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NITRITE CONTENTS IN VARIOUS MASTICATORIES PREVAILING IN THE STATE OF ASSAM – A PILOT STUDY

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ABSTRACT

Background: The habit of using masticatories is quite common among the population of the state Assam. There are numerous types of masticatories available throughout the state, most of which include areca nut in different forms, accompanied with beetle leaves and tobacco leaves and other associated substances added for flavor. **Aim:** To determine the level of nitrite present in different types of masticatories prevailing in the state. **Materials & Methods:** The classical Griess method was used for the estimation of nitrite in different types of these masticatories prevailing in the state Assam (n=35) using the basic diazotization principle and spectrophotometric determination. **Statistical Analysis used:** The results obtained were analyzed statistically by using SPSS V.16 software. **Results:** The result of the study indicate the amount of nitrite in different types of these masticatories, among which highest levels of nitrite was found in raw areca nut with beetle leaves without lime. The presence of lime decreases the amount of nitrite ($p<0.05$) whereas, the presence of beetle leaves and unprocessed areca nut increases the amount of nitrite ($p<0.05$) in the masticatories used by the people of the state. **Conclusion:** From the study conducted, it can be concluded that the presence of lime reduces the amount of nitrite in the masticatories, whereas the presence of beetle leaves and unprocessed areca nut contribute higher amount of nitrite in the masticatories.

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INTRODUCTION

Cancer prevalence in India is estimated to be approximately 2.5 million, in which around 8, 00,000 new cases reported and 5, 50,000 deaths reported per year [1]. Among the all the possible causes, the chronic use of betel quid found to be one of the strongly associated reason for its occurrence [2]−[4]. Consumption of betel leaves, areca nut and tobacco in different forms were very much common among different states of India including Assam, Orissa and Madhya Pradesh [5] – [10]. Betel quid chewing may cause high exposure to carcinogenic tobacco-specific nitrosamine and this level is much more if compared to a smoker [11] [12].

From the different studies conducted so far, it was confirmed that high level of nitrosamine is present in Asian foods as compared to Western foods [13]. Although, nitrite not being acting directly as a toxic substance indeed, but during the last few years more stress has been given in these area as this nitrite may lead to the formation of N-nitrosamines which possess teratogenic, mutagenic activities and thus acting as potent carcinogens [14] – [16]. Among different nitrosamines, two of them namely N-nitrosodiethylamine (NDEA) and N-nitrosodimethylamine (NDMA) were considered potent carcinogenic to humans reported by International Agency for Research on Cancer (IARC) [17]. NDMA is generally metabolized by cytochrome P-450 enzymes and it generates methyldiazonium ions and subsequent DNA adducts like predominantly *N*7-methylguanine and *O*6-methylguanine [18]. Similarity has been found between this mode of action and the crucial role of O6-methylguanine DNA-methyltransferase in rodents and humans [19].

In the present study, we have tried to gather different types of masticatories use by people of Assam and to find out the level of nitrite presence in them. As we have already mentioned the practice of chewing betel leaves, areca nut and tobacco is quite common among the people of the state and that may play a role in the generation of different types of potent carcinogen in human. After collecting the samples, they were being processed and were finally subjected to nitrite determination following the Griess method and Association of analytical communities (AOAC) official method of analysis of nitrite determination which is based on the diazocoupling reaction between sulphanilamide and N-(1-napthyl) ethylene diamine dihydrochloride [20]

Table 1: Sample code with description of the samples

Code	Description of the Sample
S-1	*Raw Areca nut* with *Betel leaves* without *Lime*
S-2	Raw Areca nut with Betel leaves with Lime
S-3	Raw Areca nut with Betel leaves, lime & *tobacco leaves*
S-4	*Fermented Areca nut* with Betel leaves without Lime
S-5	Fermented Areca nut with Betel leaves with Lime
S-6	Fermented Areca nut with Betel leaves with Lime and Tobacco leaves
S-7	*Dried Areca nut* with Betel leaves without Lime
S-8	Dried Areca nut with Betel leaves with Lime
S-9	*Mitha Paan*
S-10	*Zarda Pan*
S-11	Shikhar *pan masala* without *Zarda*
S-12	Shikhar pan masala with Zarda
S-13	Rajanigandha *pan masala* without Zarda
S-14	Rajanigandha pan masala with Zarda
S-15	Bubloo Supari
S-16	Sweety *Supari*
S-17	Parag pan masala
S-18	Dilruba pan masala without Zarda
S-19	Dilruba pan masala with Zarda
S-20	Kuber sweet Supari
S-21	Vimal pan masala with zarda
S-22	Vimal pan masala without zarda
S-23	Kamla Pasand pan masala with zarda
S-24	Kamla Pasand pan masala without zarda
S-25	Raj Niwas pan masala with zarda
S-26	Raj Niwas pan masala without zarda
S-27	Pan Bahar pan masala with zarda
S-28	Pan Bahar pan masala without zarda
S-29	Chutki Pan masala
S-30	Dilbaag pan masala with zarda
S-31	Dilbaag pan masala without zarda
S-32	Saras pan masala with zarda
S-33	Saras pan masala without zarda
S-34	Manikchand pan masala with zarda
S-35	Manikchand pan masala without zarda

MATERIALS AND METHODS

Chemicals: Sodium Nitrite, Phosphoric acid, Zinc sulphate were obtained from Himedia, India. Napthyl-ethylene diaminedihydrochloride (NED) and Sulphanilamide was obtained from Sigma Aldrich.

Sample description: All the 35 types of samples gathered from the different part of the Kamrup district of Assam. The samples were listed in the table1, where few of the names were highlighted in italics as they have different local names. The local names were described as: Raw Areca nut: Kesa Tamul, Betel Leaves: Paan, Lime: Suun, Tobacco Leaves: Sada Gura, Fermented Areca nut: Burha Tamul, Mitha Paan: Locally made blend of Betel leaves, Dried Areca Nut, Catechu, Lime, Clove, Cardamon and flavoring agents, Zarda Paan: Same as Mitha Paan but here chewing Tobacco leaves are mixed, Paan masala: product containing blend of Dried Areca nut, Catechu, Lime and other flavoring agents, Zarda: Chewing Tobacco, Rajanigandha, Shikhar, Dilruba, Parag, Vimal, Kamla Pasand, Raj Niwas, Pan Bahar, Chutki, Dilbaag, Saras, Manikchand: Commonly available brand of paan masala, Supari: Dried Areca nut, Bubloo, Sweety, Kuber: Product containing Dried Areca nut coated with sweeteners and flavoring agents.

Sample collection: The samples for the study were chosen based on availability and frequency of use by the peoples of the state. The samples were collected for a period of 6months in between October, 2015 to October 2016. Each type of sample was obtained at 5 separate random sources within Kamrup district of Assam.

Sample collection protocol: The protocols of the study include collection of each type of sample from five random sources for every six month and were divided into batch of six. Afterwards, the next step involved mixing of collected sample of same type obtained from five different sources for every month. The samples were crushed and mixed using a steel grinder (Classic, Phillips). These steps were followed for all the 35 different samples and further these samples were subjected to chemical treatment following the Griess method of nitrite determination.

Methodology: 10gm of crushed sample were taken from and mixed with 10ml of distilled water and crushed properly. Then the crushed sample was diluted to four fold with distilled water and $1/20^{th}$ volume of $ZnSO_4$ was added to the very same. This solution thus obtained was subjected to centrifugation at 9,500 rpm for 5 minutes and 5ml of supernatant was collected to which 5 ml of Griess reagent added and incubated at dark for 30 minutes, where the Griess reagent was made by mixing 1%NED and 1% Sulphanilamide in 5%Phosphoric acid. Standard curve was plotted by using Sodium nitrite after incubating it with Griess reagent and the concentrations of nitrite in the sample were determined at 540nm using UV-VIS spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). The aforementioned procedure was followed for all the 35samples divided in six batches and the average nitrite content for each sample was calculated [21] – [23].

Table 2: Sample code with amount of Nitrite present

Sample Code	Concentration (µg/gm)	Sample Code	Concentration (µg/gm)
S-1	234.36	S-19	186.64
S-2	128.04	S-20	202.56
S-3	129.56	S-21	131.61
S-4	206.72	S-22	128.95
S-5	148.44	S-23	136.76
S-6	182.6	S-24	132.48
S-7	199.44	S-25	145.83
S-8	106.48	S-26	140.11
S-9	139.52	S-27	143.66
S-10	169.72	S-28	141.79
S-11	114.6	S-29	119.5
S-12	129.56	S-30	148.95
S-13	141.6	S-31	140.11
S-14	142.32	S-32	149.22
S-15	108.16	S-33	139.21
S-16	129.56	S-34	138.64
S-17	115.28	S-35	130.14
S-18	178.12		

RESULTS AND DISCUSSION

The concentration of nitrite of the all the 35samples were found and they were presented in table2 and graphically in figure2 were expressed in µg/gm. From the result we have found that, the amount of nitrite production in the masticatories decreases in the presence of lime ($p<0.05$) and the presence of betel leaves in different types of masticatories contributes to the production of nitrite ($p<0.05$) represented in figure1(a) and 1(b). It has been also found that, the amount of nitrite is more in raw/fermented areca nut containing masticatories as compared to that of masticatories containing processed areca nut ($p<0.05$) represented in figure1(c). From our study conducted, we have found nitrite amount in different masticatories commonly used by the people of the state. Among the twenty different types of masticatories used for the study, highest amount of nitrite was found in raw areca nut

with betel leaves without lime, which was followed by fermented areca nut with betel leaves without lime and one brand of sweet supari. And, subsequently, one of the commonly used pan masala with zarda and the very same brand without zarda. The lowest amount of nitrite was found in dried areca nut with betel leaves with lime.

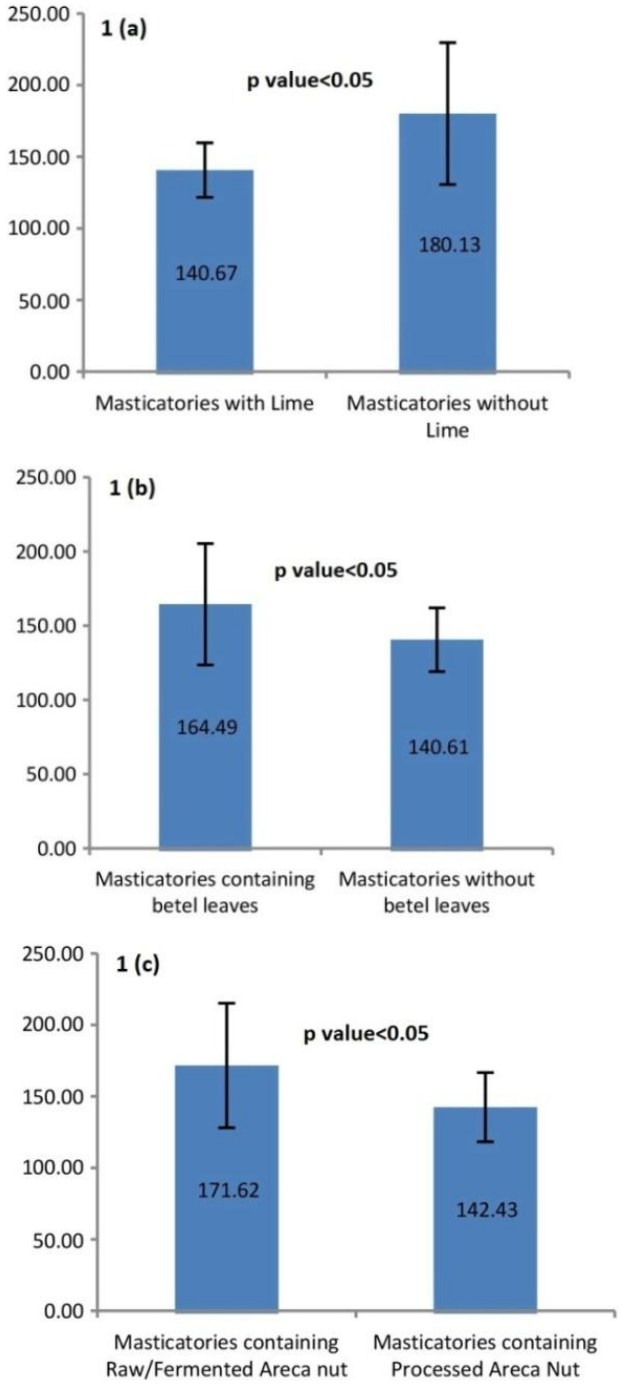

Figure:1 Graphical representation of mean nitrite content in a). Masticatories with lime and masticatories without lime, b). Masticatories containing betel leaves and masticatories without betel leaves, c). Masticatories containing Raw/ fermented Areca nut and Masticatories containing processed Areca nut.

Although in the present study, the amount of masticatories were taken in an amount of 10gm but this amount varies from person to person intake and frequency of intake of this items. The most important part of this result reflects a fact regarding the lime and the nitrite concentration released. The lime is in generally used by people to reduce the acrid taste of the areca nut, but here our result depicts that, the nitrite concentration decreases with the lime in present with different forms of areca nut and betel leaves. The nitrosamines are formed by the reaction of secondary or tertiary amines with suitable nitrosating species, as the nitrite ion by itself are very weak nitrosating agent, but under the acidic condition they get converted to nitrous anhydride, which reacts to form nitrosamine [24].

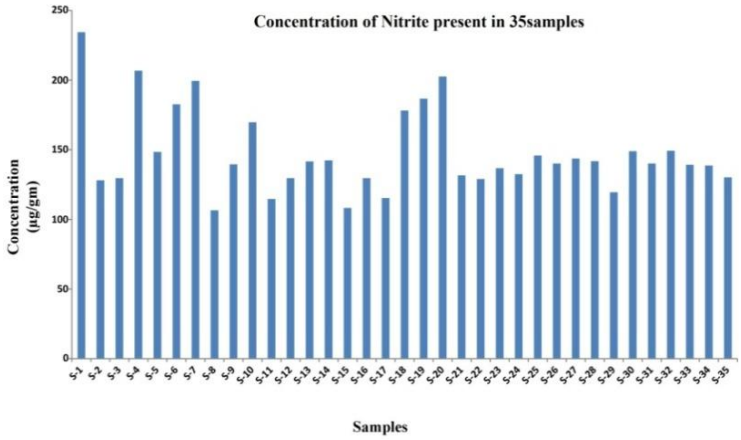

Figure:2 Graphical representation of average amount of nitrite of 35 different masticatories

Thus we can also say that, the presence of lime or increase of the pH may decrease production of some harmful nitrating agent within the human body. But by virtue of this research study we are not promoting any locally available brand of pan masala, flavored supari barnd, habit of chewing pan (both mitha & zarda), or any other forms of masticatories. As we know the ill effects of tobacco, areca nut etc, this piece of work trying to grab the attention of public and all related people towards these ill practices and the risk associated with them.

CONCLUSION

From the study, it can be concluded that the presence of lime in the masticatories decreases the production of nitrite irrespective of the presence of areca nut, betel leaves and tobacco leaves.

The amount of nitrite found to be higher in masticatories containing betel leaves and in masticatories containing raw/fermented areca nut.

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Nil

CONFLICT OF INTEREST
The authors declare no conflict of interest

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