

Possible Cumulative Effect of Spice-Consumption on Mortality Rate and the Number of Genomic Variants (Sequence Reads) of SARS-CoV-2: The USA vs. Higher Spice-Intake Countries

Rama Yusvana

Faculty of Engineering Technology (B-SMAT Focus Group), Tun Hussein Onn University of Malaysia (UTHM), Pagoh Education Hub, 84600 Pagoh, Muar, Johor Malaysia

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ABSTRACT

Background: The COVID-19 mortality rate of the USA was significantly higher than in countries that traditionally consume spicier diet. This study delves into the intriguing relationship between dietary spice consumption and SARS-CoV-2 virus genomic variants, aiming to shed light on potential factors contributing to differing mortality rates across countries with different amount of spice consumption, years before the pandemic began.

Objective: To investigate the possibility that the mortality rate, the number of genomic variants and the type of mutation in the SARS-CoV-2 virus are the results (or cumulative effect) of years of dietary spice-consumption.

Methods: Early in the pandemic (up to November 2020), selected 'Sequence Reads Archive' or SRA of DNA sequences were downloaded from the National Center for Biotechnology Information (NCBI) SRA database (<https://www.ncbi.nlm.nih.gov>) with keyword 'SARS-CoV-2'. A total 100 samples of DNA sequences from infected individuals of both lower and higher spice-intake countries were analyzed for the number of genomic variants (sequence reads) and the major type of mutations between the two groups (i.e., USA vs. India, Bangladesh, Malaysia, Qatar and Egypt respectively) using bioinformatics platform 'Galaxy' (<https://usegalaxy.eu/>).

Results: Data shows that the approximate spice consumption in the USA has been at all-time lower than the other spice-intake countries. In addition, the latter countries have a lower-mortality rate than the USA. Infected individuals from the US also have 15x higher number of variants in the 'Spike' protein region of the virus than individuals from higher-spice group. Most of the mutations in the US were of missense-type. In contrast, frameshift-type mutation predominates in the higher spice-intake individuals resulting in apparent lower-mortality rate.

Conclusions: It is possible that after long-term consumption of immune-supporting spices as part of regular dietary nutrition, the higher spice-intake nations could be more resistant to the viral infection compared to the people who consumed American diet.

Keywords: spices, herbs, COVID-19, American diet, natural immunity, genomic variant analysis, SARS-CoV-2, frame-shift mutation, missense mutation, mortality-rate.

INTRODUCTION

The recent WHO 'Traditional Medicine Global Summit 2023' reaffirmed global commitments towards indigenous knowledges, biodiversity and 'Traditional, Complementary and Integrative Medicine (TCIM)'. Traditional medicines (including herbal) have been used in the maintenance of health and well-being, and in efforts to prevent, diagnose, improve or treat physical illnesses (1). The herbal medicines typically include ingredients such as garlic (2), turmeric (3), ginger (4), cinnamon (5), dill (6) and others that in culinary world are often referred to as spices. They were certainly used by many countries during the COVID-19 pandemic for

their health and well-being. One study mentioned that nations with higher consumption of spices have shown lesser COVID-19 cases and higher recovery rates (7). In this article, the author uses the word ‘herbs’ and ‘spices’ interchangeably and assumes that both have properties that extend beyond simply providing flavor and color to food.

Up to September 2023, USA remains the highest in the total number of reported cases of COVID-19 and among the top 10 with highest mortality rate in the world (Source: WHO and Worldometers). The fatality number could also reflect the overall performance or capability of our innate and adaptive immune response to sufficiently activate natural killer cells (for the pathogen), macrophages and ultimately produce specific antibodies that are capable of neutralizing the virus attack (8). Despite various factors such as genetics, age, and medical condition, higher mortality rate indicates lower performance or capability of the immune response in the infected population to overcome the potential damage that the pathogen could cause in the body.

Many studies have been conducted to show that our immune system can be further supported (or ‘boosted’) with limited but daily consumption of various culinary herbs and spices such as curcumin, basil, garlic, and oregano into our diet (9-12). Johnson and Narayana have reviewed potential role of spices in offering innate and adaptive immunity to human body (7). They found that turmeric acts as an anti-viral agent through inhibition of viral entry into cells, suppression of viral replication and modulation of cytokines. Herbs such as curcumin has been proposed to interfere directly with the receptor / ligands such as S1 subunit of SARS-CoV-2 (13) that mediate entry of the virus into the host cells as well as modulation of associated inflammatory events (9, 14). Others propose that routine consumption of culinary herbs and spices simply ‘boost’ or support the production of antibody in response to pathogen attack such as bacteria and virus through mechanism that is still relatively unknown (12). The study assumes that there is no serious underlying medical condition such as cancer.

However, the virus often mutates their nucleic acid sequence to evade host-defense mechanism. Mutation is common for all pathogenic viruses including coronavirus (15, 16). The more mutation occurs, particularly on the Spike protein domain, the more likely the host cells need to produce ‘new batch’ of antibody that is capable of handling conformational changes (if any) due to the mutation or limited duration of the previously-acquired immunity (17, 18). If this happens, those who incorporate more herbs and spices into their diet would likely to recover sooner than those with a smaller number of spices in their diet. The COVID-19 data Table from Worldometer (www.worldometers.info) showed that per 3rd November 2020, the Recovery Rate (i.e., Total Recovered / Total Cases) of USA was calculated to be around 64.3%, whereas India, Qatar and Egypt for example, were in the range 92.0 – 97.8 % recovery rate. This is presumably due to the antibody ‘boosting’ effect mentioned above, leads to the faster production of ‘newly-adapted’ batch of antibody as part of the host adaptive immune response.

To shed light on possible factors contributing to the significant difference in mortality between the two groups (low vs. high spice-intake countries), the author investigated the approximate amount of spice-consumption in some nations from 2014, or about six (6) years before the WHO declared the world in a state of pandemic (March 2020) using publicly available data. The author then depicted the cumulative mortality rate since the onset of COVID-19 pandemic (up to September 2023). Subsequently, the author investigated the extent of mutation (through genomic variant analysis) as well as the type of mutations occurring between SARS-CoV-2 samples from infected individuals living in ‘low spice-intake’ country such as USA (Group 1), and from infected individuals living in ‘high spice-intake’ countries such as India, Bangladesh, Malaysia, Qatar and Egypt (Group 2).

The grouping of spice-level is based on a study that, despite the growing interest of the American people towards Asian and Mediterranean cuisines, American diets typically contain far less amount of culinary herbs and spices than typical foods from Asia (e.g. India, Bangladesh), Southeast Asia (e.g. Malaysia), Middle-eastern / Arabic food (e.g. Qatar and Egypt) and the Mediterranean (19). This may be due to reasons such as safety concerns, conflicts in scientific literatures about the precise role of the active ingredients in the diet and its safe amount for cancer prevention, etc. Regardless, the author has considered USA in the ‘Low spice-intake’ (group 1), whereas India, Bangladesh, Malaysia, Qatar and Egypt are in the ‘High spice-intake’ (group 2).

METHODS

Data collection for countries spice consumption

Data collection on the global spices market size volume per capita (in kg), specifically from high spice-intake countries such as Thailand, Malaysia, Kuwait, India, Saudi Arabia, Bangladesh, United Arab Emirates (UAE), and Oman, were collected over the period spanning 2014 to 2022. Similar data was also collected from USA (for comparison purposes). To ensure the reliability, the dataset was sourced from the Food and Agriculture Organization (FAO) of the United Nations, which is renowned for its comprehensive and authoritative data on various aspects of global agriculture and food industries. Access to the FAO data was facilitated through the publicly accessible data repository provided by Report Linker (<https://www.reportlinker.com/dataset>). The data collection process involved the retrieval of annual data points for spices market size in volume terms and corresponding population figures for each of the selected countries. Subsequently, the spices market size per capita can be obtained by dividing the total market volume by the respective population data.

Data collection for countries mortality rate

The primary source of mortality data for this study was from the World Health Organization (WHO) COVID-19 Dashboard, a widely recognized repository for global COVID-19 statistics. Access to the WHO data was facilitated through the publicly accessible data repository provided by 'Our World in Data' (<https://ourworldindata.org/coronavirus>) specifically for Coronavirus. The data collection spanned from March 2020 to August 2023, encompassing a comprehensive view of the pandemic's progression over time. The data included cumulative confirmed COVID-19 deaths per million people in countries traditionally known to have high spice-intake in their diets (namely Indonesia, Morocco, Kuwait, India, Qatar, Egypt, Afghanistan, Bangladesh, United Arab Emirates, Pakistan, and China). The data also included, the USA, for comparison purposes.

Selection of sample SARS-CoV-2 genome from infected individuals.

Samples of the viral genome sequence from population with different spice-intake were obtained directly from the dataset of infected population in the specified country of origin. This can be achieved by randomly accessing the Sequence Reads Archive (SRA) database from a specific geo-location. There are many countries in the middle-east, Asia and south-east Asia regions having relatively higher spice-intake level. However, not many of them have uploaded SARS-CoV-2 genome sequence from infected individuals early in the pandemic (up to November 2020) in the form of FASTQ format for SRA (Sequence Reads Archive) analysis. For a reasonable visibility during reporting, only 50 DNA sequences were analyzed from these regions (plus additional 50 from USA for comparison). The data included 19 from India, 17 from Qatar, 7 from Malaysia, 5 from Bangladesh and 2 from Egypt. These countries are traditionally known to have relatively higher-spice intake in their diets, than the west such as USA.

Acquirement of 'Sequence Reads Archive' (SRA) data

Acquirement of SARS-CoV-2 'sequence reads' data and subsequent processing and analysis of the data were based on recommendation provided in the training section of Galaxy project (20, 21). Randomly selected 'Sequence Reads Archive' or SRA of DNA sequences were downloaded from the National Center for Biotechnology Information (NCBI) SRA database website (<https://www.ncbi.nlm.nih.gov>) with a keyword 'SARS-CoV-2'. At the time of collecting the data, the search resulted in 117,770 of SRA entries for all the sequencing works involving SARS-CoV-2 virus. The results were then filtered for 'FASTQ' file-type only, by clicking the link to this file type. The filter gave 25,775 entries of SARS-CoV-2 analysis having the FASTQ format. The filtered results were then sent to the 'SRA Run Selector' page where they were further screened based on the 'geo-location' or 'country of origin' of the entries (e.g., Malaysia, etc.). This gives a list of results of the sequencing experiments with individual SRR accession number from the specified country of origin. The selected SRA profiles were then checked to ensure that they were all derived from the correct organism (i.e., SARS-CoV-2), all were collected from human host (i.e., Homo sapiens), isolated from the nasopharyngeal and oropharyngeal swab, and the host disease is Covid-19. The average spot length should be around 100 bases or

more; otherwise, different map alignment tool should be used. The selected SRR entries were then processed into an ‘Accession List’ text file and downloaded to a local hard drive.

Uploading of SRA sequences to bioinformatics platform

The downloaded ‘Accession List’ text file containing desired SRA experiments of choice were uploaded to Galaxy server (<https://usegalaxy.eu>) for processing using ‘Faster Download and Extract Reads in FASTQ’ tool which retrieves the ‘sequence read’ datasets and the corresponding quality score for each nucleotide read for all the runs that were listed in the SRA dataset. The results were shown in the history panel on the right column of the Galaxy platform. They can either be of type ‘Paired-end’ data collection (folder) or ‘Single-end’ data collection.

Genomic variant analysis

Detailed steps for genomic variant analysis, such as annotation and variant calling are described in full in the **Supplementary Methods**, based on recommendation provided in the training section of Galaxy project ([20](#), [21](#)).

Statistical analysis between independent samples (groups)

The independent samples *t*-test was used to compare the means of two independent groups (lower spice-intake vs. higher spice-intake) to determine whether there is statistical evidence that the associated population means are significantly different at significance level ($\alpha = 0.05$). Using Microsoft Excel ‘Two-Sample Assuming Unequal Variances’ independent samples *t*-test, the null hypothesis used was that the means of the groups are essentially the same and that there is no significant difference between the two groups. If the t_{stats} value is greater than the $t_{critical}$ value or the *P*-value is less than the α value, the author would reject the null hypothesis and determine that the two groups are statistically and significantly different. Similarly, Microsoft Excel ‘Analysis of Variance’ (ANOVA) single factor tool was used to compare the means of more than 2 groups. In this case, the authors would like to know if there is at least one inequality among several groups specifically selected for analysis of variance as described in the results or discussion section.

RESULTS

Countries spice consumption

Figure 1 shows the data on the accumulated spices market size volume per capita (in kg) from 2014 to 2022, as sourced from the Food and Agriculture Organization (FAO) of the United Nations. It provides valuable insights into the consumption patterns of spices in various countries. Over this period (including the COVID-19 pandemic period), we can observe several noteworthy trends and variations in spice consumption.

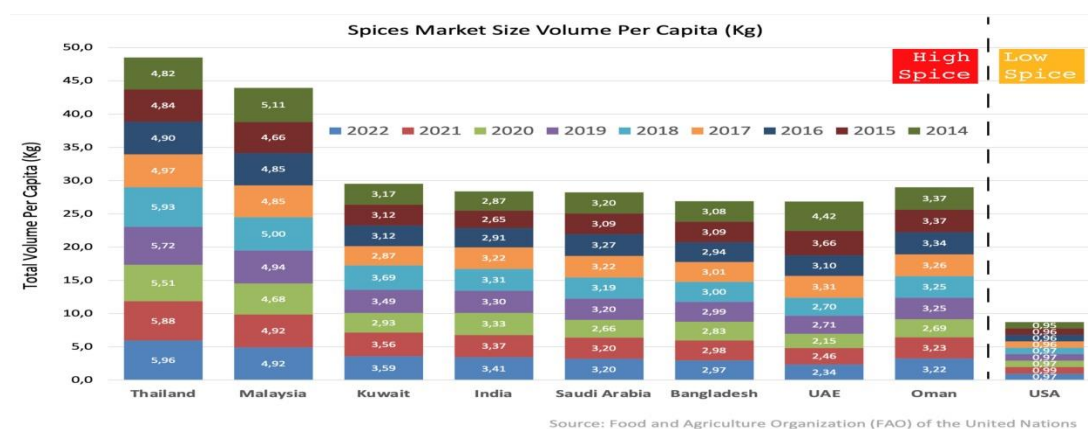


Figure 1. Annual spice market size volume per capita (in kg) from several countries representing high dietary spice-intake (Thailand, Malaysia, Kuwait, India, Saudi Arabia, UAE, Oman and Bangladesh) vs. low spice-intake country (USA) spanning the period from 2014 to 2022 and sourced from Food and Agriculture Organization (FAO) of the United Nations (Artwork modified from ‘Report Linker’ at <https://www.reportlinker.com>)

One striking observation is the consistent and robust growth in spice consumption in countries like Malaysia and Thailand respectively. Thailand has experienced steady growth from 4.82 kg per capita in 2014 to 5.96 kg in 2022. Similarly, Malaysia has shown a consistent amount in market size (or consumption) around 4.92 kg to 5.11 kg per capita over the same period. India, with its rich culinary traditions, has also seen a notable rise from 2.87 kg (in 2014) to 3.41 kg per capita (in 2022). These upward trends could be attributed to factors such as increasing awareness of the health benefits of spices and globalization, which has exposed people to diverse cuisines.

On the other hand, some countries, like Saudi Arabia, Oman and Bangladesh, have seen relatively stagnant spice consumption every year. This might be due to the prevalence of a more consistent or traditional cuisine in these regions. Oman's per capita consumption, for instance, has remained almost unchanged around 3.2 kg per capita throughout the years. In contrast, country like Kuwait has shown moderate growth from 3.17 kg in 2014 to 3.59 in 2022, and UAE on the other hand, has shown a decrease from 4.42 kg per capita (in 2014) to 2.34 kg in 2022, which may indicate a gradual shift in dietary preferences. Overall, **Figure 1** shows that the total accumulated amount of spice consumption in these regions is similar around 28 kg per capita. However, Thailand and Malaysia accumulated around 46 kg per capita in nine (9) years period. The data underscores the global nature of the spice market and the influence of cultural, economic, and health-related factors on spice consumption patterns in these regions.

In contrast however, the USA has consistently maintained a relatively low per capita spice consumption compared to the other countries mentioned above. This could reflect a cultural preference for milder flavors or less reliance on spices in American diet. Over the period from 2014 to 2022, the data reveals that spice consumption in the American diet has remained relatively low and stable. The United States consistently recorded one of the lowest per capita spice consumption levels among the countries in Asia or Gulf regions. In 2014, the per capita spice consumption in the USA was 0.95 kg, and by 2021-2022, it had only marginally increased to 0.97-0.99 kg. This trend suggests that American dishes, while diverse and influenced by various global flavors, has generally maintained a preference for milder and less spice-intensive cuisine compared to countries with more robust spice traditions. The relatively modest increase in spice consumption during this period may be attributed to the growing interest in international cuisines and a heightened awareness of the health benefits associated with certain spices, leading to a gradual incorporation of spices into American cooking, albeit at a slower pace compared to other nations.

Countries COVID-19 mortality rate

Figure 2 shows the progression of mortality rate (i.e., per million people) of several countries in the world since March 2020 for comparison purposes. The cumulative confirmed COVID-19 deaths in the listed countries provide a compelling insight into how, different nations have managed the pandemic's impact on their populations over time. The United States, with 589 deaths per million people, six months after the pandemic (September 2020), saw a significant increase to 3,332 deaths per million by September 2023. This sharp rise reflects the challenges the country faced in controlling the virus, including initial waves, vaccine distribution, evolving variants and quite possibly, lifestyle of the people (e.g., diets). Despite the concerted efforts, COVID-19 continued to exert a substantial toll on the American population.

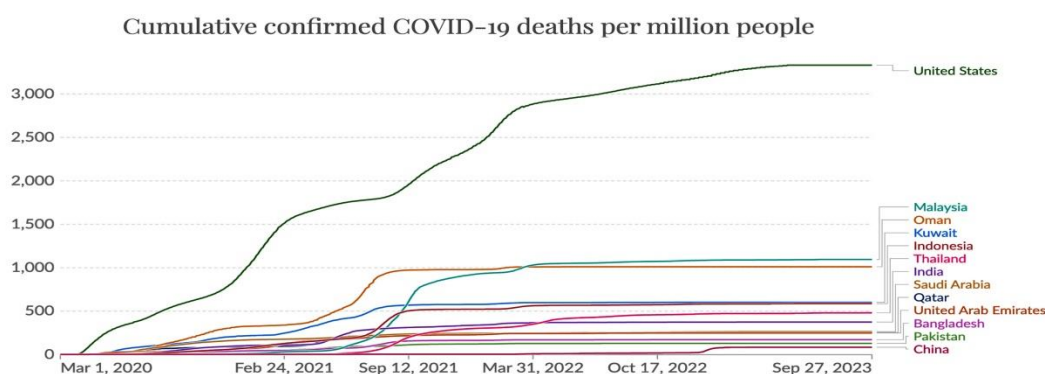


Figure 2. Cumulative data of confirmed COVID-19 deaths per million people in several countries of the world. Data was obtained from WHO COVID-19 Dashboard through 'Our World in Data' website. The site stated that

due to varying protocols and challenges in the attribution of the cause of death, the number of confirmed deaths may not accurately represent the true number of deaths caused by COVID-19. (Artwork modified from ‘Our World in Data’ at <https://ourworldindata.org/covid-deaths>)

In contrast, some countries such as Oman, Kuwait, and Saudi Arabia, exhibited relatively low death rates in September 2020. However, these nations also experienced gradual increases in COVID-19 deaths per million over time, indicating the persistence of the virus and the ongoing efforts needed to manage it effectively. Qatar, with a notably lower starting point, showcased a similar trajectory, highlighting the fact that even nations with successful initial responses to the pandemic, still faced challenges in keeping the virus under control. Similarly, countries like Malaysia, Oman, Thailand, and India, initially reported lower death rates in September 2020, but their death rates increased gradually over time, but still significantly lower than USA three years later (September 2023) as shown in **Figure 2**.

The data underscores the complexity of managing the pandemic in densely populated regions and the need for sustained public health measures and ‘immune-boosting’ campaigns to mitigate its impact, whether naturally (via traditional medicine) or in combination with vaccination program. Ultimately, this dataset illustrates the dynamic and evolving nature of the COVID-19 pandemic's impact on different countries, that could be influenced by a myriad of factors including public health measures, existing medical condition and lifestyle (including diets) of the people, for years before the start of the pandemic (an example was shown in **Figure 1**). Other factors may include healthcare infrastructure and the emergence of several new variants due to the extent of mutation in the viral genome as shown in the following sections of the report.

Genomic variants coded for the Spike ‘S’ proteins region of SARS-CoV-2 virus

Figure 3 shows the distribution of SARS-CoV-2 genomic variants (sequence reads) from samples of the two groups of countries (i.e., low and high spice-intake), both in terms of the total number of (sequence reads) variant and variation in the number of the two main types of mutation in this region (i.e., frameshift and missense mutations). These genomic variants specifically coded for the Spike ‘S’ protein region of SARS-CoV-2 virus.

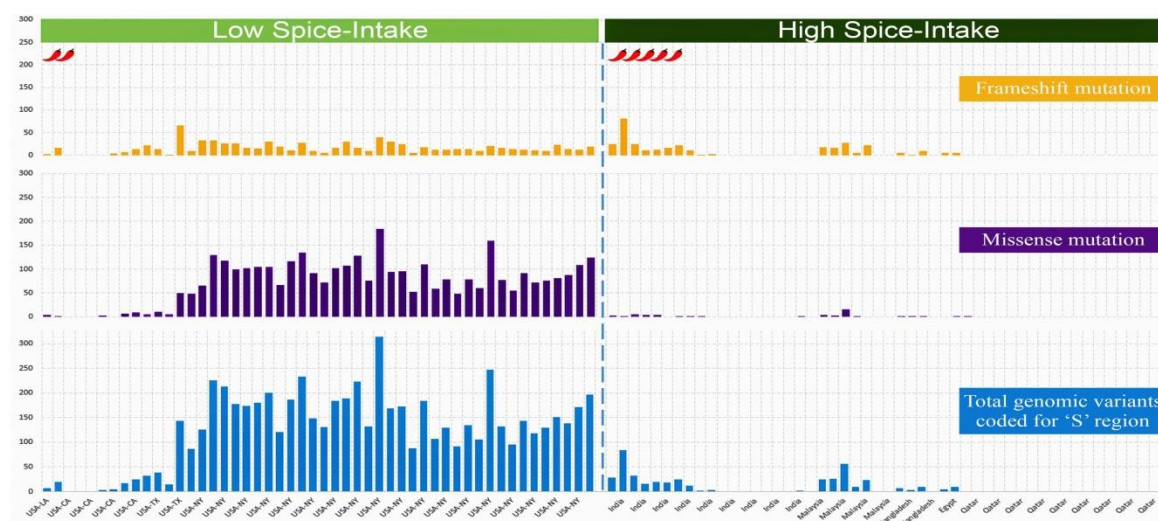


Figure 3. Side-by-side comparison of the number of genomic variants that coded specifically for the Spike (S) protein segment of SARS-CoV-2 virus. The first (top) row shows the number of frameshift-type mutation in the segment. The second (middle) row shows only the number of missense-type mutation coded for the ‘S’ region. The third (bottom) row shows the total number of genomic variants (sequence reads) coded for the ‘S’ protein region. All the lower spice-intake group is on the left panel and the higher spice-intake group is on the right panel.

Figure 3 shows an observable difference in genomic variants between the low spice-intake (left) vs. the high spice-intake (right) countries in that there are a lot less genomic variations in the ‘S’ Spike-protein region of the SARS-CoV-2 viral genome in countries with higher consumption of (herbs and) spices such as India, Malaysia, Qatar and Bangladesh. Statistical analysis using independent sample (Student’s) t-test at 0.05 significance level

(α) gave the t_{stats} values that are higher, in all cases, than the $t_{critical}$ values for both the number of variants in the ‘S’ regions and the mutations of type frameshift or missense mutations ($t_{stats} = 10.53 > t_{critical} = 2.00$ at $df = 53$ for S_glycoprotein; $t_{stats} = 3.87 > t_{critical} = 1.98$ at $df = 97$ for frameshift mutation; and $t_{stats} = 10.60 > t_{critical} = 2.01$ at $df = 49$ for missense mutation). This provides evidence that the mean values of these groups are statistically different and that there are a lot more genomic variations (or mutations) in the ‘S’ regions of SARS-CoV-2 from infected people living in lower spice-intake country.

Although there is apparently a cluster of lower SARS-CoV-2 genomic variations derived from a limited number of samples from the States of Los Angeles (LA), California (CA) and Texas (TX) as shown in the left end region of **Figure 3**, the statistical analysis using the t-test (‘two-samples assuming equal variance’ method) still shows a higher number of variations in the ‘S’ region (of type: missense mutation) in the US clusters than the other 50 samples from the 5 higher spice-intake countries ($t_{stats} = 2.19 > t_{critical} = 2.00$ at $df = 61$ for the ‘S’ protein region and $t_{stats} = 3.22 > t_{critical} = 2.00$ at $df = 61$ for missense-type mutation).

In addition, Analysis of Variance (ANOVA) of thirty-seven SARS-CoV-2 sequence data collected by ‘NYU Langone Health’ during the months of March – April 2020 covering a total of 6 areas (Brooklyn, Manhattan, Queens, Nassau County, Suffolk County and New Jersey) suggested that there was no significant difference among the means of the data from the 6 areas, both in terms of the number of SARS-CoV-2 variants in the ‘S’ protein region of the virus and the types of mutation (frameshift and missense) at 0.05 significance level (α). Analysis of Variance of the 6 areas gave the P_{values} of 0.918, 0.781 and 0.961 (i.e., $P_{values} > \alpha$) for the number of variants in ‘S’ region, frameshift-type mutations and missense-type mutations respectively (Between Groups $df = 5$; Within Groups $df = 31$). The analysis showed that the means of the number of variants in the ‘S’ segment of the SARS-CoV-2 genome from these USA regions (New York, LA, Texas, and California.) were not statistically different.

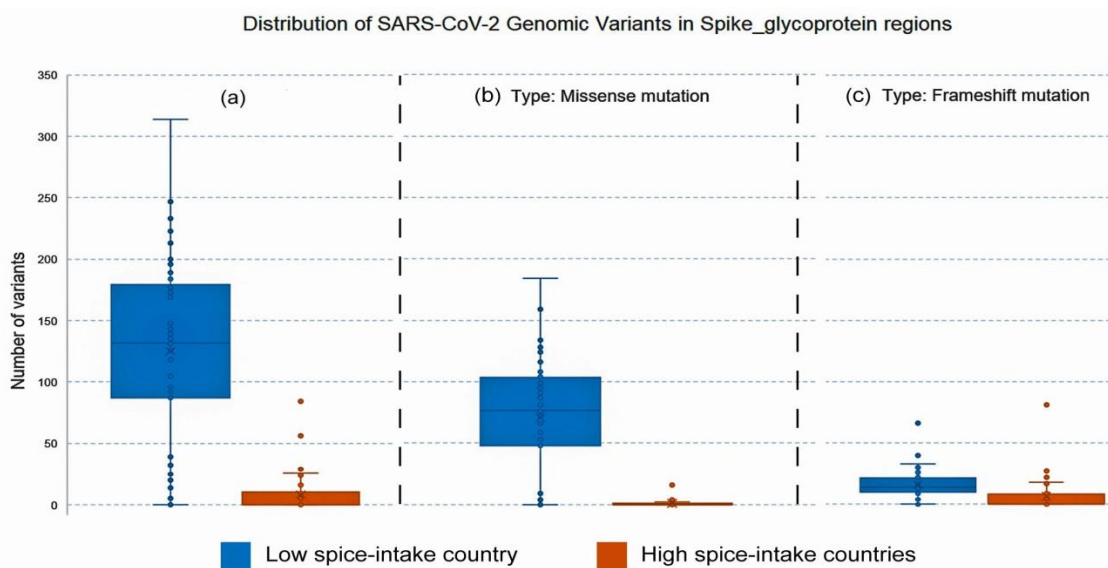


Figure 4. ‘Box and Whisker’ plot describing the quartile (range) and average number of genomic variations (sequence reads) that coded for the ‘S’ Spike protein region of SARS-CoV-2 virus from sample of infected individuals living in low spice-intake country (USA) in blue color and high spice-intake countries (India, Bangladesh, Malaysia, Qatar and Egypt) in orange color. The plot shows (a) Total number of genomic variants (b) Number of missense-type mutation (c) Number of frameshift-type mutation.

Figure 4 shows an ‘alternative view’ of the range of genomic variation (sequence reads) or mutation of the nucleic acids that coded for the Spike (‘S’) protein region of SARS-CoV-2 genome using a ‘Box and Whisker’ plot. The ‘box’ shows where the middle (50%) portion of the data is (i.e., the interquartile range). The two ends of the box mark the first quartile (the 25% mark) and the third quartile (the 75% mark) of the number of genomic variants data. The lower ‘whisker’ part is the minimum (the smallest) number of variants in the dataset and the upper ‘whisker’ part is the maximum (the largest) number of variants in the dataset. Finally, the median is normally represented by a horizontal bar in the center of the box. However, because more than half of the number

of variants (both in the ‘S’ region and the 2 types of mutation) in high spice-intake countries have a value of zero, the median values for these variables are also zero. Therefore, the average (mean) values could also be used for comparison purposes instead of the median values. For example, the average numbers of genomic variants coded for the ‘S’ region of the SARS-CoV-2 virus are 125 and 8 in the low and high spice-intake countries respectively. Therefore, based on these numbers, the mutation rate for the ‘S’ region of the SARS-CoV-2 virus obtained from the low (herbs and) spices region is estimated to be 15 times higher than the mutation rate in countries where consumption of (herbs and) spices are higher. As reported previously, the missense-type mutation predominates in the low spice-intake country over the other types of mutation as also shown in **Figure 3**.

Genomic variants coded for the entire protein regions of SARS-CoV-2 virus

Figure 5 and **Figure 6** show the extent of SARS-CoV-2 genomic variations in the infected samples from the low and high spice-intake countries respectively. The graphs show the variants that occur in the entire viral genome including ORF1ab, S, M, N, and E regions as described previously (22). The horizontal (X) axis shows the total number of variants (sequence reads) for different types of mutation (color-coded) stacked side-by-side. The Y-axis shows the NCBI accession number (begin with SRR) for each sample followed by city or country name (**Figure 6**).

The distribution of variants in the entire regions or segments of the viral genome (i.e. including ORF1ab, S, M, N, and E) for SARS-CoV-2 has been reported previously (23) including the total number of distinct variants and the major types of mutations based on analysis of variants from 68 countries all together. Despite the difference in the method used, this report analyzed only 100 genome sequences of SARS-CoV-2 from a selected number of countries and divided into 2 groups based on their likely dietary spice-intake (high and low) and focused the analysis in the Spike (S) protein regions mainly, due to the critical role played by this region.

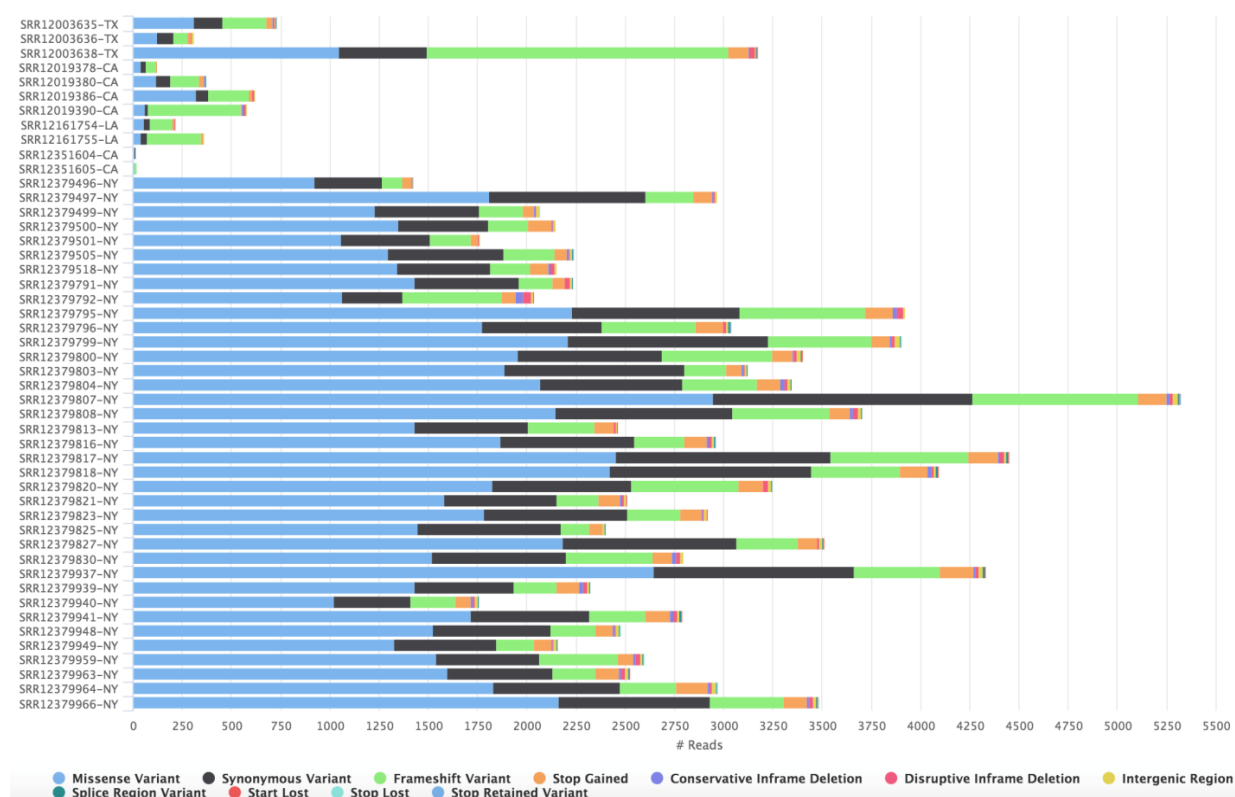


Figure 5. A snapshot of genomic variation in the entire genome of the SARS-CoV-2, based on Sequence Reads Archive (SRA) from several states of the US (mainly New York, California, Los Angeles and Texas) representing low spice-intake group. The number of variant reads (x-axis) is in the range of up to 5,500. The types of mutation (such as frameshift mutation, missense, synonymous, etc.) are also shown for each SRA, with missense-type mutation predominates. The plot was automatically generated online using MultiQC® tool of the Galaxy (Europe) platform

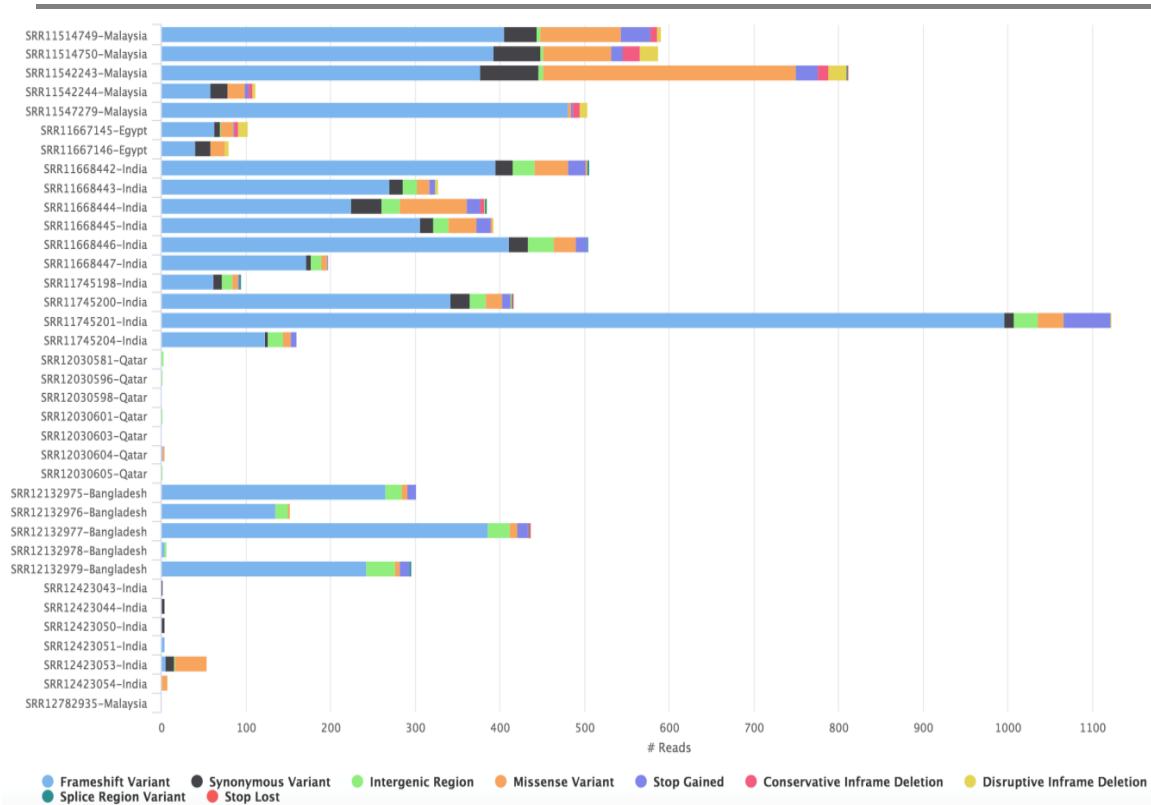


Figure 6. A snapshot of genomic variation in the entire genome of the SARS-CoV-2, based on Sequence Reads Archive (SRA) from Asia / Middle-eastern countries (India, Bangladesh, Malaysia, Qatar, and Egypt) representing high spice-intake group. Out of 50 samples, only those having genomic variants were displayed, in this case, only 36 data were displayed. The rest shows no genomic variants. The number of variant reads (x-axis) is in the range of up to 1,100 (as opposed to 5,500 in the USA). The types of mutation (such as frameshift mutation, missense, synonymous, etc.) are also shown for each SRA, with frameshift mutation predominates. The plot was automatically generated online using MultiQC® tool of the Galaxy (Europe) platform

Apart from within-group variation, the between-group variation is clearly visible. The total (i.e., all segments of SARS-CoV-2) genomic variation in the USA is generally higher than in the high spice-intake countries (India, Bangladesh, Malaysia, Qatar and Egypt). The major types of mutation in each sample are also shown in the colored-graph with the missense-type mutation predominates over the other types of mutation (frameshift, synonymous, etc.) in the low spice-intake country (USA). This is in agreement with the finding by other group (23). On the other hand, the frameshift-type mutation seems to predominate within the high spice-intake countries.

Figure 5 and **Figure 6** however, do not depict which regions (ORF1ab, S, M, N and E) show the most variants or how the number of variants distributed across the viral genome. The information for ‘S’ region was shown in **Figure 3** and **Figure 4**. It is important because each region contains exons of different roles, either structural or non-structural and has been reported previously (23-25). Of particular importance is the ‘S’ segments that code for Spike protein structure which plays a key role in the host receptor recognition and cell membrane fusion process (26). Knowing key differences in this region in relation to relative amount of spice-intake of the infected-individuals could provide an insight of how environmental factors affect the number and type of variants and its impact towards fatality rate.

Most of the SRAs in **Figure 5** were from New York city. This is because the rate of infections in the city, with its high population density, has exceeded every other state, and as of April 20, 2020, it has more than 30% of all the US COVID-19 cases. The city has emerged as an epicenter of the pandemic, with over 120,000 confirmed cases and over 13,000 deaths due to confirmed or probable COVID-19 death as of April 17, 2020 (27). In addition, majority of NCBI Sequence Reads Archive (SRA) with FASTQ format from USA was from New York city. In fact, some of the SARS-CoV-2 data from New Jersey was also sequenced by a sequencing center in New York city (NYU Langone Health).

DISCUSSION

The use of spices and herbs have played a significant role against viral (e.g. SARS-CoV-2) infections (28-30). The relation between spice consumption and recovery from COVID-19 is not exactly clear. However, Bousquet et al. (31), proposed a potential interaction between Nrf2 and TRP (Transient Receptor Potential)-inducing foods, containing spice compounds such as allicin, capsaicin, and curcumin, which could shed light on how dietary choices play a role in addressing COVID-19. It appears to involve a process facilitated by Reactive Oxygen Species (ROS), in conjunction with TRP channels. This process is regulated by Nrf2, a nuclear factor (erythroid-2 derived)-like pathways. Spicy foods might have the effect of reducing sensitivity in TRP channels. The rapid desensitization by spices is likely to reduce disease severity. In addition, Nrf2 agonists expand the 'duration of action' of spices. Therefore, relatively high doses of spices regularly consumed in Asian or sub-Saharan countries could reduce COVID-19 infection and/or severity. This might explain the distinct difference in mortality rate as shown in **Figure 2**.

Furthermore, samples of infected population from low spice-intake country (USA) showed higher number of genomic variants in the 'Spike' protein region (**Figure 3** and **Figure 4**). It is possible that the higher mutation rate of the virus has overwhelmed the host immune response as indicated by lower-recovery / higher-mortality rate. Alternatively, very low (or limited) immune pressure could drive the virus to have fast viral adaptation (32) resulting in high genomic variants. There is no direct evidence linking the 'low' spice-intake of infected individuals and the 'higher number of variants' in the viral genome (or *vice versa*). However, spice-intake has been linked to improved immune response of individuals in general (9-14). In fact, the US National Institute of Health (NIH) website (<https://www.ncbi.nlm.nih.gov/books/NBK92774>) shows a list of herbs and spices for cancer prevention and treatment (33). Therefore, governments and medical professionals, particularly from low spice-intake countries should promote more culinary herbs and spices as part of daily diets of the people.

The network pharmacology studies suggests that Chinese herbal formulas (34), including the lung-cleaning and toxicity-excluding (LCTE) soup have played an important role in treating the COVID-19 pandemic in China (35). In addition, those with cancer are at higher risk of having severe COVID-19 (36). Cancer is one of co-morbidity factors that seem to greatly increase the mortality risk together with cardiovascular, diabetes mellitus and chronic lung diseases (37). The studies further confirmed the importance of daily intake of herbs (and spices) for prevention and treatment of cancer which is one of the risk factors for severe COVID-19 cases.

The number of SARS-CoV-2 genomic variants at both within (**Figure 3** and **Figure 4**) and outside of the 'S' region (**Figure 5** and **Figure 6**) were higher in patients from low spice-intake than in high spice-intake. Various factors could contribute to the mutation, including variations in replication dynamics (38), which may be due to the capability of RNA-proofreading enzyme in their genomes. In addition, cell-derived RNA editing enzymes often become a contributing factor to induce point mutation in the viral genome including RNA viruses (39). RNA editing enzymes often have substrate specificity and context preferences around target sites (40). However, these causative factors for mutations may have no bearing on the difference in the relative amount of spice-intake in the infected-individuals. More researches are needed to link the amount of spice-intake with the number of genomic variants.

The higher number of missense-type mutations in the 'S' regions of SARS-CoV-2 genome as shown in **Figure 3** and **Figure 4** have also been reported previously (23). Of particular importance that has been documented worldwide is the D614G spike protein mutation (41), which has emerged as a predominant clade in Europe and was spreading worldwide. *In silico* analyses on the spike protein structure suggests that the missense-type mutation is most likely neutral to protein function as it relates to its interaction with the human ACE2 receptor. Each missense mutation is a single point mutation that results in a change of 1 amino acid due to different nucleotide (codon). The substitution may affect the stability of the protein 3D structure (42). More researches are needed, especially on the virulence of the virus because of missense-type mutation.

The functional consequences of these mutations on SARS-CoV-2 infectivity are still largely unknown, despite our ability to predict the effect mutation on the stability of the protein 3D structure, based on simulation of the physics (vibrational entropy changes) (43). However, it has been reported that point mutation bias in SARS-CoV-2 variants results in an increased ability to stimulate inflammatory responses (44) which correlates with

enhanced production of cytokines (such as TNF- α and IL-6) that could lead to cytokine storm which has been reported to cause severe pneumonia (45) or at least an increase in the severity of symptoms in SARS-CoV-2 patients. Therefore, higher rate of genomic variations could contribute to the higher morbidity (amount of the disease) or mortality (number of deaths) in the population. *In vitro* study has also shown that mutations observed in SARS-CoV-2 have the potential to impact viral pathogenicity through blood vessel-related damages which include human lung cancer cell (38) which might explain high fatality rate in the group as shown in **Figure 2** above.

Data from this study (**Figure 1**) suggests that prolonged (and controlled) consumption of immune-boosting spices in dietary practices could enhance resistance to viral infections, potentially explaining the contrasting outcomes observed between regions with distinct dietary habits, such as the USA and countries with higher spice intake (**Figure 2**). The successively low spice consumption in the USA since 2014 seems to have contributed to the high mortality rate in the country. These findings underscore the significance of dietary choices in bolstering immune responses and warrant further investigation into the link between spice consumption and viral resistance.

In summary, regularly consuming culinary (herbs and) spices into our diets does not prevent individuals from exposure to SARS-CoV-2 (or any other viruses) and the subsequent COVID-19 (or other viral diseases). However, it should help assisting our body's immune response in fighting the virus (assuming there are no underlying serious medical conditions mentioned above). The study does not suggest sudden lifestyle changes. Therefore, the consumption should follow advice from the local doctors or medical professionals. At the (community or) country level, higher spice-intake should result in lower mortality or higher recovery rates subject to age distribution, genetics or medical condition of the population. Limited number (100) of analyzed SRA sequences is one of the limitations in this study. However, it allows visibility of all elements in a bar plot as shown in **Figure 5** and **Figure 6**, as well as statistically-acceptable sample size. Based on the result of analysis in this report, the higher number of genomic variants (with predominantly missense-type of mutation) in the USA could be linked with the higher mortality rate of the population as shown in **Figure 2**.

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