Kombucha: Determining the Likelihood of Secondary Fermentation And Increased Ethanol Content Via Stated Sugar Content on Product Labels.

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Abstract

Background: Kombucha products are now a common, and popular beverage. Increasingly, Kombucha beverages are outpacing popularity of other carbonated beverages on the market, such as soda pop. This increase is seen by many as a positive change of consumer interests, as Kombucha has much less sugar content than many soda pop alternatives. However, Kombucha products are fermented beverages, and therefore are apt to contain ethanol, which may be a hazard for certain at risk populations. This study aims to investigate how information provided on product labels may or may not allow for increased consumer control by making an educated guess about potential ethanol content.

Methods: Kombucha products were placed into 2 groups, Group A (high sugar content), and Group B (low sugar content). Signs of secondary fermentation was then monitored by placing the bottles and cans into incubators at an ideal fermentation temperature. NCSS Statistical Software was used to determine whether or not there was a statistically significant difference between groups.

Results: The researcher has inferred that reading sugar content from labels of Kombucha is likely not an effective method to ascertain potential for secondary fermentation leading to higher than expected levels of ethanol. There was not a statistically significant difference between groups A and B where $P = 0.366$ and power for the test at P value $= 0.01$ is 0.042. Sample sizes were small, and there were potential errors in experimental design.

Conclusion: Kombucha remains a healthy choice for consumers. This research reveals that Kombucha, as has been found in previous research, can undergo secondary fermentation when temperature abused leading to higher than expected levels of ethanol in the product. Furthermore, consumers may not be able to rely on nutritional labels as a means to ascertain whether potential health hazards regarding ethanol content exist. Recommendations include increased detail on packaging labels to further elucidate potential for higher than expected ethanol content in Kombucha products.

Keywords: Kombucha, product labels, secondary fermentation, functional beverage, temperature abuse.

Introduction

A fascination with carbonated beverages extends long into human history, and has traditionally been associated with health benefits (Shachman, 2004). The word seltzer originates from a German village, Niederseltsers, where locals would bathe in and drink naturally carbonated spring water for its supposed health regenerating powers (Shachman, 2004). In relation to carbonated beverages, the history of fermented beverages goes back as long as civilization has existed, and some anthropologists believe that the desire to reliably produce ethanol through fermentation may have been a driving force behind the emergence of agriculture (Mike & Sue, 2012). The development of Kombucha through history is complex, and begins in ancient China alongside the equally long and complex history of the establishment of tea drinking (Chakravorty, Bhattacharya, Bhattacharya, Sarkar, & Gachhui, 2019). This paper will not cover these extensive topics, but rather will explore how Kombucha relates to modern consumer behavior, and more specifically with hazards surrounding secondary fermentation, where higher than expected alcohol content is known to occur when raw Kombucha in a can or bottle is time and temperature abused. Building on previous research, this study aims to determine which brands may be the safest choice for those consumers who wish to reap the numerous health benefits of Kombucha tea (particularly reduced levels of sugar content when compared to soda pop), while avoiding possibly dangerous levels of ethanol.

Literature Review

The western obsession with Kombucha may have its origins in a 1992 article written in Austria by T. Valente, who was looking into the intriguing health benefits purported by the complex bacterial and yeast driven fermentation within Kombucha (Spedding, 2015). The trend has not receded, where the beverage remains a significant aspect of the functional foods health trend (foods which have complex health benefits such as polyphenols and glucuronic acid) which has reached high levels of popularity as evidenced by the attached photo (photo from a popular grocery chain in Vancouver, Canada). Kombucha producers pay more to have

Red Outline Indicates Kombucha Brands. Photo taken by David Parker, 2020.

their products placed at eye level, and the high diversity of products is evidence in itself of high demand. Moreover, the cooler represents the only cooled section for beverages within this particular store, whereas conventional carbonated beverages (soda pop) have been relocated to shelves which are not refrigerated. Furthermore, recent market research predicts the global Kombucha market will grow by 18% during the period of 2020 – 2024, despite and in part due to the Covid 19 crisis (Technavio, 2020). Reported overall health benefits are stated as being the driving force for this increase in the market, and an interest in functional beverages which are promoted to increase ones immune function (among other promoted health benefits) are becoming more popular with consumers (Technavio, 2020). Overall, with increasing demand, and a projected growth into the future there will undoubtedly be a wider array of Kombucha beverages in the market than there are today. This increase in product diversity will demand a comprehensive regulatory environment to ensure product safety as well as an educated consumer population whom are able to make informed decisions. Furthermore, while there is evidence that supports the health benefits of consuming Kombucha, there are associated risks and hazards that should be communicated.

This research project aims to continue previous investigations into the likelihood of commercially produced Kombucha bottles and cans capacity for secondary fermentation, which may cause certain brands to contain higher than the expected levels of ethanol. Previous research by James Chhay at the British Columbia Institute of Technology (BCIT), and work completed by the British Columbia Centers of Disease Control (BCCDC) have found many different brands which contained ethanol levels >1% ABV. Any beverage in Canada with an ABV >1% is considered an alcoholic beverage and is subject to different regulations, and taxes (Government of British Columbia, 2020). Where Chhay found that secondary fermentation at room temperature abuse conditions contributed to risk of higher than expected ABV, BCCDC found that 31.5% of Kombucha brands purchased in Vancouver, Canada tested higher than the regulated 1% ABV level, and 70% of Vancouver based processors were found to have the capacity for secondary fermentation (McIntyre & Jang, 2020). To expand upon this previous research, this work will investigate if known factors such as sugar content can be predictors for secondary fermentation and thus possibly higher than expected levels of ethanol when stored in high temperature conditions (which for the purposes of this experiment is close to 35C). The use of high temperature conditions are to simulate what may be experienced by bottles or cans under moments of time and temperature abuse. Conditions which may be possible in a poorly managed warehouse during summer months (N. Reiben, personal communication, December 2020).

While the shift from conventional carbonated beverages (e.g. Mountain Dew) to functional beverages will likely have an overall positive health outcome on most populations, there are risks associated with Kombucha and related fermented carbonated beverages that consumers should be aware of. Alcohol content which is above regulatory standards and high acidity are the main hazards mentioned in the literature (BCCDC, 2020; Chhay, Chen, & Kuo, 2014; Mcintyre & Jang, 2020). Furthermore, it is not yet fully understood what public perception is regarding alcohol hazards and Kombucha consumption, and many manufactures in British Columbia produce Kombucha which does contain higher than expected levels of ethanol (BCCDC, 2020).

The process of fermentation is known to increase nutrient content, and can be considered as a way to add value to what is already a healthy beverage, as well producing alcohol as a by-product (Chakravorty et al., 2019). Kombucha is made from fermenting tea using a complex arrangement of yeasts and bacteria in what is known as the Symbiotic Culture of Bacteria and Yeast (SCOBY), also known as "The Mother" (Villarreal-Soto, Beaufort, Bouajila, et al 2018). The SCOBY is duplicated during each fermentation batch, producing a "baby", which is used in subsequent batches, or sold or given to others wishing to ferment their own batches (Villarreal-Soto et al., 2018). The process has been perfected over of thousands of years, and has a long history and use as a functional beverage in Asia and has only recently been produced at commercial scales (Chakravorty et al., 2019; Spedding, 2015). Spedding (2015) mentions how this scaling up process from pilot projects to commercial scale productions feature many challenges and scaling up a project is not linear (due to inherent complexities and differences that arise with each batch). Errors can be made by the manufacturer which may increase the risk of certain hazards, such as very low pH, or higher than desired ethanol content. In theory, the process of fermentation which includes yeasts that produce alcohol are outcompeted by other yeasts and certain species of bacteria which produce acidic acid, lactic acid and glucuronic acid resulting in an acidic beverage that is <.5% ABV. But in practice, ABVs are usually higher than .5% (Mcintyre & Jang, 2020). Another possible issue is that pH is commonly used as an indicator that fermentation has completed, which can sometimes be as low as pH 2.4 (Villarreal-soto et al, 2018). Not only does this low pH present a hazard, which has in some cases caused acidosis in susceptible populations, it is also an insufficient technique at determining the end point of fermentation (Villarreal-soto et al, 2018, Spedding 2015). Overall, the complex array of nutrients formed, amino acids and myriad of other inherent qualities produce a final product that does have proven health benefits, but can be also be hazardous in certain vulnerable populations (Jayabalan, Malbaša, Lončar, Vitas, & Sathishkumar, 2014).

Due to the increasing demand for Kombucha products available today, it is important that companies take seriously food safety plans they create which aim to produce a product that is controlled for alcohol levels and that precautions have been taken into account regarding secondary fermentation as it leaves the place of manufacture and makes its way onto shelves in retail stores. The time interval between the products being bottled, stacked onto pallets, and ending up in refrigerators or coolers across British Columbia is identified as a critical step in the products food safety plan

(BCCDC, 2020). As mentioned by the BCCDC in their recent updated report, cold holding throughout the supply chain after the product is complete ensures that the live cultures in the bottle do not begin secondary fermentation (BCCDC, 2020).

Factors Contributing to Secondary Fermentation

Certain manufactures use methods to reduce secondary fermentation, and the problem of higher than expected ethanol is acknowledged by several producers. Some factors which may increase the risk of secondary fermentation are the addition of flavoring agents containing sugars which are added post fermentation; lack of yeast specific preservatives; and when pasteurization is not performed (Mcintyre & Jang, 2020).

Other methods companies have been known to use to reduce secondary fermentation are the use of centrifugal chambers to remove active yeasts, or cold fermentation processes which encourage types of yeasts tend to die off over time (Mcintyre & Jang, 2020).

Regarding exploding cans, or bottles, as temperatures rise within the can or bottle, the dissolved carbon dioxide trapped within the fluid of the beverage will dissociate and move into the headspace (the area of empty space in the sealed bottle), thereby increasing the pressure inside the bottle possibly leading to an explosion or rupture depending on how long and at what temperature the environment the bottle is in (Brewyourbucha, 2018). Under the conditions which will be seen with this experiment, a temperature of 35C should not cause a can or bottle to rupture without secondary fermentation. A can of coke has an internal pressure of ~50psi, where an average can of coke can handle upwards of 100psi before rupture. Another factor is secondary fermentation (or bottle conditioning), which produces CO_2 as a by-product. While it is not certain that all bottles will undergo secondary fermentation, it is more likely with live cultured, unfiltered Kombucha bottles as there remains both yeast and residual sugars within the fluid which are required for secondary fermentation to take place (BCCDC, 2020). The last factor is heat and time, which are to be controlled within the incubator.

Materials and Methods

A total of 48 cans and bottles of 10 different popular brands of Kombucha were purchased from Whole Foods, Save on Foods, Donald's Grocery, and McGill Grocery in Vancouver over a period of 2 days in order to locate products of different lot numbers and flavours within each brand. The distribution of bottles and cans was based on product availability, where cans were limited within the current market.

The incubators used were what were available from BCIT tech, as well as a homemade version using a drying oven as the underlying structure. Temperature differences between both incubators was ± 1 ºC. For more information on these incubators, see (Parker, 2021).

The bottles and cans were separated into 2 groups by taking readings from product labels to assume internal residual sugar content. Group A contained products which contained > 20g/L of assumed sugar content and Group B < 20g/L of assumed sugar content. 20g/L was chosen as a dividing point as available literature referred to 20g/L of sugar as being a minimum amount of required sugar for yeasts to use as a food source. This literature was based on the production of beer and wine, and not Kombucha, of which literature was not available.

Products were incubated for approximately 4 weeks, and due to time constraints the experiment was ended prematurely. Secondary fermentation was monitored for by observational changes in product packaging, either in the form of ruptures, explosions, expansions, or excessive pressure upon opening.

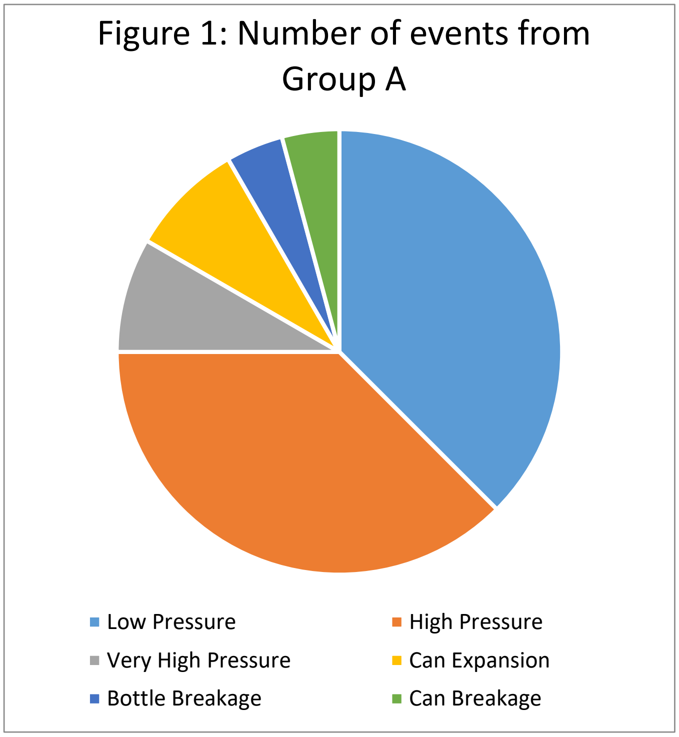
NCSS software was used to determine whether or not differences observed were statistically significant, and descriptive statistics were utilized for further explanatory purposes (NCSS Statistical Software, 2021).

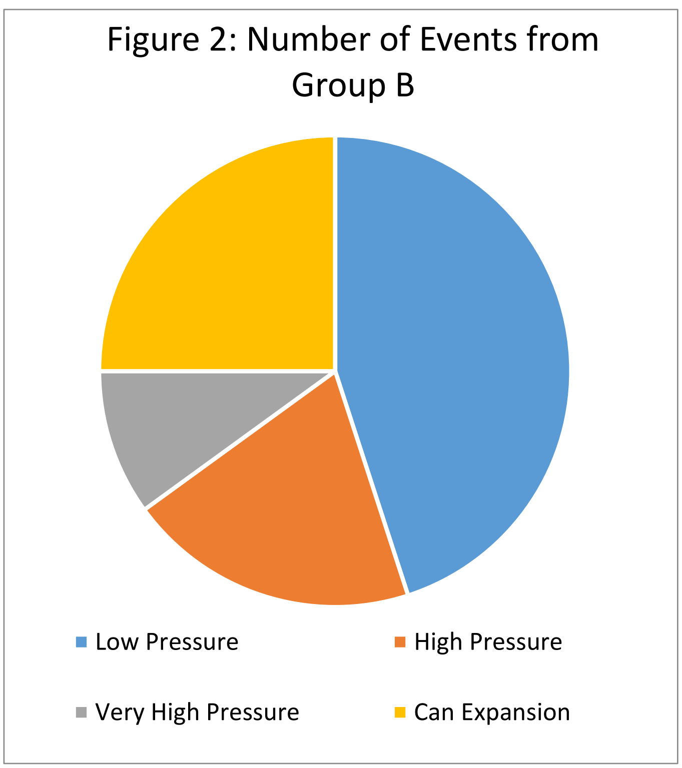
Results

Data collected was ratio numerical data in the form of length of time it took for a given bottle or can of Kombucha to show evidence of secondary fermentation. Additionally, non-numerical multichotomous ordinal data (e.g., Low pressure, High Pressure, Very High Pressure, Can expansion, Bottle Breakage, Can Rupture) was collected at the end of the experiment. Furthermore, Non-numerical multichotomous data was collected where the researcher was looking for leaking from blown caps, rupture of cans, explosions of glass bottles, and expansion of cans.

Descriptive Data

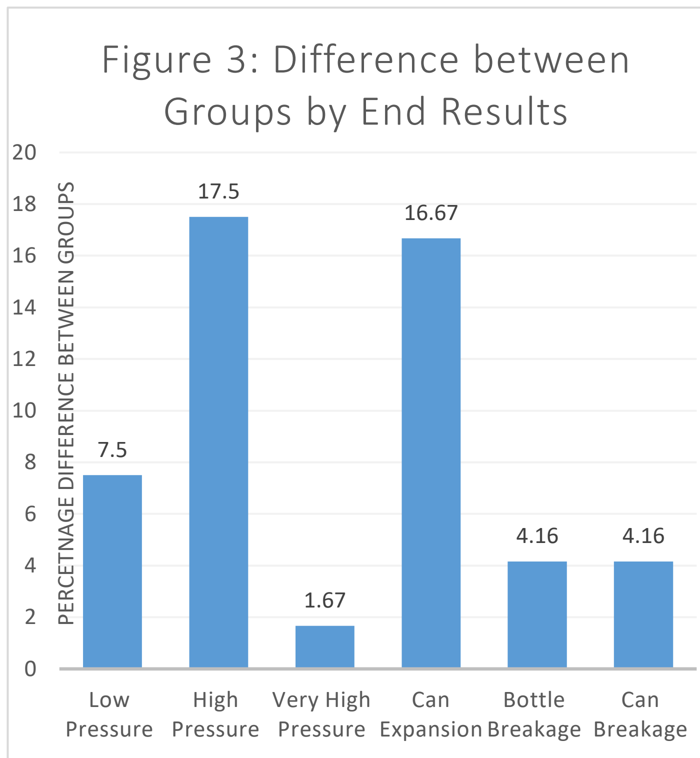
Overall, one glass bottle from group A (Dr. Brew Watermelon) was recorded as an explosion. One can ruptured from group B. The majority of end results for both groups were low pressure, with 37.5% of results for Group A, and 45% of results for Group B. Second most common recording was high pressure upon opening, encompassing 37.5% of results for group A, and 20% of results for group B. See Fig 1 and 2 for a visual representation.





Very high pressure was recorded about equally for both groups, with 8% and 10% respectively. Group B did not have any instances of glass or can breakage.

Differences between Groups A and B can be inferred by looking at the produced graphs, where Figure 3 reveals two visible factors of difference between groups A and B. Group A had 17.5% higher occurrence of high pressure cans than in Group B, however conversely, there was a 16.67% higher occurrence of can expansion in Group B than in Group A. All control samples were categorized as low pressure upon opening.



Inferential Statistics

Examining differences between the two groups in relation to time it took for a change such as rupture, or expansion to take place can be done using a 2 sample T test using 2021 trial version of NCSS

(NCSS Statistical Software, 2021). The following null hypothesis was created. A threshold P value of 0.01 was chosen to reduce the probability of a type I error, or that the null hypothesis is true when in reality

H_0 = That there is no statistically significant difference between groups A and B regarding risk of secondary fermentation.

H_a = That there is a statistically significant difference between the groups A and B regarding risk of secondary fermentation.

By looking at the NCSS printout, the tests for assumptions under normality test Kurtosis and Omnibus reject H0 of normality at a=0.01 with an answer of No in both positions. Therefore the data is not normally distributed, and the researcher will look at the Man Whiney U and Sum of Ranks test. Doing so reveals a P value 0.366. P = 0.366 is > P 0.01, therefore do not reject Ho and conclude that there is no statistically significant difference between Groups A and B. Power for the test at P value = 0.01 is 0.042, which is below .8 and therefore indicating possible type II error, which can be alleviated with a larger sample size.

Discussion

Inferential statistics have revealed that predicting secondary fermentation by monitoring for rupture or explosions is not likely to be determined by examining the label and assuming sugar levels. However, the experimental design of this project, as discussed in the limitations section of this paper, may have been a major limiting factor in regards to this determination. It may still be possible to determine risk of secondary fermentation and therefore increased levels of alcohol content by reading product labels by examining other aspects not mentioned by the researcher. Furthermore, descriptive statistics may provide evidence to the statement that product labels are not a good indicator of secondary fermentation or higher levels of alcohol content, as seen in the high number of positive results in Group B (the expanded cans), where products in group B were not expected to undergo secondary fermentation due to low residual sugar content. However a beta error may be present due to low sampling sizes, as in the case of expanded cans, the cans represented one brand, which may have been improperly labeled (contained more residual sugar than is stated). Additionally, previous experiments have shown that secondary fermentation is common amongst Kombucha products under time and temperature abuse conditions, however previous research does not specify underlying factors which may be used as predictors for secondary fermentation, with the exception of all products being raw and unfiltered (Chhay et al., 2014; Mcintyre & Jang, 2020). Another component is industry awareness, which, according to McIntyre & Jang, 2020, mentioned that several companies proclaim that they were either actively or looking into undertaking measures to reduce the likelihood of secondary fermentation from occurring in their products. It is not clear from these results that companies have undertaken these precautions, as 7 out of 10 brands exhibited some form of evidence that secondary fermentation occurred. Furthermore, methods which are known to prevent secondary fermentation, such as pasteurization or high levels of filtration are not commonly used, as consumers prefer products which are live and unfiltered (Mcintyre & Jang, 2020).

Limitations

The limitations with this study are based on three main components, which will be summarized below. First are errors with the experimental design, second are limitations in accessing laboratory equipment, and third are limitations in available time.

First, the experimental design did not take into account how differences between brands and associated evidence of secondary fermentation will be documented in relation to packaging designs which are developed to prevent or limit signs of secondary fermentation. Through personal correspondence with a manager of a local brewery, there are bottling standards and specifications which are likely adhered to regarding bottle and can manufacturing (B. Hewitt, Personal Correspondence, March 2021). Furthermore, Kombucha companies in North America are likely to purchase these products to package their beverages. Due to this, monitoring for signs of secondary fermentation by observing for physical changes may not be representative of whether or not secondary fermentation is or is not taking place, and therefore this experimental design may not be effective in ascertaining brands which may or may not have increased levels of ethanol.

Secondly, limitations with available laboratory equipment in the lab and time constraints may have had impacts to the number or form of end results observed. The incubators used were not capable of holding consistent temperatures, and incubator 2 had rather large temperature fluctuations. Finally, the desired incubation time of 5 weeks was not achievable by the researcher.

Knowledge Translation

Despite the aforementioned limitations, the results which were gathered does appear to show, to some degree, a likelihood that packaging designs do not reflect actual contents of the products, specifically in regards to sugar content. Furthermore, results reveal that consumers may not be able to refer to product labels in order to determine potentially higher than expected levels of ethanol.

This research may be part of a rationale towards greater accountability, and increased accuracy pertaining to product labeling.

Furthermore, and as will be mentioned in the next section, future research can help to determine what aspects of product labels may be most instructive for consumers wishing to avoid higher than expected levels of ethanol in Kombucha products.

Potential Areas of Future Research

- Research into what the minimum amount of residual sugar is required for Kombucha to initiate secondary fermentation, at what temperature, and at what time, would be important data when shipping and storing Kombucha within the supply chain. Complex interactions with between sugar content, alongside bacteria and yeast content may add to this research.
- Research into what, if any aspects of currently existing product label information may inform consumers as to actual risk of higher than expected levels of ethanol in Kombucha products, not including pasteurized or sugar free products.
- A survey into how Kombucha products are handled throughout supply chains in North America would assess what the level of risk is within current industry standards. This could also be informed by further experimentation regarding temperatures and time conditions which Kombucha can be held at safely, informing warehouse logistics.
- Assess possible differences between brands which have undergone temperature abuse conditions compared to those which have not undergone temperature abuse conditions and their relation to functional properties of Kombucha. There have been some reports in the literature that point to increases in alcohol content and decreases in levels of enzymes, micro nutrients and other functional properties of Kombucha.
- Assess the overall health profile of high alcohol Kombucha, in relation to low alcohol brands. Currently, certain Kombucha brands are being marketed as an alcoholic beverage and are simultaneously marketed as a healthy beverage while containing up to 8% ABV. These beverages, due to their alcohol content pose a known health hazard if consumed irresponsibly, but research into possible positive health effects is missing in the literature. A comparison between functional properties of low alcohol Kombucha to that of high alcohol Kombucha would fill this knowledge gap.

Conclusion

Kombucha remains a healthy choice for consumers, particularly when other options include high sugar sodas. The increase in popularity of Kombucha will likely have overall health benefits for populations that's consume them, as they have confirmed health benefits aside from containing low sugar in comparison to most other carbonated beverages on the market (probiotics, other functional enzymes) (Chakravorty et al., 2019). However, this research does reveal that, as has been previously elucidated, that Kombucha, if temperature abused can undergo secondary fermentation potentially leading to alcohol content higher than desired. Furthermore, this research reveals that consumers may not be able to rely on nutritional labels as a means to ascertain whether or not there may be higher than expected levels of alcohol in the product, or provide assurance that will not undergo secondary fermentation if temperature. Currently, pasteurized, or sugar-free products provide this assurance.

Acknowledgements

The researcher would like to thank Dale Chen from BCIT for his patience and encouragement throughout the writing process. Special thanks to Fred Shaw from BCIT for assisting in taking temperature readings and monitoring the incubation process where the researcher was not available. Additional thanks to Sung Sik Jang from BCCDC for providing valuable resources throughout.

Competing interest

The authors declare that they had no competing interests while conducting this study.

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