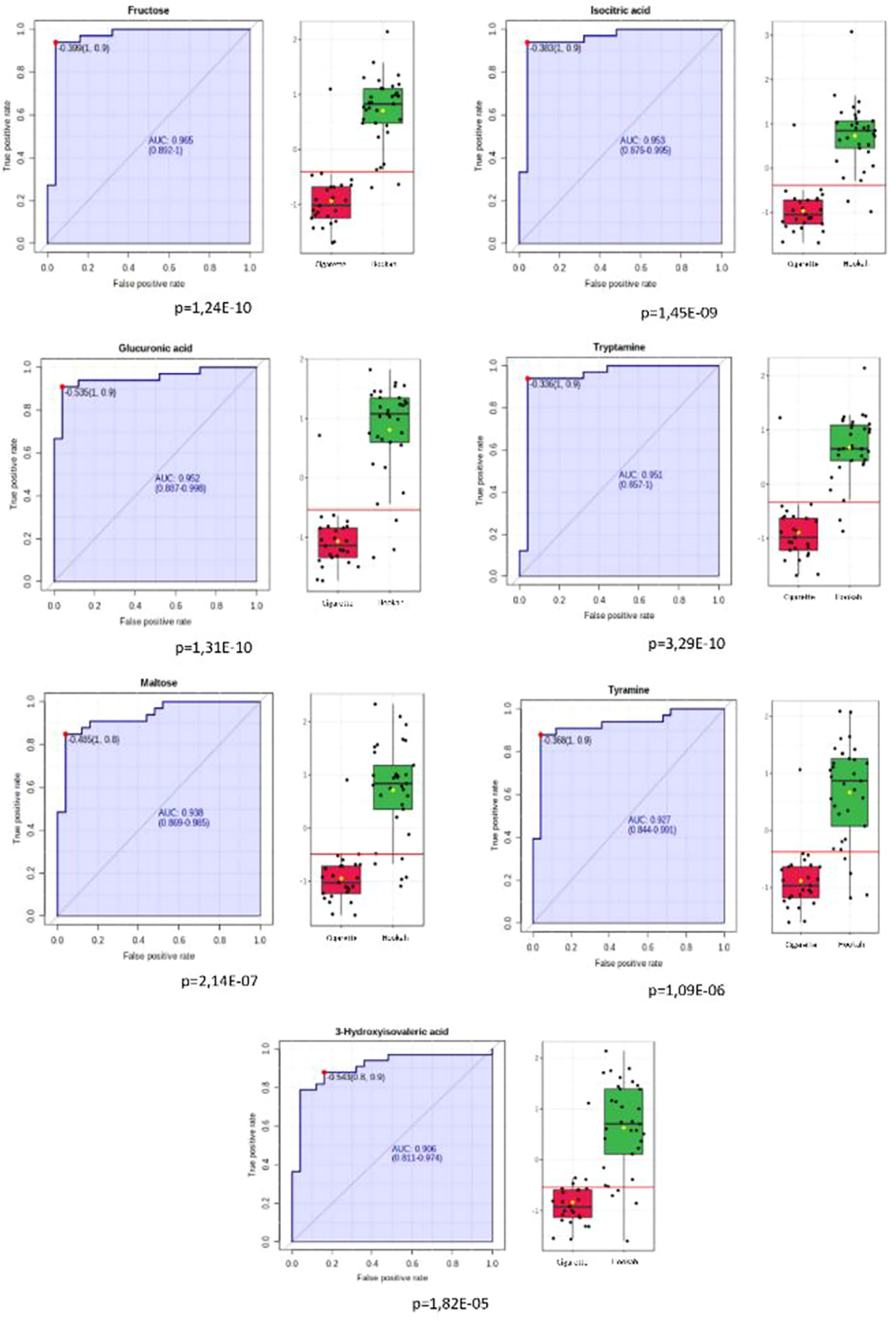

Figure 3. Univariate ROC curve and box plot of significant metabolites in the comparison of control versus cigarette, control versus hookah, and cigarette versus hookah.

tyramine were observed in the control group compared to the hookah group and higher levels in the hookah group compared to smokers of manufactured cigarettes, suggesting that hookah tobacco consumption may be even more susceptible to decomposition by monoamide oxidase.

Another hypothesis arose is that the increased levels of tyramine would be related to the large release of catecholamines during smoking, since tyramine acts in the production and the release of catecholamines.[57]

Phenylalanine was higher in the control group compared to the hookah group. This essential amino acid is a precursor of the amino acid tyrosine and catecholamines. Some psychotropic drugs contain phenylalanine. This amino acid is found in protein-rich foods such as meat, cottage cheese, and wheat germ. Another dietary source is artificial sweeteners containing aspartame. Phenylalanine is found in the human brain, plasma, saliva, urine, feces, blood, breast milk, sweat, and cerebrospinal fluid. When present at high concentrations in the body, this

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ACS Omega 2023, 8, 36845−36855

amino acid can act as a neurotoxin and as a metabotoxin. Phenylalanine has been associated with colorectal, pancreatic, breast and oral cancer, leukemia, epilepsy, dengue, heart attack, phenylketonuria, viral and bacterial infections, and periodontal diseases, among others.[58]

Takeda et al. studied the composition of the human salivary metabolome in smoker healthy patients. The authors detected phenylalanine in nonsmokers and, at lower concentrations, in the saliva of smokers.[59] Dame et al., who used different evaluation and identification methods in their characterization study of human saliva, also detected the presence of phenylalanine.[60] These data agree with the present study showing that hookah tobacco consumption reduced the concentrations of this metabolite in saliva.

Increased levels of fructose were observed in the hookah group compared to the cigarette group. This metabolite belongs to the class of organic compounds known as c-glycosyl compounds. Although fructose is a hexose, a 6-carbon sugar, it generally exists as a 5-member hemiketal ring, known as furanose, a structure responsible for the long metabolic pathway and high reactivity compared to glucose. Fructose is one of the three most important sugars and is found in many foods, such as honey, fruits, berries, melons, beets, sweet potatoes, and onions. The metabolite can be found in saliva, feces, urine, blood, and cerebrospinal fluid. Associations with colorectal cancer, type 2 diabetes mellitus, and eosinophilic esophagitis have been reported.[61]

Dame et al. also observed the presence of fructose in their study,[60] in addition to the view of Talhout et al. who describe the fate of sugars during smoking, particularly their effect on smoke composition. The authors explain that sugars are natural components of tobacco and that their levels can vary according to the curing process.[51] However, some manufacturers add sugars, claiming that they serve as a humectant and as a flavoring agent. Fructose is one of the most frequently used sugars. The addition of sugars also contributes to promote tobacco consumption by neutralizing the unpleasant taste and impact of smoke on the throat. In addition, sugars confer a sweet taste and pleasant smell, which particularly attract starting smokers.[51]

The authors also mention that, in addition to the adverse health effects caused by increasing tobacco consumption, products with high levels of sugar produce even higher levels of toxic components in smoke. During tobacco burning, sugars are pyrolyzed, generating highly toxic or carcinogenic degradation products such as formaldehyde, acetaldehyde, acetone, acrolein, 2-furfural, and other furans. According to the authors, these compounds are generally even more toxic when inhaled compared to their toxicity after ingestion since the respiratory system lacks the detoxifying metabolic pathways present in the digestive tract.[51] These findings are in line with data from our study in which high fructose levels were found in the hookah group compared to the cigarette group, since the tobacco used in hookah smoke is famous for its diversity of flavors, a fact that explains this increase.

Isocitric acid was higher in the hookah group compared to smokers of manufactured cigarettes. This metabolite belongs to the class of organic compounds called tricarboxylic acids and derivatives. It is found in all living species and is formed from citrate with the help of the enzyme aconitase. Isocitric acid was detected in different foods such as blackcurrant, wild celery, soursop, and apple and is a potential biomarker for the consumption of these foods. Isocitrate can be found in semen, urine, saliva, blood, and cerebrospinal fluid and has been associated with diseases such as anoxia, Alzheimer's disease, front temporal dementia, polycystic kidney disease, and eosinophilic esophagitis.[62]

Vickery and Abrahams studied the effect of cultured tobacco leaves on D-isocitrate solutions and noted that, although usually not present in tobacco leaves at detectable levels, D-isocitric acid is the main product of citric acid.[63] Dame et al. also detected the presence of isocitric acid in human saliva.[60] Zhang et al. investigated patients with esophageal squamous cell carcinoma and identified an increase in isocitric acid. The authors explain that citric acid and isocitric acid are intermediates of the tricarboxylic acid cycle and of the glyoxylate and dicarboxylate metabolic pathway and suggest that energy metabolism is involved in the regulatory mechanism of sensitivity to neoadjuvant therapy for esophageal cancer.[64] Our study demonstrated a higher concentration of isocitric acid in the hookah group compared to the cigarette group, suggesting that hookah smoking is more harmful in terms of the production of organic acids, with possibly greater implications for the tricarboxylic acid cycle.

Glucuronic acid was higher in the hookah group compared to the cigarette group. This metabolite is a carboxylic acid. In humans, it is often associated with toxic substances and hormones. Glucuronic acid can be found in feces, urine, and blood.[65] In the study of Li et al., glucuronic acid was detected in both tobacco leaf samples analyzed.[41]

Substances foreign to the organism, known as xenobics, are subject to one or several metabolic processes that constitute the oxidation of phase 1 and the conjugation of phase 2. These metabolic processes happen to eliminate the xenobics substances from the body more easily, and, among them, the glucuronidation is, in quantitative terms, considered the most important conjugation reaction.[66,67] Bezabeh et al.[68] studied human bile and observed highly elevated levels in bile samples of patients with pancreatic cancer. The authors highlighted that glucuronic acid is synthesized in the liver and is involved in a series of important detoxification pathways, removing a variety of drugs, environmental toxins, and carcinogens from the human body.[56] Our results suggest that hookah appears to increase the release of free glucuronic acid even when compared to cigarettes, which is an important finding whose cause, however, is still unknown. Additionally, studies with menthol cigarettes show that beyond several other metabolic implications associated with toxicity of carcinogens, menthol inhibited rates of NNAL-O-glucuronidation and NNAL-N-glucuronidation.[69,70]

Tryptamine was higher in the hookah group compared to the group of cigarette smokers. It is a tryptophan catabolite converted by the gut microbiota and belongs to the class of tryptamines and derivatives. Tryptamine is a monoamide compound and a common precursor molecule of many hormones and neurotransmitters. Outside the human body, tryptamine was found in foods such as vegetables of the onion family, acerola, Japanese walnuts, and green zucchini and may therefore serve as a potential biomarker for the consumption of these foods. Tryptamine can be found in blood, feces, and urine. Associations with colorectal cancer and irritable bowel syndrome have been reported.[71]

Songstad et al. observed high accumulation of tryptamine in transgenic tobacco.[72] There is a wide variety of tobacco crops, a fact that may explain the difference in tryptamine levels

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observed in our study, which were higher in the hookah group than in the cigarette group, as also reported in the literature.

3-Hydroxyisovaleric acid was higher in hookah smokers compared to cigarette smokers. This metabolite belongs to the class of organic compounds known as hydroxylated fatty acids. It can be found in feces, urine, blood, and cerebrospinal fluid and has been associated with diseases such as lung, colorectal, and pancreatic cancer and periodontal diseases. High concentrations of this metabolite are observed in smokers. 3-Hydroxyisovaleric acid is a normal human metabolite excreted in urine and a byproduct of the leucine degradation pathway. Its production starts with the conversion of 3-methylcrotonyl-CoA to 3-methylglutaconyl-CoA in the mitochondria by the biotin-dependent enzyme methylcrotonyl-CoA carboxylase. Some factors such as biotin deficiencies and certain lifestyle habits such as smoking can reduce the activity of methylcrotonyl-CoA carboxylase, which can lead to the accumulation of 3-methylcrotonyl-CoA that is converted to 3-hydroxyisovaleryl-CoA by enoyl-CoA hydratase. Increased concentrations of 3-methylcrotonyl-CoA and 3-hydroxyisovaleryl-CoA can lead to interruption of the esterified CoA ratio, and consequent mitochondrial toxicity.[73] At sufficiently high concentrations, 3-hydroxyisovaleric acid can act as an acidogen, with several adverse effects in many organ systems and an increase in these levels is associated with several inborn errors of the metabolism. Additionally, 3-hydroxyisovaleric acid is an organic acid and high levels of organic acids in blood, urine, brain, and other tissues cause general metabolic acidosis.[73]

Sealey et al. studied smokers and nonsmokers and observed that biotin catabolism is accelerated in female smokers. The authors explain that the demonstration of increased excretion of 3-hydroxyisovaleric acid, which accompanies biotin depletion, indicates that biotin status is reduced by the accelerated conversion of biotin to inactive metabolites.[74] In our study, the levels of 3-hydroxyisovaleric acid were upregulated in the hookah group compared to the cigarette group, which seems to demonstrate the greater harm of hookah in terms of biotin depletion in the body.

Further studies are necessary to validate and standardize the metabolic profile of cigarettes and hookah smokers in order to ensure the reliability of the salivary biomarkers. Another aspect that should be taken into consideration in future studies is the analysis of altered metabolic pathways in order to refine our understanding of the metabolic profiles of smokers.

In the future, the creation of databases with data on alterations in the salivary metabolome will be of great value to the identification and early diagnosis of diseases. Some limitations presented in the present study should be taken into account when developing new studies, such as a better assessment of the patients' diet, which could help to better understand its influence on the salivary metabolome.

CONCLUSIONS

In this study, the evaluation of the salivary metabolome of hookah smokers showed separation between the groups, especially between the control versus hookah groups and cigarette versus hookah groups, and it seems to demonstrate that the use of hookah tobacco is more damaging to health. In the near future, studies relating the metabolic profile with clinical data are needed.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c03683.

Experimental Section; Analysis of salivary metabolites; Results; Table S1. Sixty-five metabolites identified in 70% of the samples; Figure S1. Control versus cigarette. (A) Dendrogram. (B) Heatmap; Figure S2. Control versus hookah. (A) Dendrogram. (B) Heatmap; Figure S3. Cigarette versus hookah. (A) Dendrogram. (B) Heatmap; Figure S4. Volcano plot: control versus cigarette ($p \leq 0.05$; FDR < 0.05). Higher metabolites cigarette group are in red and lower in blue; Figure S5. Volcano plot: control versus hookah ($p \leq 0.05$; FDR < 0.05). Higher metabolites in hookah group are in red and lower in blue; Figure S6. Volcano plot: cigarette versus hookah ($p \leq 0.05$; FDR < 0.05). Higher metabolites cigarette group are in red and lower in blue (PDF)

AUTHOR INFORMATION

Corresponding Author

Mônica Ghislaine Oliveira Alves − *Technology Research Center (NPT), Universidade de Mogi das Cruzes, Mogi das Cruzes 08780-911, Brazil; Department of Biosciences and Oral Diagnosis, Institute of Science and Technology, São Paulo State University (UNESP), São Paulo 01049-010, Brazil;* orcid.org/0000-0002-3411-9443; Phone: +55 12 981278200; Email: mgoliveiraalves@gmail.com; Fax: +55 1239479010

Authors

Ellen Greenfield − *Technology Research Center (NPT), Universidade de Mogi das Cruzes, Mogi das Cruzes 08780-911, Brazil*

Mariana de Sá Alves − *Department of Biosciences and Oral Diagnosis, Institute of Science and Technology, São Paulo State University (UNESP), São Paulo 01049-010, Brazil*

Fernanda Rodrigues − *Technology Research Center (NPT), Universidade de Mogi das Cruzes, Mogi das Cruzes 08780-911, Brazil*

Júlia Oliveira Nogueira − *School of Dentistry, Universidade Mogi das Cruzes, Mogi das Cruzes 08780-911, Brazil*

Letícia Ferrari da Silva − *School of Dentistry, Universidade Mogi das Cruzes, Mogi das Cruzes 08780-911, Brazil*

Henrique Pereira de Jesus − *School of Dentistry, Universidade Mogi das Cruzes, Mogi das Cruzes 08780-911, Brazil*

Desiree Rosa Cavalcanti − *School of Dentistry, Universidade Mogi das Cruzes, Mogi das Cruzes 08780-911, Brazil*

Bruna Fernandes do Carmo Carvalho − *Department of Biosciences and Oral Diagnosis, Institute of Science and Technology, São Paulo State University (UNESP), São Paulo 01049-010, Brazil*

Janete Dias Almeida − *Department of Biosciences and Oral Diagnosis, Institute of Science and Technology, São Paulo State University (UNESP), São Paulo 01049-010, Brazil*

Maria Anita Mendes − *Dempster MS Lab, Department of Chemical Engineering, Polytechnic School, University of Sao Paulo, Sao Paulo 05508-900, Brazil*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.3c03683

Notes

The authors declare no competing financial interest.

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