Determining the accuracy of colorimetric pH testing compared to potentiometric methods

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ABSTRACT
Background: Bacterial growth in foods can be prevented by applying various controls to the food product, including adjusting the acidity of the food. Research has indicated that a pH level of 4.6 or lower will be effective to prevent most bacterial growth. In order to verify this level has been achieved pH test strips (colorimetric) or a digital calibrated pH meter (potentiometric) can be used. This study attempted to quantify the degree of accuracy that pH test strips have compared to the calibrated pH meter.
Method: MColorpHast™ pH indicator strips with a pH range of 0-14 were tested against a calibrated Extech pH100 meter. In this study 40 samples of rice were acidified to varying levels. Each sample was measured with both colorimetric and potentiometric method. Results were compared to determine the level of accuracy of the pH test strips. As well, test strips were used to measure pH in a variety of different coloured preserves.
Results: A two-tailed test showed that there was a statistically significant difference between the readings from the pH test strips and the digital pH meter (P=0.0003).
Conclusion: Based on the results, it can be concluded that both methods of measurement are not equally accurate. A calibrated pH meter will give more accurate readings of pH levels and should be used in most cases to confirm food safety with a high degree of confidence. In testing dark coloured jellies and preserves, pH test strips should not be relied on as they will be stained by the food, making the colorimetric reading difficult to determine accurately.

Keywords: *pH, test strips, calibrated pH meter, food safety, acidity, accuracy, colorimetric, potentiometric, comparison.*

INTRODUCTION

Food of all varieties may provide a medium for the growth of unwanted pathogens that can cause illness to a consumer. There are a number of ways food can become contaminated, beginning at its harvest all the way through to when it is served and consumed. To prevent contamination of the food, a variety of controls can be applied that will inhibit pathogenic growth and reduce the risk of a foodborne illness. Factors that influence the growth of harmful microorganisms include the nutrient availability of the food, the temperature and length of time for which the pathogens are allowed to grow, the acidity of the food, the presence of oxygen, and the water activity or moisture content of the food (1). All of these factors can be used simultaneously to prevent growth. The more controls in place, the greater the prevention against microbial growth. Using multiple factors for preventing pathogen growth is known as the "hurdle concept"; the more controls in place, the more barriers the bacteria will have to overcome for growth (2). While it is important to consider all factors that impact levels of contamination on food, the level of acidity as a control for microbial growth will be the focus of this study.

Acidity and alkalinity is measured on a pH scale that ranges from 0 to 14, with a pH of 0 being the most acidic, a pH of 7 being neutral, and a pH of 14 being the most alkaline. Microorganisms require a specific pH range for optimal growth; they will not grow outside of that pH range (2). When the pH range of the food is controlled, we can selectively inhibit pathogenic growth by selecting the range at which bacteria do not readily grow. The majority of pathogens grow best at a neutral pH of around 7 (2). When the pH drops below 4 the majority of pathogenic growth is inhibited (2). Therefore having a higher acidity can effectively prevent pathogen proliferation. Some foods have a naturally high acidity which will help protect them from contamination. Meat and fish for example tend to have a more neutral pH which makes them susceptible to pathogens (2).

To ensure that proper pH levels are being met, measurements can be taken with pH litmus paper strips or a calibrated potentiometric pH meter. The purpose of this research was to determine whether pH test strips can be depended on for giving accurate pH readings.

EVIDENCE REVIEW

Foods may be classified as either high or low acid foods. High acid foods have a pH of 4.6 or lower, whereas low acid foods have a pH greater than 4.6. A pH level of 4.6 is often referred to because this is the point at which growth of *Clostridium botulinum* is effectively inhibited (2). When canning foods, pH is commonly used as a control. *C. botulinum* is a major concern for contamination and growth in canned goods, which is why its pH range for growth is often relied upon. If the food has a pH greater than 4.6, other control factors such as temperature control should also be implemented for prevention of bacterial growth.

In the processing of foods pH can be controlled with the addition of pH adjusting agents. In Division 15, Part B of the federal Food and Drug regulation Table 10 outlines the wide variety of food additives that may be used as pH adjusters (3). Foods that are commonly controlled by lowering the pH are known as acidified or pickled foods. Acidified foods are those that have initially low-acid to which acids or acidic foods are added to lower the pH and control bacterial growth (4). Foods that are commonly acidified include pickled beets, red bell peppers, pears, some tropical fruits that are naturally less acidic, green olives, tomato salsa, or foods that are being canned into a hermetically sealed container (4). In hermetically sealed containers *C. botulinum* is of particular concern as they are a spore forming bacteria that vegetate in the absence of oxygen.

Another example in which acidity is increased in a food to help curb pathogenic growth is in the preparation of sushi rice. Rice is particularly susceptible to the microorganism *Bacillus cereus* which is a spore forming organism that can vegetate after being cooked at high temperatures, creating a risk for foodborne illnesses. Sushi rice is traditionally held at room temperature so the addition of vinegar, which has a low pH, is used to lower the overall pH of the rice and establish a control for pathogenic growth since temperature is not being used as the control (5). It has been found that a pH of around 5.0 will sufficiently prevent the growth of *B. cereus* (6). As part of the processing of these foods it is imperative that the processing facilities, service establishments, and those performing food safety inspections verify that appropriate pH levels are being met with reliable pH measuring equipment.

Other health factors to consider with regards to pH levels

With regards to the acidity and alkalinity of foods there are other health concerns to be aware of when acidifying foods. When dealing with acidic foods such as fruit juice, maple syrup or tomatoes, it is important not to store these items in metal containers for extended periods of time. The acid in these foods can dissolve the metal container they are being held in and could lead to potential metal food poisoning (7). A food processor or handler should be aware of the pH levels of the foods they are dealing with so they can choose appropriate containers and avoid metal poisoning.

Other health concerns around acidic foods include the potential for dental erosion with a highly acidic diet (8). Whether a more acidic or alkaline diet is better for your overall health has yet to be determined. Some studies have shown that having a more alkaline diet may reduce morbidity and mortality from chronic diseases, however more research is needed in this area (9).

Measuring the pH: potentiometric vs. colorimetric

Potentiometric measurement of acidity involves using an electric calibrated pH meter that consists of an electrode probe and a digital display. Colorimetric measurement involves the use of dyed paper that changes colour depending on the pH level. To accurately measure the pH of food by either method requires preparation of a uniform sample where any excess oils are removed or decanted from the food (10). For solid foods, a blender should be used to make a slurry by adding 50 ml of distilled water for every 100 grams of food. Once finished blending, a pH measurement can be taken by either dipping the electrode of the calibrated pH meter or the paper test strip into the slurry (10). It is also important to maintain a consistent temperature while measuring pH levels, as the pH values will fluctuate with temperature. When measuring strongly acidic items the pH level (3 or lower) will not fluctuate with temperature, however neutral and alkaline foods will have a pH level that is temperature dependant (11). When calibrating the pH meter a consistent temperature should be maintained.

Using colorimetric measurement with paper pH strips can be an inexpensive and a quick way to roughly determine the acidity of a food. They are stable and generally do not have explicit expiration dates. To maintain their working condition they should not be stored in direct sunlight or humid areas (12). Paper strips indicate the pH level by changing colour. They come with a colour scale that helps the user determine the pH range by matching the colour of the strip. Papers can be purchased in rolls or strips and may indicate wide or narrow pH ranges (13). The most sensitive pH test strips that are available can indicate differences of up to 0.2 pH units (13).

With potentiometric methods, the pH meter must first be calibrated. To do so, two standard buffer solutions must be used and should be at the same temperature (13). After rinsing the electrode with deionized water and drying it, it can be submerged into the first standard buffer, then the meter should be adjusted so that it matches that of the pH of the buffer. It should be placed into the same buffer twice more to ensure the successive readings are also accurate. Repeat this procedure with a second standard buffer of a different pH to complete the calibration; this is known as two point calibration (13). It is recommended to use a pH meter that has an accuracy within 0.02 units (10).

Guidelines for acidification and pH measurement

In the province of British Columbia there are no specific regulations that require monitoring and maintaining certain pH levels in specified foods. There are several guidelines that recommend pH levels should be below 4.6, particularly for foods that are commonly prepared at home for sale at markets such as jellies, jams, pickled vegetables, and salsas (14).

The British Columbia Centre for Disease Control (BCCDC) also has specific recommendations for pH levels in sushi rice that is cooked then kept at room temperature (15). At these temperatures rice is at greater risk for pathogenic growth and as such vinegar must be added to lower the pH and put in place another barrier to bacterial proliferation. The BCCDC recommends acidifying the room temperature sushi rice to a pH of 4.2 to ensure pathogenic growth is inhibited (15).

The Manual of Procedures for Meat Hygiene issued by the government also gives recommendations for those involved in food processing and inspection (16). It is recommended that paper pH strips be used for checking foods while they are being processed, or when finished. The only time it is not recommended to use pH strips is when the pH is 4.0 or higher (16). At this point the accuracy of the paper pH strips cannot be relied upon to guarantee the product has a pH less than 4.6. Verifying their accuracy in this study will determine whether or not this is an appropriate recommendation.

Other applications of pH control

When canning foods, verifying a proper pH has been met is an important control point to stop pathogenic growth, especially for preventing *C. botulinum*. The quality of the food such as flavour, colour, and texture can also be impacted by pH levels, so monitoring levels will be of value to the food processor (17). Testing the pH of a food is a useful quality control check not only for prevention of microbe growth but also for maintaining desired flavour. This gives food processors multiple reasons to ensure proper acidity is being met.

Strengths and limitations

In the research around pH as a food safety control, it has been well established that pH is effective for prevention of bacterial growth. Sufficient evidence has shown that lowering pH is an effective food safety control. As a result specific pH limits have been determined for various food pathogens. For example, maintaining a pH below 4.6 will effectively control most bacterial growth. It is generally known that calibrated pH meters will provide more accurate readings compared to paper test strip. However there is little research that has quantified how accurate or inaccurate paper strips are.

Gaps in the research and knowledge

To date there is little research with regards to comparing the accuracy of pH test strips to that of a calibrated pH meter. One study looked at comparing the accuracy of four different portable pH meters compared to pH paper for measuring the acidity of dog urine. The study found that the calibrated pH meters provided near perfect accuracy, whereas the paper strips had poor to moderate accuracy (18). With regards to testing the pH of foods there is insufficient literature to conclude whether or not pH strips provide sufficiently accurate measurement to confer absolute food safety. As they are the less expensive method of measurement, knowing whether they can be relied on is important as they are more easy to use for the general population and food establishments.

METHODS

This experiment involved using 40 different samples of cooked rice that had been made into a slurry, with each sample measuring exactly 50mL. Regular white rice was cooked in a rice cooker, then blended into a uniform slurry with a handheld blender. Distilled water was added to the slurry to aid with the blending without impacting the pH; 50mL of water can be used for every 100mL of food without impacting the pH value (3). The slurry was then separated into 40 different beakers. Each beaker contained 50mL of rice. Each rice sample was acidified independently with different amounts of white vinegar to create varying levels of pH across all of the samples. A Pasteur pipette was used to add the white vinegar to the rice sample, starting with 0.5mL of vinegar for the first sample. Increments of 0.5mL of white vinegar were added to subsequent samples. This resulted in the 40th sample receiving 15.0mL of white vinegar. All ingredients were kept at room temperature prior to mixing to ensure each sample is the same temperature, and prevent fluctuations in pH readings that can result from temperature differences (11). A digital food grade thermometer was used to ensure each sample was the same temperature. The pH of the samples were first read using the calibrated pH meter, and then again with the pH paper strips. The pH meter was calibrated using two buffer solutions, one with a pH of 4.0 and the other with a pH of 7.0.

To further verify the accuracy of pH test strips across different food products, a variety of jarred preserves were tested. The specific purpose was to determine whether coloured food items can stain the test strip to the point where the ability to accurately read the strip has been compromised.

The null hypothesis for this study is that readings obtained from pH test strips are equally as accurate as the readings obtained from a calibrated pH meter. The alternative hypothesis is that readings obtained from the pH test strips are either higher or lower than the readings from a calibrated pH meter.

RESULTS

The data produced from conducting this experiment is entirely numerical. Both the calibrated pH meter and the pH test strips produce numbers from the pH scale which ranges from 0 to 14. The mean pH reading from the calibrated pH meter was compared to the mean pH reading from the pH test strips. The standard deviation helps to elucidate how the data is spread around the mean. The data from this experiment was analyzed using NCSS 11 (19). As the data compares the means from two independent groups an Independent t-test was used for analysis. The descriptive statistics from the experiment are shown in Figure 1 (below).

pH Measure	Count	Mean	Median	Standard Deviation
Meter	40	3.22	3.2	0.26
Strip	40	3.07	3	0.35

Figure 1: A table outlining the descriptive statistics from the study.

At the time of the experiment, the laboratory in which the study was conducted held a stable temperature of 20˚C throughout the duration of the study. The data was successfully collected from all 41 samples using both the pH test strips and the pH meter. Looking at the Test of Assumptions, the Kurtosis Normality shows that normality was rejected. Therefore this must be analyzed using non-parametric test results from the Mann Whitney U test. The two-tailed test was examined because it is unclear whether the pH readings from the test strips would be higher or lower than that of the pH meter. A P-value of 0.0003 was achieved from the statistical analysis. Therefore the null hypothesis is rejected, and it can be concluded that there is a statistically significant difference in pH readings between the pH strips and the pH meter. This signifies that both methods of measurement are not equally accurate and pH test strips cannot be relied upon as confidently as a pH meter for confirming safety in foods that use acidity as a control for pathogen growth.

There is strong practical significance from this result as it confirms the accuracy of pH test strips to be less reliable than the pH meter. Therefore suggesting the use of pH meters over pH strips may be more practical for food processors. The weakness from this test is that the power is relatively low at 60%, when it should be 80% or over to have an acceptable power. In order to improve this result the number of samples to be measured could be increased.

From examining the impact that different coloured preserves have on staining the pH test strip, another weakness of test strips was uncovered. The test strips that were dipped in the cherry and blackberry preserves were found to be sufficiently stained to make accurately reading the results impossible. Those that were dipped in the lighter pepper jelly and marmalade remained unstained and could still be read accurately.

Figure 2: Shows the extent of staining from different coloured preserves.

DISCUSSION

In comparing the two available methods for measuring acidity of food, using pH test strips or a calibrated pH meter, this study found that the pH meter offers more consistently reliable results. It could not be statistically concluded that pH test strips deliver as accurate readings as a calibrated pH meter. Despite this finding, there are still instances in which pH test strips may be useful to determine a food product's relative safety.

The standardly used pH level to confirm that pathogenic growth will be inhibited is 4.6. Food products should be below this level in order to be deemed capable of curbing microbiological growth (2). Due to the nature of the measuring process using pH test strips, one can only determine the pH range that the food product falls within. Test strips cannot determine the pH level down to a specific decimal point, whereas the pH meter is able to do this. Therefore the pH test strips should only be relied upon to confirm a pH range. A pH meter should be used in place of test strips when confirming that the level is a below a specific point such as 4.6. This practice is confirmed by the standard operating procedure recommended in The Manual of Procedures for Meat Hygiene issued by the federal government. The manual recommends pH strips be used for checking foods while they are being processed, or when finished. The only time it is not recommended to use pH strips is when the pH is 4.0 or higher (15).

This study looked at an additional component of the colorimetric method, to determine if coloured foods would impact the ability to accurately read the test strip. It was found that lighter coloured foods such as rice slurry and light preserves did not impede the ability to read the strips whereas dark jams stained the strip such that it could not be read accurately. The British Columbia Guideline for the Sale of Foods at Temporary Markets recommends that products such as salsa, jams, and jellies should have a pH of 4.6 or lower (14). Due to the discoloration impact that some of these products have on pH test strips, this study supports using a pH meter as opposed to test strips for verifying the pH of dark coloured products.

There is little research that determines the accuracy level of pH strips, however the findings from this experiment do align with another study that compared pH meters to test strips for measuring the acidity of dog urine. That study found that the calibrated pH meters provided near perfect accuracy, whereas the paper strips had poor to moderate accuracy (17). With regards to testing the pH of foods there is insufficient literature to conclude whether or not pH strips provide sufficiently accurate measurement to confer absolute food safety. This study aimed to fill that gap in research.

RECOMMENDATIONS

Due to the relative inaccuracy of pH test strips compared to using a calibrated pH meter for measuring acidity levels, it is recommended that pH test strips only be used in certain cases. In order to confirm the final pH of a prepared food product, a calibrated pH meter should be used as it will give a more accurate reading down to several decimal points. The pH test strips may be used more as an indicator that the acidity of food falls within a specific desired range. This could be used to confirm the general pH range of a food product while it is being prepared, which will help the producer determine whether adjustments to the acidity need to be made.

Due to the staining of the pH test strips that result from being dipped in dark jams and jellies, they cannot be relied on for accurate readings with these products. To test the pH of a dark coloured and staining food product, only a calibrated pH meter should be used.

LIMITATIONS

In the execution of this study there were factors that could be improved upon to increase the validity of the results. For measuring the pH of the prepared rice slurry, pH test strips that measure the full range of the pH scale were used. Test strips with narrower pH ranges do exist, and are able to measure the level of pH to a more specific degree. There is potential that these strips would provide more accuracy than the ones used in this study, however the lab was unable to provide the narrow ranged strips.

Another limitation in this study is the lack of research around this topic. In order to definitively determine whether pH strips should be used as a reliable method of measuring pH, more research is needed.

FUTURE RESEARCH

1. The pH of the rice slurry was observed to increase slightly over time. Perhaps more time is needed for the pH to settle, as it seemed to become less acidic over time. A study could determine the length of time needed for the pH level to stabilize
2. Compare a variety of different strips, using different brands and pH ranges, to determine whether some are better than others.
3. Another study could determine if there a way to measure darkly coloured preserves without staining the test strip beyond the ability to read it accurately.

CONCLUSION

It has been well established that monitoring pH levels for various foods is used as an important control for pathogenic growth. To date, few studies have quantified the level of accuracy that paper pH strips can produce. Food safety guidelines recommend only using pH strips in certain circumstances because of unknown or unreliable accuracy. This study has determined that the calibrated pH meter will offer more accurate and therefore reliable results than a pH test strip. The findings from this study support the reasoning behind only using pH test strips as a rough indication of the pH level of a certain food product, and not to try and determine the exact pH level of the product with the strips. The pH test strips are better for quick measurements to verify it is within a certain desired range, but if one wants to look at finer measurements of pH levels they should not be relied upon. A pH meter would be more suitable in this case.

The pH test strips may also be unreliable when testing certain food products that may stain the strip, making the colorimetric results difficult to read. For these cases, measuring foods such as dark jams and salsa, it should be noted that a pH meter will be more reliable. The pH was found to have risen after the vinegar sat in the rice for extended periods of time. More research can be done in this area to determine how much time is needed for the pH to reach a stable level. Food operators should be aware that pH levels may increase shortly after adding vinegar to the rice, therefore more stringent monitoring procedures may be necessary.

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COMPETING INTEREST

The authors declare that they have no competing interests.

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