The Effect of Probiotics as a Starter Culture for Producing Yogurt

SungHoo Jegal[1], Helen Heacock[2], Aljosa Trmcic[3], Fred Shaw[4]

1 Lead Author, School of Health Science, British Columbia Institute of Technology, Burnaby, BC

2 Supervisor, School of Health Science, British Columbia Institute of Technology, Burnaby, BC

3 Supervisor, BC Centre for Disease Control, Vancouver, BC

4 Contributor, School of Health Science, British Columbia Institute of Technology, Burnaby, BC

Abstract

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Background: Many comprising studies showed that probiotics can manifest antimicrobial activity. Due to positive health effects of probiotics, they have been added in a fermentation of various foods to increase the nutrient content and to improve the quality of the foods. Furthermore, probiotics are used as a starter culture for several fermented foods like a yogurt. Probiotics may contain strains that are capable of initiating fermentation of the foods, however, a safety of the foods is not certain. Therefore, the study is done to analyze use of probiotics as a starter culture for a yogurt.

Methods: The study was designed to analyze the pH pattern of three different yogurt groups (control, commercial starter culture, and probiotics). Each group had three samples that were made using Dairyland 2% milk and corresponding cultures. The control group samples were not inoculated with any culture. The commercial starter culture group samples were inoculated with Yogourtmet Freeze-Dried Starter and the probiotics group samples were inoculated with probiotic capsule, Jamieson 10 Billion Probiotic. The samples were incubated for 7 hours and every 45 minutes the pH was measured using Hanna Professional Portable Yogurt pH Meter.

Results: The statistical analysis of the pH measurement showed significant different between the control groups and other two groups. The control group samples pH decreased a bit, but it was not enough to turn the samples to a yogurt.

The pH pattern of the commercial group samples showed rapid decrease in pH after 180 minutes and the average pH of the last reading was 4.10. The pH of the probiotics group samples decreased linearly, and the average pH of the last reading was 4.58.

Conclusions: The commercial starter culture and the probiotics group samples initiated fermentation and enough acidification occurred to decrease the pH below 4.6. With 7 hours of incubation period, the probiotics group samples just met the pH that makes the yogurt safe to consume. Therefore, the use of the probiotics as a starter culture for producing yogurt can be suggested with adequate incubation period.

Keywords: Yogurt, pH, probiotics, starter culture, fermentation, acidification

Introduction

Around the world, fermented foods are consumed, and there are various kinds of fermented foods such as yogurt, kefir, kombucha, sauerkraut, kimchi, miso, and cheese. These foods are prepared in various ways, and most of them are traditional foods that many people enjoy. The fermented foods, including fermented beverages, have probiotics that are known to be beneficial to humans when consumed. Because of growing demands for probiotics, many kinds of probiotic supplements are available in pill and capsule form. Many homemade and commercially produced fermented foods use probiotics as a starter culture to boost the speed of the fermentation, enrich the nutrient contents, and to control the quality of the foods. There has been much research done on probiotics and their clinical effects on humans but not much done on the safety of fermented foods in respect to pH. The study will focus on the administration of probiotics and relative pH level of yogurt.

Literature/Evidence Review

History of Fermentation

Fermentation of food has a long history, and the means of fermentation was the preservation of foods in the past. Fermentation does not only apply for foods, but it is also incorporated with

alcoholic beverages. The earliest report of fermentation began in the Neolithic Chinese and ancient Roman era between 7000 BC and 6000 BC. Many of the countries started to produce fermenting beverages and foods in the early period.

Especially in Asian culture, people developed a series of fermented foods and beverages for an everyday meal (Barbosa-canovas, 2016). Wine is popular alcoholic beverages produced by the fermentation process of fruit, honey, and rice; it has been conducted since 7000 BC (Chambers and Pretorius, 2010). Furthermore, the traditional fermented foods and beverages were frequently consumed for therapeutic purposes even though people did not have scientific evidence that describes the mechanism of the probiotics in the body (Ozen and Dinleyici, 2015).

Fermented Food Safety

Fermentation practices were carried out throughout the world. It has been such a long time since the development and production of indigenous fermented products. There are now a tremendous number of different types of fermentation foods around the world (Franz et al., 2014). In the past, the major purpose of fermentation was mostly related to preservation and people did not understand the mechanisms of fermentation. However, development in technology revealed health effects of fermented foods. Studies were done on foods to identify microorganisms that are responsible for fermentation and beneficial health effects (Lourenes-Hasttingh and Viljoen, 2001). Development of technologies and growth of interest in fermentation foods lead to a new way of fermentation techniques (McNeil and Harvey, 2008). Many factors may affect the safety of the foods. Water activity, pH, preservatives, temperature, and competitive microflora contribute to the fermentation, and a combination of these factors make the food safe to consume (Mclntrye, 2016). In the case of fermented foods, pH is the critical factor in determining the safety of the foods. pH of the fermented foods decreases due to the microbial activities (Russell and Diez-Gonzalez, 2008). There are no specific legislations or regulations regarding production of

fermented foods. However, Mclntrye, food safety specialist from BCCDC created Fermented Food Safety advisory document. According to the document, the fermentation should occur within 24-72 hours and should reach a pH of 4.6 within 72 hours; pH of final products should not pass below 3.2. The water activity should be 0.85 or less and must be stored at temperature of 4 degree Celsius or less. Due to the fact that fermentation occurs spontaneously and requires bacterial growth, it is vital to reduce or eliminate pathogenic microorganisms that can be present in the raw source of the fermentation. Therefore, preparation and process of the fermentation must be conducted in a way that it will minimize cross contamination and avoid undesired microorganism in the foods or beverages.

History of Probiotics

Russian bacteriologist Eli Metchnikoff discovered the health effect of the probiotic in 1908. He found scientific evidence of the beneficial health effect of lactic acid bacteria in fermented milk and recommended people to drink fermented milk in order to prolong life (Lourens-Hattingh and Viljoen, 2001). Meanwhile, in 1899, Henri Tissier isolated bifidobacteria from the feces of breast-fed infants by displacing the microorganisms responsible for gastric upsets (Lourens-Hattingh and Viljoen, 2001; Lee and O'Sullivan, 2010).

Probiotics

The amount of microbiota in the gastrointestinal (GI) tract differs between individuals but approximately 10 - 100 trillion symbiotic microbiota has existed in the human gut (Ursell et al., 2012). Some of the microorganisms are pathogenic, and some are commensal; some of the commensal microorganisms are the same strains as the probiotics and have the same beneficial properties (Martin et al., 2013). The term probiotic refers to a viable bacterial microorganism which can have beneficial effects on humans (Kechagia et al., 2013). Theses friendly microorganisms are usually incorporated in the fermented foods. Due to the health effects of probiotics, demand for probiotics has increased drastically during the past two decades (Mattila-Sandholm et al., 2001). To

satisfy the growing demand for probiotics, industries have been working on isolating and mass-producing specific strains of probiotics (Fijan, 2014). The effects of the probiotics on human are well studied. However, the mechanisms, how they react with certain organisms still needs to be studied.

Clinical Effect of the Probiotics

Most of probiotics are consumed orally and go through the human digestive system. Once probiotics are in the body, they must be able to survive in the extremely low pH condition of a stomach and make their way to rest of GI tract. Therefore, in order to have a beneficial effect, probiotics must express certain desirable properties. The probiotics must be stable and viable in the digestive system which means that they are acid and bile tolerant, adhesive to mucosal and epithelial surfaces, and able to compete with pathogenic bacteria (Kechagia, 2013). Currently, many promising studies claim a significant scientific evidence of the beneficial effect of probiotics. Many people know that probiotics are good for the digestive system, but their potentials for clinical use are far beyond just digestive aids. The microbial balance and immune system in humans can be improved by consuming adequate amounts of probiotics. More specifically, they can be used to prevent and treat Helicobacter pylori infection, prevent systemic infections, manage inflammatory bowel diseases, prevent and treat atopic diseases, prevent and manage allergic diseases, and postoperative infections (Kelesidis and Pothoulakis, 2012; Gill and Guarner, 2004). Also, they have functions of anticarcinogenic and antimutagenic activities, mitigation of lactose intolerance symptoms, reduction in cholesterol and blood pressure level, prevention of bacterial vaginosis and urinary tract infection, retainment of mucosal integrity, and improvement of periodontal health (Franz et al., 2014). Sometimes they are used as a starter culture of the fermentation.

Association of Food-borne Diseases and Fermented Products

Although fermented products are considered as safe from foodborne diseases, outbreaks caused by fermented products are reported

sometimes, and most of the outbreaks are due to mishandling of the initial and final products, or from improper processing of the fermentation. For example, according to Matargas et al. (2015), Salmonella enterica and Listeria monocytogenes are the pathogens that can be found in the raw materials of Italian fermented sausages cacciatore and Felino. If initially, the raw materials such as pork and chicken contain a large amount S. enterica or L. monocytogenes, the final sausage products are likely to contain unacceptable levels of the pathogens that are a health hazard to humans. However, if pH of the sausage decreased fast enough to a certain level and maintained at that pH, then it will inactive the pathogens (Matargas et al., 2015).

Methods and Materials

Materials

The equipment required was a stainless saucepan, a Taylor instant read dial thermometer, a spatula, a whisk, 500ml glass beakers, glass shots (50ml), and an oven (Christensen, 2018). Dairyland pasteurized 2% inorganic milk was selected to make yogurt with two different starter cultures and one without starter culture. The commercial yogurt starter culture, Yogourtmet Freeze-Dried Starter, and probiotic capsule, Jamieson 10 Billion Probiotic, were used as a starter culture.

A

B

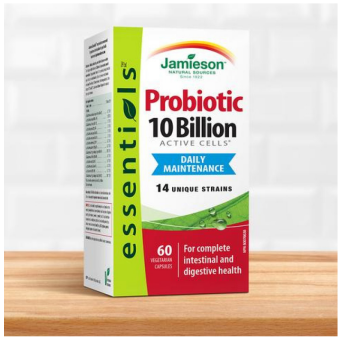

Fig. 1. Selected starter cultures for the experiment (A) Yogourtmet Commercial Starter Culture (Yogourtmet, n.d.). (B) Jamieson Probiotic Capsules (Jamieson, n.d.).

Ingredient of Starter Cultures

The ingredients of Yogourtmet are skim milk powder, sucrose,

Lactobacillus bulgaricus, Streptococcus thermophilus, and Lactobacillus acidophilus. It contains 100 billion live and active bacteria per 100g serving (Yogourtmet, n.d.).

Jamieson 10 Billion Probiotic contain 14 strains of probiotics: Bifidobacterium lactis, Lactobacillus paracasei, Bifidobacterium breve, Lactobacillus gasseri, Lactobacillus rhamnosus, Lactobacillus rhamnosus, Lactobacillus acidophilus, Lactobacillus plantarum, Bifidobacterium longum, Bifidobacterium bifidum, Lactobacillus casei, Lactobacillus reuteri, Lactococcus lactis, and Bifidobacterium infantis. It contains 10 billion CFU of probiotics (Jamieson, n.d.).

Preparation of Yogurt

The experiments designed to repeated three times to increase the consistency and reliability of the results. As seen in the Fig. 2, there were 3 different groups, and each group consisted of 3 samples, therefore 9 samples were prepared for each experiment and in total 27 samples were prepared for the 3 experiments. The first group was the control. The control group samples were not inoculated with a starter culture and set for spontaneous fermentation. The second group samples were inoculated with the commercial starter culture (Yogourtmet). The third group samples were inoculated with the probiotics (Jamieson). Before making yogurt, all the equipment used for making yogurts were sterilized with boiling water to produce the accurate results. 1L of 2% milk was poured into the pan and heated to 83°C (Wells, 2018). Once the temperature of the milk reached 83°C, just before boiling point, the heat was turned down to low and simmered for 5 minutes. The heated milk was then cooled down to 44°C in an ice bath. While cooling down the milk, it was stirred gently to prevent skin forming (BC Dairy Association, n.d.). After the temperature of the milk reached 44°C, 250ml of milk was transferred into 3 different 500ml beakers. Appropriate starter cultures were added to the beakers (1.25g of the commercial starter culture and 3 probiotic capsules) and mixed well. Each inoculated and non-inoculated 250ml of milk were then poured into 3 clean glass shots and set in oven for 7 hours. The oven was pre-

heated at 40°C. The procedure was repeated 3 times.

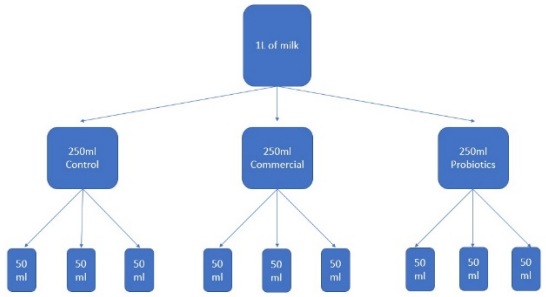

Fig. 2. Flow chart of the samples for an experiment.

Measuring pH of the samples

The pH of samples was measured repeatedly every 45 minutes during 7 hours of the incubation period using a Professional Portable Yogurt pH Meter (HI-98164) from Hanna Instruments. To increase the accuracy of the measurement, a three-point calibration was made with three standard buffer solutions with pH of 4.01, 7.01, and 10.1 (Hammel et al., 2016). The electrode was rinsed with distilled water and placed into the buffer solution. The CAL button was pressed, and the pH reading appeared in the display. Up or down keys were used to adjust the buffer value. When the pH reading became steady, a CFM functional key appeared and was pressed to confirm the first calibration value. Second and third calibrations were done followed by same procedure as the first calibration. Once all the calibrations were done, the instrument saved the calibration values and returned to normal mode (Hanna Instrument, n.d.). The standard buffer solution of pH 4.01, 7.01 and 10.1 were used to calibrate because the data were mostly measured from pH of 3 to 6. The electrode was cleaned with distilled water between calibrations to rinse off all traces of the previous buffer solutions to prevent the possible measurement errors and produce valid and reliable data.

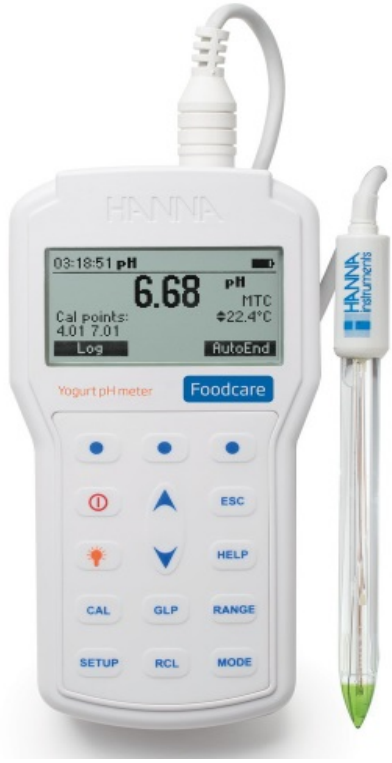

Fig. 3. Hanna Professional Portable Yogurt pH Meter (Hanna Instrument, n.d.).

Justification of methods

Spontaneous fermentation was used for the true control to see if bacteria in milk is enough to initiate a fermentation. The commercial starter culture was chosen for the second group because they are largely available, and it is specifically used for making yogurt at home. For the third group, probiotics (Jamieson 10 Billions Probiotic) was chosen because it contains strains of bacteria that are capable of turning milk into yogurt such as Lactobacillus (Sarvari et al., 2014).

Each group has 3 samples; therefore, 10 readings of each sample will give 30 data points for each group which is enough to do statistical analysis. The experiment is repeated 3 times (3 times for control, 3 times for the commercial starter, and 3 times for probiotics). By repeating experiment 3 times, consistency of the results can be verified.

The amount of commercial starter culture used was decided based the product instructions. The amount of probiotics used was decided based on how much people usually use to make yogurt at home.

Alternative Method

Instead of measuring pH for every 45 minutes for 7 hours, all samples can be measured once after incubation period to see if the 7 hours of incubation period is enough for yogurt to reach pH below 4.6. The groups can be the same: control, commercial starter, and probiotics. Each group will have 30 samples. All the procedures will be repeated 3 times thus will be measuring 90 samples of pH of each group.

Results

Descriptive Statistics

The collected data were continuous numerical data. Each group of yogurts consisted of 3 samples and pH of each sample was measured and recorded every 45 minutes for 7 hours after the samples were placed in the oven for the incubation. Each sample consisted of 10 readings of pH measurements, and therefore each group has 30 readings of pH measurements. Below table.1A, 1B, and 1C are the summary of the mean,

median, standard deviation and max/min of the variables for each of the three experiments.

A

	Mean	Median	SD	Max/Min
Control pH	6.519	6.505	0.05558	6.67/6.43
Commercial Starter pH	5.452	5.775	0.9576	6.65/4.02
Probiotics pH	5.477	5.482	0.6639	6.62/4.54

B

	Mean	Median	SD	Max/Min
Control pH	6.509	6.490	0.05558	6.65/6.42
Commercial Starter pH	5.430	5.710	0.9576	6.69/4.03
Probiotics pH	5.487	5.385	0.6639	6.68/4.53

C

	Mean	Median	SD	Max/Min
Control pH	6.485	6.485	0.07623	6.63/6.36
Commercial Starter pH	5.420	5.645	0.9548	6.61/4.09
Probiotics pH	5.481	5.415	0.6651	6.63/4.54

Table. 1. Descriptive Statistics of the first experiment (A), the second experiment (B), and the third experiment (C).

All the sample groups presented the same trend where pH decreased as incubation time increased. More specifically, samples with the commercial starter and the probiotics decreased pH rapidly within the 12 hours period. The standard deviation for the control group was the smallest followed by the probiotics group and the commercial starter group. On the other hand, an opposite trend of mean and median values was observed from the trend of standard deviation.

ANOVA Inferential Statistics

All the statistical analyses were carried out through NCSS software (Heacock, 2018 & NCSS, 2018). A one-way ANOVA test (appendix A) was conducted to see if there were differences in means of different groups (Heacock, 2018). The null hypothesis (Ho) was there is no difference in pH between means of the control, commercial starter, and probiotics. The alternative hypothesis (Ha) was at least two means of pH of the control,

commercial starter, and probiotics are different.

The first and second experiment reports (appendix A) showed that the data were parametric and unequal variances therefore Welch's test was read. The p-values for the both experiments were 0.000001 which were less than 0.05 therefore null hypothesis was rejected and it could be concluded there statistically significant differences between at least two means of the groups. The power values were 99.9%. Therefore, one could be confident that the results were true. There were not likely to be alpha errors and beta errors. Based on the Scheffe's Multiple-Comparison Test, the control group was different from the commercial starter group and the probiotics group while the commercial starter group and the probiotics group did not have a statistically significant difference.

The third experiment report (appendix A) showed different results from the first and the second experiment. The data were non-parametric and unequal variances; therefore, Kruskal-Wallis One-Way ANOVA was read. The p-value was 0.000001. It is significantly smaller than 0.05, therefore, the null hypothesis was rejected and concluded that there was a statistically significant difference between at least two means of the groups and there were not likely to be an alpha error. The power value was 99.9% indicating that results were most likely true and beta errors were not likely to exist. Like the first and second experiment, Scheffe's Multiple-Comparison Test remarked the control group was different from the commercial starter group and the probiotics group while the commercial starter group and the probiotics group didn't have statistically significant difference.

Repeated Measures ANOVA Inferential Statistics

Repeated Measures ANOVA test (appendix B) was used to compare means of pH of the groups within the time at which each measurement was made. There were three null and alternative hypotheses. The null hypotheses were that there is no significant difference between the pH of the groups (control, commercial, probiotics), there is no significant difference between the pH of the different measurement times (0,45, 90,

135, 180, 225, 270, 315, 360, 405 minute), and there is no significant difference between the pH of the groups within the different measurement times. The alternative hypotheses were that there is significant difference between the pH of the groups, there is significant difference between the pH of the different measurement times, and there is significant difference between the pH of the groups within the different measurement times.

Based on the Analysis of Variance Table (appendix B), the p-values between the pH of the groups, the pH of the different measurement times, and the pH of the groups within the different measurement times were 0.0000001, therefore, the null hypothesis were rejected and concluded that there were a statistically significant difference between the pH of the groups, the pH of the different measurement times, and the pH of the groups within the different measurement times. The power values were 99.9%, therefore, the results were likely to be true, and alpha and beta error were not likely to exist.

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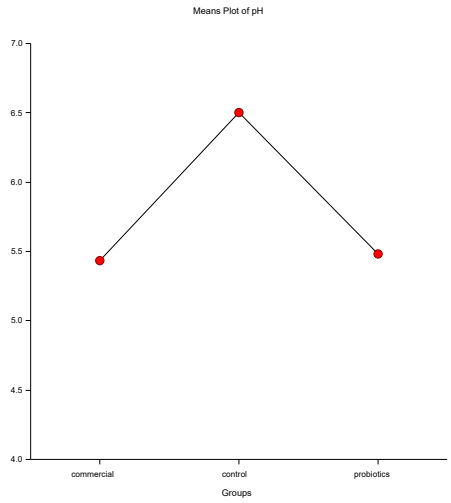

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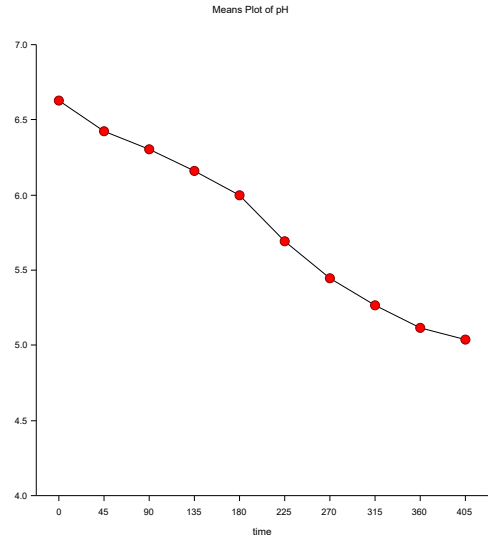

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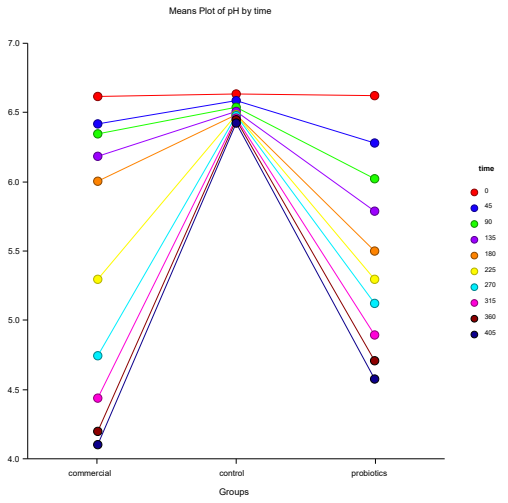

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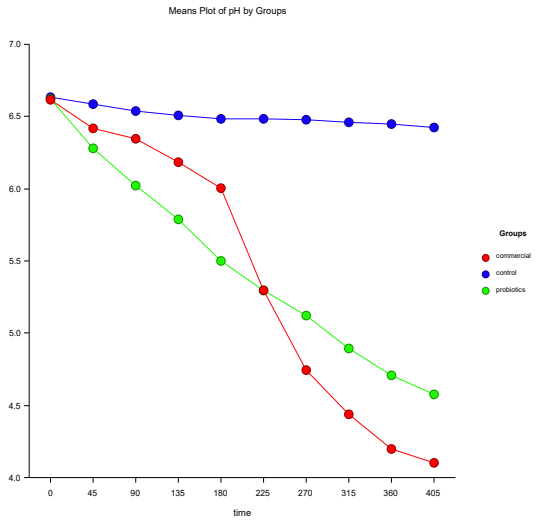

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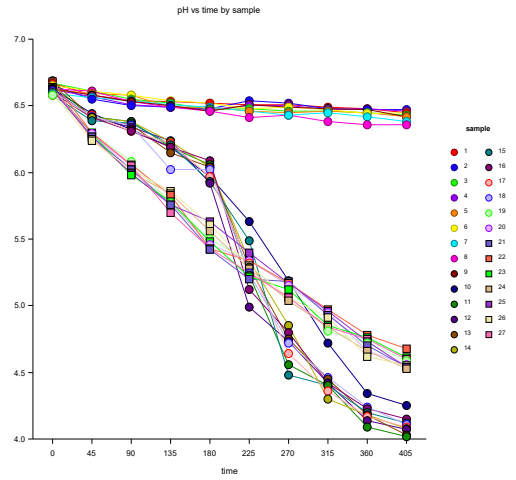

Fig. 4. Repeated Measures ANOVA test results plots. The means of the pH were plotted between the groups (A), at the different measurement times (B), at the different measurement times within the groups (C), for the groups within the different measurement times (D), and for all the individual sample within the different measurement times (E).

As seen in Fig. 4A, the control group had the highest pH mean and the commercial starter group had the lowest pH mean. The overall trend of means of the pH was plotted in Fig. 4B. As seen in the Fig. 4B, the means of pH decreased over time. Fig. 4C and D clearly showed the comparison between the means of pH related to the groups and the different measurement times.

As shown in the Fig. 4C and D, the means of pH of the control and the probiotics group gradually decreased over time, however, the commercial starter culture group decreased the pH rapidly after 180 minutes of the incubation. Until 225 minutes of the incubation, the probiotics group pH means were lower than the means of the pH of the commercial starter culture group. After 225 minutes of the incubation, the commercial starter culture group pH means became lower than the probiotics group pH means (Fig. 4C and 4D). Similar pH changes of the samples were observed within the different groups (Fig. 4E).

The control group samples did not coagulate. The commercial starter group samples coagulated and formed firm yogurt. The probiotics group did coagulate but did not form firm yogurt. Average pH of all the control group samples after 7 hours of incubation was 6.43, 4.10 for the commercial starter group, and 4.58 for the probiotics group. The commercial starter group samples started to coagulate at 225 minutes and smelled like yogurt. Most of them completed coagulation at 270 minutes, formed thick creamy texture. The probiotics group samples started to coagulate at 180 minutes and smelled like yogurt at 225 minutes. However, even at 315 minutes the samples did not complete coagulation. At 405 minutes, the coagulations were not firm, they broke easily and did not formed thick creamy texture like the commercial starter group samples.

Discussion

Decrease in pH was observed in the all groups. The overall trends of decrease in pH of each samples had consistency within the groups. The control group pH gradually decreased over time. Pasteurized milk contains many bacteria that may contribute in a fermentation process (FDA 2019). However, due to not enough fermentation process, milk did not turn into a yogurt. Fermentation of milk require certain strains of bacteria such as Lactobacillus bulgaricus and Streptococcus thermophilus (Sodini et al., 2004). It was not certain that if the pasteurized milk used in the experiment, contains such bacteria furthermore, it is not sure if the bacteria survived the heating process of the procedure. However, as seen in the results one can

suspect that the milk did not contain bacteria that are capable of initiating fermentation.

The results confirmed that the use of the commercial starter culture ensures an adequate pH for the safety of yogurts. As seen in the Fig. 4D, the first half of the incubation period, the commercial starter culture group decreased pH gradually but after 180 minutes pH started to decrease rapidly and as shown in the Fig. 4E, by 315 minutes most of the pH of samples were below 4.6 which is the level of pH that makes the fermented food safe (McIntrye, 2016). The commercial starter culture, Yogourtmet, contains the strains of bacteria (Lactobacillus bulgaricus, Streptococcus thermophilus, and Lactobacillus acidophilus) that are capable of initiating fermentation of milk into a yogurt (Yogourtmet, n.d.; Sodini et al., 2004). Inoculation of bacteria abled rapid decrease in pH of yogurt. The average pH of the last reading was 4.1 which is below 4.6 and therefore comply with Fermented Food Safety Guideline (McIntrye, 2016).

The use of probiotics capsules for yogurts fermentation decrease the pH at the level where it makes the yogurts safety to consume. The probiotics group showed gradual decrease in pH throughout the incubation period. The probiotics (Jamison, n.d.) used in the experiments contains many strains of probiotics such as a Lactobacillus acidophilus which is also contained in the commercial starter culture (Yogourtmet, n.d.) used in the experiment. Even though the probiotics contained many strains of bacteria, the formation of yogurt and degree of decrease in pH was not as good as the yogurt made with the commercial starter culture.

The decrease in pH occurs during fermentation because of microbial activities. In yogurt, lactose is converted into lactic acid by Lactobacillus bulgaricus, and streptococcus thermophilus and accumulation of lactic acid decrease the pH of the product (Russell and Diez-Gonzalez, 2008). The commercial starter culture contained all the strains that were necessary for a fermentation process to initiate, however, even though probiotics did not contain the strains that are capable of initiating a fermentation process, it was able to form coagulants.

Limitation

Due to the design of the experiments required certain equipment (incubation oven, heating stove, basic kitchen utensils, pasteurized milk, commercial starter culture, probiotics supplement and pH meter), the experiments were done at the BCIT lab. The incubation oven used was little small to occupy large sample size. Therefore, the amount of milk used in each sample had to be reduced to fit the incubation oven. This may contribute an error in the experiment because inoculants may not be evenly distributed throughout the samples. This problem could have been solved if the experiment was conducted at the different location where it has big enough oven that can occupy larger volume of samples. However, the pH meter was not available to be used outside of the BCIT lab, therefore, samples must be kept in small volume.

The experiment required 7 hours of incubation period and due to the small size of incubation oven, the experiment had to be conducted for 3 days. Furthermore, the lab and the equipment were shared with other students, therefore, an arrangement was made with other students to utilize the lab. Like mentioned in the discussion, the probiotics used in the experiment contained Lactobacillus acidophilus which was also found in the commercial starter culture. However, within the designed incubation period, the average final pH measurement just met the standard safety pH level of 4.6. In order to see if the probiotics group further decreased the pH and formed more uniform texture of yogurt, the experiment need to last more than 7 hours.

Future Research

- Different brands of probiotics can be used as a starter culture for a yogurt and see if there are any difference in pH.
- Different fermented food can be selected to see if use of probiotics and commercial start culture makes difference fermentation process for other foods.
- Inoculate samples with the strains that are known to initiate fermentation (Lactobacillus bulgaricus, Streptococcus thermophilus, and Lactobacillus

acidophilus) individually and see pH difference in different strains.

Conclusion

The study showed that probiotics can be used as a starter culture for producing yogurt. Decrease in pH pattern was observed in all the groups. The pH changes pattern of the probiotics group showed gradual decrease in pH that indicates the fermentation process was in progress; the average of last pH reading after 7 hours of incubation period was 4.58 which was within the standard safe pH level. Recommendation can be provided to public that when making a yogurt using probiotics as a starter culture, ensure to incubate a inoculated milk for more than 7 hours to produce yogurt that is safe to consume. The commercial starter culture contained strains that were capable of initiating fermentation and the probiotics also contained a strain (Lactobacillus acidophilus) that was capable of initiating fermentation and contained in the commercial starter culture. Compare to the probiotics group, the pH of the commercial starter culture group decreased in more rapid rate. There was a significant difference between the pH of the groups within the different measurement times.

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Competing Interest

The authors declare that they have no competing interests.

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Appendix A

One-Way Analysis of Variance Report

Dataset Untitled
Response control1,commercial1,probiotics1

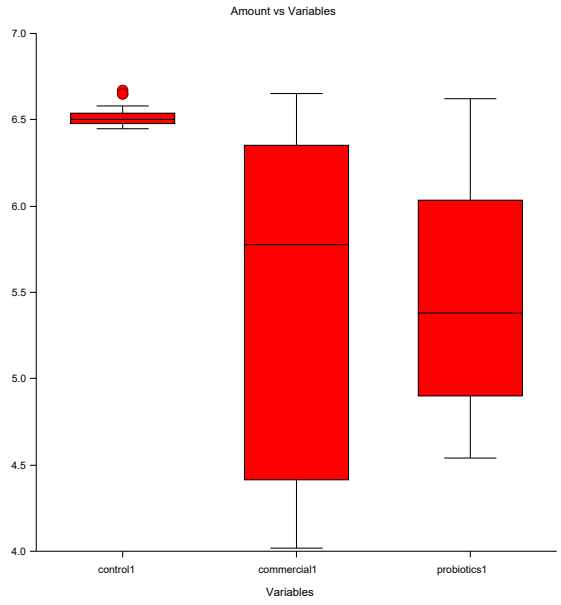
Tests of the Normality of Residuals Assumption

Normality Attributes	Test Value	Prob Level	Reject Normality? (α=0.20)
Skewness	-0.5784	0.56297	No
Kurtosis	-0.9917	0.32135	No
Skewness and Kurtosis (Omnibus)	1.3180	0.51736	No

Tests of the Equality of Group Variances Assumption

Test Name	Test Value	Prob Level	Reject Equal Variances? (α=0.20)
Brown-Forsythe (Data - Medians)	41.1358	0.00000	Yes
Levene (Data - Means)	68.0091	0.00000	Yes
Conover (Ranks of Deviations)	49.9248	0.00000	Yes
Bartlett (Likelihood Ratio)	123.2258	0.00000	Yes

Box Plot Section



One-Way Analysis of Variance Report

Dataset Untitled
Response control1,commercial1,probiotics1

Expected Mean Squares Table

Model Term	DF	Term Fixed?	Denominator Term	Expected Mean Square
A (...)	2	Yes	σ²	σ² + sA
Error	87	No		σ²

Note: Expected Mean Squares are for the balanced cell-frequency case.

Analysis of Variance Table and F-Test

Model Term	Means? DF	Sum of Power Squares (α=0.05)	Mean Square (α=0.05)	F-Ratio	Prob Level	Reject Equal Means? (α=0.05)
Between	2	22.25606	11.12803	24.5315	0.00000	Yes 1.00000
Within (Error)	87	39.46505	0.4536213			
Adjusted Total	89	61.72112				
Total	90					

Welch's Test of Means Allowing for Unequal Variances

Model Term	Numerator DF	Denominator DF	F-Ratio	Prob Level	Reject Equal Means? (α=0.05)
Between Groups	2	39.06	54.0931	0.00000	Yes

Kruskal-Wallis One-Way ANOVA on Ranks

Hypotheses
H0: All medians are equal.
H1: At least two medians are different.

Test Results

Method	DF	Chi-Squared (H)	Prob Level	Reject H0? (α=0.05)
Not Corrected for Ties	2	39.8480	0.00000	Yes
Corrected for Ties	2	39.8677	0.00000	Yes

Number Sets of Ties 17

Multiplicity Factor 360

Group Detail

Group	Count	Sum of Ranks	Mean Rank	Z-Value	Median
control1	30	2102.50	70.08	6.3124	6.505
commercial1	30	1000.00	33.33	-3.1241	5.775
probiotics1	30	992.50	33.08	-3.1883	5.38

One-Way Analysis of Variance Report

Dataset Untitled
Response control1,commercial1,probiotics1

Normal Scores Tests

Hypotheses
H0: All group data distributions are the same.
H1: At least one group has observations that tend to be greater than those of the other groups.

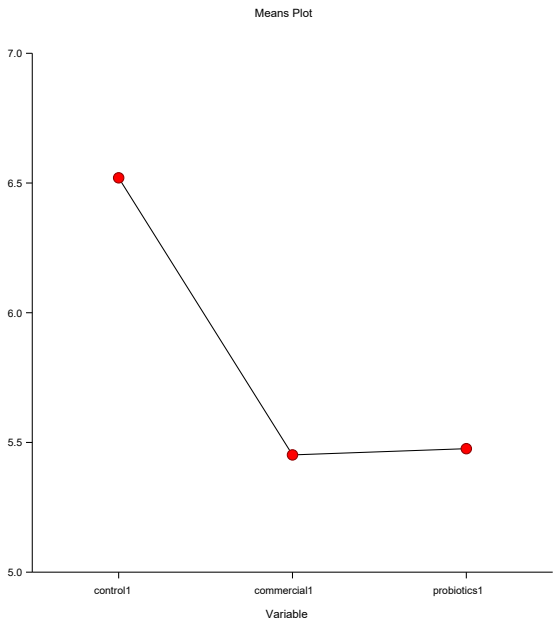
Results

Test	DF	Chi-Squared (H)	Prob Level	Reject H0? (α=0.20)
Terry-Hoeffding - Expected Normal Scores	2	32.7469	0.00000	Yes
Van der Waerden - Normal Quantiles	2	33.1910	0.00000	Yes

Descriptive Statistics

Group	Count (ni)	Mean	Effect	Median	Standard Deviation	Standard Error √(MSE/ni)
All	90	5.816222	5.816222			
A:						
control1	30	6.519333	0.7031111	6.505	0.05558053	0.1229663
commercial1	30	5.452	-0.3642222	5.775	0.9576242	0.1229663
probiotics1	30	5.477334	-0.3388889	5.38	0.6638754	0.1229663

Plots of Means Section



One-Way Analysis of Variance Report

Dataset	Untitled
Response	control1,commercial1,probiotics1

Scheffe's Multiple-Comparison Test

Response: control1,commercial1,probiotics1
Term A:

Alpha=0.050 Error Term=S(A) DF=87 MSE=0.4536213 Critical Value=2.4905

Group	Count	Mean	Different From Groups
control1	30	6.519333	commercial1, probiotics1
commercial1	30	5.452	control1
probiotics1	30	5.477334	control1

Notes:
This report provides multiple comparison tests for all possible contrasts among the the means. These contrasts may involve more groups than just each pair, so the method is much stricter than need be. The Tukey-Kramer method provides more accurate results when only pairwise comparisons are needed.

Tukey-Kramer Multiple-Comparison Test

Response: control1,commercial1,probiotics1

Term A:

Alpha=0.050 Error Term=S(A) DF=87 MSE=0.4536213 Critical Value=3.3796

Group	Count	Mean	Different From Groups
control1	30	6.519333	commercial1, probiotics1
commercial1	30	5.452	control1
probiotics1	30	5.477334	control1

Notes:
This report provides multiple comparison tests for all pairwise differences between the means.

Kruskal-Wallis Multiple-Comparison Z-Value Test (Dunn's Test)

Variable	control1	commercial1	probiotics1
control1	0.0000	5.4495	5.4866
commercial1	5.4495	0.0000	0.0371
probiotics1	5.4866	0.0371	0.0000

Regular Test: Medians significantly different if z-value > 1.9600
Bonferroni Test: Medians significantly different if z-value > 2.3940

One-Way Analysis of Variance Report

Dataset	Untitled
Response	control1,commercial1,probiotics1

Procedure Input Settings

Autosaved Template File
C:\Users\Sunghoo\Documents\NCSS 12\Procedure Templates\Autosave\One-Way Analysis of Variance - Autosaved 2019_2_17-15_15_54.t5

Variables Tab

Input Type	Response Variable(s) and a Factor (Grouping) Variable
-- Variables ----------	
Response Variable(s)	control1, commercial1, probiotics1
Factor Variable	<Empty>
-- Comparisons ----------	
Planned Comparisons	None

Reports Tab

-- Select Reports ----------	
Assumptions (Normality and Equal Variance)	Checked

Reports	
EMS Report	Checked
ANOVA Report	Checked
Welch's Test	Checked
Kruskal-Wallis / Van der Waerden / Terry-Hoeffding Tests	Checked
Means Report	Checked

·· Alpha ……

Test Alpha	0.05
Assumption Alpha	0.20

-- Multiple Comparison Tests ----------

Bonferroni Test (All Pairs)	Unchecked
Bonferroni Test (Versus Control)	Unchecked
Dwass-Steel-Critchlow-Fligner Test	Unchecked
Duncan's Test	Unchecked
Dunnett's 2-Sided (Versus Control)	Unchecked
Dunnett's Lower 1-Sided (Versus Control)	Unchecked
Dunnett's Upper 1-Sided (Versus Control)	Unchecked
Dunnett's Confidence Intervals	Unchecked
Fisher's LSD Test	Unchecked
Hsu's M.C. with Best	Unchecked
Kruskal-Wallis Z Test (Dunn's Test)	Checked
Newman-Keuls Test	Unchecked
Scheffe's Test	Checked
Tukey-Kramer Test	Checked
Tukey-Kramer Confidence Intervals and P-Values	Unchecked

·· Multiple Comparison Alpha and Decimals ……

MC Alpha	0.05
MC Decimals	All

One-Way Analysis of Variance Report

Dataset	Untitled
Response	control1,commercial1,probiotics1

Procedure Input Settings (Continued)

Report Options Tab

-- Report Options ----------

Variable Names	Names
Value Labels	Data Values

-- Decimal Places ----------

Means and C.I. Limits	Auto (Up to 7)
Std Deviations and Std Errors	Auto (Up to 7)

P-Values	5
Test Statistics	4
Rank Statistics	2
Fractional DF	2
α in Titles	2

Plots Tab

-- Select Plots ----------	
Means Plot	Checked
Box Plot	Checked

Storage Tab

-- Data Storage Options ----------	
Storage Option	Do not store data

One-Way Analysis of Variance Report

Dataset	Untitled
Response	control2,commercial2,probiotics2

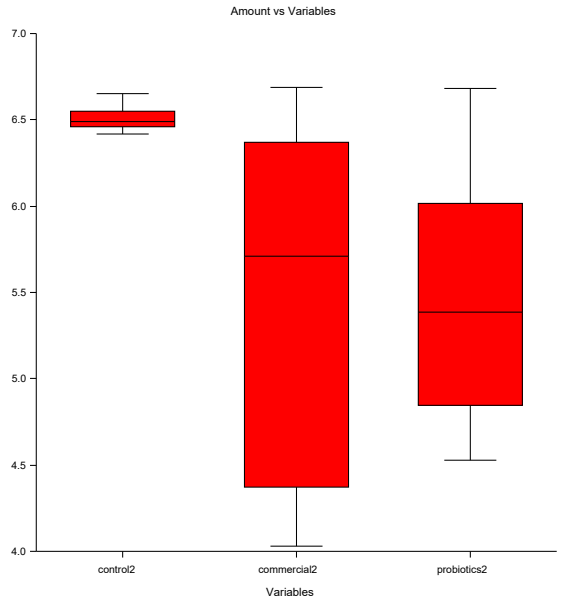
Tests of the Normality of Residuals Assumption

Normality Attributes	Test Value	Prob Level	Reject Normality? (α=0.20)
Skewness	-0.4994	0.61747	No
Kurtosis	-1.1733	0.24069	No
Skewness and Kurtosis (Omnibus)	1.6260	0.44353	No

Tests of the Equality of Group Variances Assumption

Test Name	Test Value	Prob Level	Reject Equal Variances? (α=0.20)
Brown-Forsythe (Data - Medians)	46.8517	0.00000	Yes
Levene (Data - Means)	67.1249	0.00000	Yes
Conover (Ranks of Deviations)	50.4080	0.00000	Yes
Bartlett (Likelihood Ratio)	118.5104	0.00000	Yes

Box Plot Section



One-Way Analysis of Variance Report

Dataset	Untitled
Response	control2,commercial2,probiotics2

Expected Mean Squares Table

Model Term	DF	Term Fixed?	Denominator Term	Expected Mean Square
A (...)	2	Yes	σ^2	σ^2 + sA
Error	87	No		σ^2

Note: Expected Mean Squares are for the balanced cell-frequency case.

Analysis of Variance Table and F-Test

Model Term	Means? DF Level	Sum of Squares (α=0.05)	Mean Square (α=0.05)	F-Ratio	Prob	Reject Equal Power
Between	2	22.11104	11.05552	23.6805	0.00000	Yes 1.00000
Within (Error)	87	40.61692	0.4668612			
Adjusted Total	89	62.72797				
Total	90					

Welch's Test of Means Allowing for Unequal Variances

Model Term	Numerator DF	Denominator DF	F-Ratio	Prob Level	Reject Equal Means? (α=0.05)
Between Groups	2	39.15	52.1587	0.00000	Yes

Kruskal-Wallis One-Way ANOVA on Ranks

Hypotheses
H0: All medians are equal.
H1: At least two medians are different.

Test Results

Method	DF	Chi-Squared (H)	Prob Level	Reject H0? (α=0.05)
Not Corrected for Ties	2	39.2686	0.00000	Yes
Corrected for Ties	2	39.2792	0.00000	Yes

Number Sets of Ties	15
Multiplicity Factor	198

Group Detail

Group	Count	Sum of Ranks	Mean Rank	Z-Value	Median
control2	30	2097.00	69.90	6.2653	6.49
commercial2	30	987.00	32.90	-3.2354	5.71
probiotics2	30	1011.00	33.70	-3.0300	5.385

One-Way Analysis of Variance Report

Dataset	Untitled
Response	control2,commercial2,probiotics2

Normal Scores Tests

Hypotheses
H0: All group data distributions are the same.
H1: At least one group has observations that tend to be greater than those of the other groups.

Results

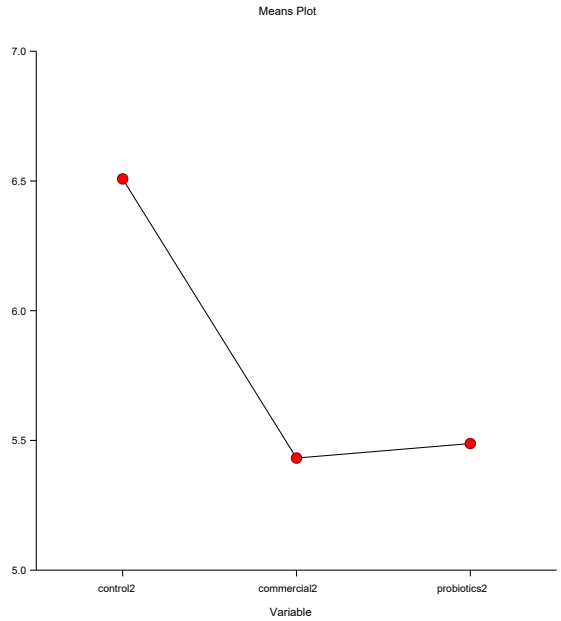
Test	DF	Chi-Squared (H)	Prob Level	Reject H0? (α=0.20)
Terry-Hoeffding - Expected Normal Scores	2	30.0151	0.00000	Yes
Van der Waerden - Normal Quantiles	2	30.8110	0.00000	Yes

Descriptive Statistics

Standard

Group	Count (ni)	Mean	Effect	Median	Standard Deviation	Error √(MSE/ni)
All	90	5.808778	5.808778			
A:						
control2	30	6.509	0.7002222	6.49	0.06166372	0.1247479
commercial2	30	5.430666	-0.3781111	5.71	0.9769302	0.1247479
probiotics2	30	5.486667	-0.3221111	5.385	0.6651229	0.1247479

Plots of Means Section



One-Way Analysis of Variance Report

Dataset	Untitled
Response	control2,commercial2,probiotics2

Scheffe's Multiple-Comparison Test

Response: control2,commercial2,probiotics2
Term A:

Alpha=0.050 Error Term=S(A) DF=87 MSE=0.4668612 Critical Value=2.4905

Group	Count	Mean	Different From Groups
control2	30	6.509	commercial2, probiotics2
commercial2	30	5.430666	control2
probiotics2	30	5.486667	control2

Notes:
This report provides multiple comparison tests for all possible contrasts among the the means. These contrasts may involve more groups than just each pair, so the method is much stricter than need be. The Tukey-Kramer method provides more accurate results when only pairwise comparisons are needed.

Tukey-Kramer Multiple-Comparison Test

Response: control2,commercial2,probiotics2
Term A:

Alpha=0.050 Error Term=S(A) DF=87 MSE=0.4668612 Critical Value=3.3796

Group	Count	Mean	Different From Groups
control2	30	6.509	commercial2, probiotics2
commercial2	30	5.430666	control2
probiotics2	30	5.486667	control2

Notes:
This report provides multiple comparison tests for all pairwise differences between the means.

Kruskal-Wallis Multiple-Comparison Z-Value Test (Dunn's Test)

Variable	control2	commercial2	probiotics2
control2	0.0000	5.4860	5.3674
commercial2	5.4860	0.0000	0.1186
probiotics2	5.3674	0.1186	0.0000

Regular Test: Medians significantly different if z-value > 1.9600
Bonferroni Test: Medians significantly different if z-value > 2.3940

One-Way Analysis of Variance Report

Dataset	Untitled
Response	control2,commercial2,probiotics2

Procedure Input Settings

Autosaved Template File
C:\Users\Sunghoo\Documents\NCSS 12\Procedure Templates\Autosave\One-Way Analysis of Variance - Autosaved 2019_2_17-15_37_13.t5

Variables Tab

Input Type	Response Variable(s) and a Factor (Grouping) Variable

-- Variables ----------

Response Variable(s)	control2, commercial2, probiotics2
Factor Variable	<Empty>

-- Comparisons ----------

Planned Comparisons	None

Reports Tab

-- Select Reports ----------

Assumptions (Normality and Equal Variance) Reports	Checked
EMS Report	Checked
ANOVA Report	Checked
Welch's Test	Checked
Kruskal-Wallis / Van der Waerden / Terry-Hoeffding Tests	Checked
Means Report	Checked

·· Alpha ……

Test Alpha	0.05
Assumption Alpha	0.20

-- Multiple Comparison Tests ----------

Bonferroni Test (All Pairs)	Unchecked
Bonferroni Test (Versus Control)	Unchecked
Dwass-Steel-Critchlow-Fligner Test	Unchecked
Duncan's Test	Unchecked
Dunnett's 2-Sided (Versus Control)	Unchecked
Dunnett's Lower 1-Sided (Versus Control)	Unchecked
Dunnett's Upper 1-Sided (Versus Control)	Unchecked
Dunnett's Confidence Intervals	Unchecked
Fisher's LSD Test	Unchecked
Hsu's M.C. with Best	Unchecked
Kruskal-Wallis Z Test (Dunn's Test)	Checked
Newman-Keuls Test	Unchecked
Scheffe's Test	Checked
Tukey-Kramer Test	Checked
Tukey-Kramer Confidence Intervals and P-Values	Unchecked

·· Multiple Comparison Alpha and Decimals ……

MC Alpha	0.05
MC Decimals	All

One-Way Analysis of Variance Report

Dataset	Untitled
Response	control2,commercial2,probiotics2

Procedure Input Settings (Continued)

Report Options Tab

-- Report Options ----------	
Variable Names	Names
Value Labels	Data Values
-- Decimal Places ----------	
Means and C.I. Limits	Auto (Up to 7)
Std Deviations and Std Errors	Auto (Up to 7)
P-Values	5
Test Statistics	4
Rank Statistics	2
Fractional DF	2
α in Titles	2

Plots Tab

-- Select Plots ----------	
Means Plot	Checked
Box Plot	Checked

Storage Tab

-- Data Storage Options ----------	
Storage Option	Do not store data

One-Way Analysis of Variance Report

Dataset	Untitled
Response	control3,commercial3,probiotics3

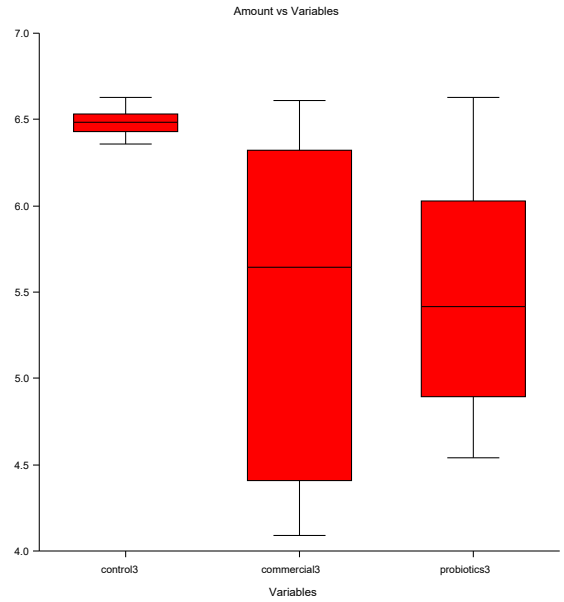
Tests of the Normality of Residuals Assumption

Normality Attributes	Test Value	Prob Level	Reject Normality? (α=0.20)
Skewness	-0.3618	0.71749	No
Kurtosis	-1.4343	0.15149	Yes
Skewness and Kurtosis (Omnibus)	2.1881	0.33486	No

Tests of the Equality of Group Variances Assumption

Test Name	Test Value	Prob Level	Reject Equal Variances? (α=0.20)
Brown-Forsythe (Data - Medians)	55.6505	0.00000	Yes
Levene (Data - Means)	71.4001	0.00000	Yes
Conover (Ranks of Deviations)	51.7383	0.00000	Yes
Bartlett (Likelihood Ratio)	105.1375	0.00000	Yes

Box Plot Section



One-Way Analysis of Variance Report

Dataset Untitled
Response control3,commercial3,probiotics3

Expected Mean Squares Table

Model Term	DF	Term Fixed?	Denominator Term	Expected Mean Square
A (...)	2	Yes	σ^2	σ^2 + sA
Error	87	No		σ^2

Note: Expected Mean Squares are for the balanced cell-frequency case.

Analysis of Variance Table and F-Test

Model Term	Means? DF Level	Sum of Power Squares (α=0.05)	Mean Square (α=0.05)	F-Ratio	Prob	Reject Equal
Between	2	21.46713	10.73356	23.6811	0.00000	Yes 1.00000
Within (Error)	87	39.43309	0.453254			
Adjusted Total	89	60.90022				

Total	90

Welch's Test of Means Allowing for Unequal Variances

Model Term	Numerator DF	Denominator DF	F-Ratio	Prob Level	Reject Equal Means? (α=0.05)
Between Groups	2	39.41	50.9836	0.00000	Yes

Kruskal-Wallis One-Way ANOVA on Ranks

Hypotheses
H0: All medians are equal.
H1: At least two medians are different.

Test Results

Method	DF	Chi-Squared (H)	Prob Level	Reject H0? (α=0.05)
Not Corrected for Ties	2	37.7231	0.00000	Yes
Corrected for Ties	2	37.7337	0.00000	Yes

Number Sets of Ties	16
Multiplicity Factor	204

Group Detail

Group	Count	Sum of Ranks	Mean Rank	Z-Value	Median
control3	30	2082.50	69.42	6.1412	6.485
commercial3	30	997.00	33.23	-3.1498	5.645
probiotics3	30	1015.50	33.85	-2.9914	5.415

One-Way Analysis of Variance Report

Dataset	Untitled
Response	control3,commercial3,probiotics3

Normal Scores Tests

Hypotheses
H0: All group data distributions are the same.
H1: At least one group has observations that tend to be greater than those of the other groups.

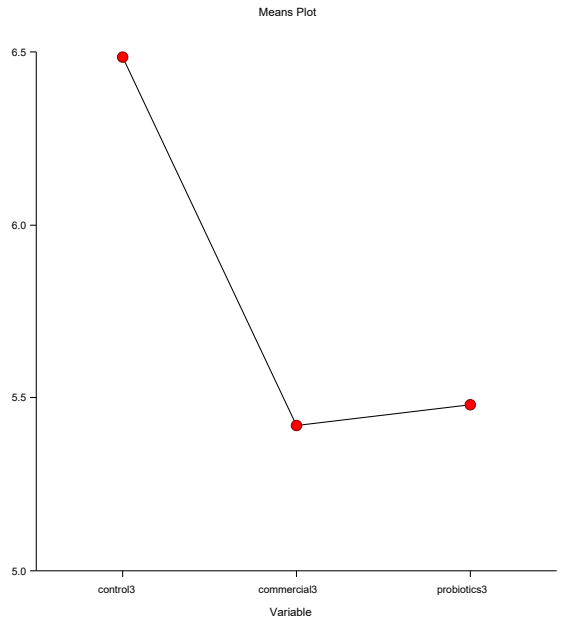
Results

Test	DF	Chi-Squared (H)	Prob Level	Reject H0? (α=0.20)
Terry-Hoeffding - Expected Normal Scores	2	30.8997	0.00000	Yes
Van der Waerden - Normal Quantiles	2	31.3611	0.00000	Yes

Descriptive Statistics

Group	Count (ni)	Mean	Effect	Median	Standard Deviation	Standard Error √(MSE/ni)
All	90	5.795556	5.795556			
A:						
control3	30	6.485333	0.6897778	6.485	0.07628139	0.1229165
commercial3	30	5.42	-0.3755555	5.645	0.9547883	0.1229165
probiotics3	30	5.481333	-0.3142222	5.415	0.6650732	0.1229165

Plots of Means Section



One-Way Analysis of Variance Report

Dataset Untitled
Response control3,commercial3,probiotics3

Scheffe's Multiple-Comparison Test

Response: control3,commercial3,probiotics3
Term A:

Alpha=0.050 Error Term=S(A) DF=87 MSE=0.453254 Critical Value=2.4905

Different From

Group	Count	Mean	Groups
control3	30	6.485333	commercial3, probiotics3
commercial3	30	5.42	control3
probiotics3	30	5.481333	control3

Notes:
This report provides multiple comparison tests for all possible contrasts among the the means. These contrasts may involve more groups than just each pair, so the method is much stricter than need be. The Tukey-Kramer method provides more accurate results when only pairwise comparisons are needed.

Tukey-Kramer Multiple-Comparison Test

Response: control3,commercial3,probiotics3
Term A:

Alpha=0.050 Error Term=S(A) DF=87 MSE=0.453254 Critical Value=3.3796

Group	Count	Mean	Different From Groups
control3	30	6.485333	commercial3, probiotics3
commercial3	30	5.42	control3
probiotics3	30	5.481333	control3

Notes:
This report provides multiple comparison tests for all pairwise differences between the means.

Kruskal-Wallis Multiple-Comparison Z-Value Test (Dunn's Test)

Variable	control3	commercial3	probiotics3
control3	0.0000	5.3649	5.2735
commercial3	5.3649	0.0000	0.0914
probiotics3	5.2735	0.0914	0.0000

Regular Test: Medians significantly different if z-value > 1.9600
Bonferroni Test: Medians significantly different if z-value > 2.3940

One-Way Analysis of Variance Report

Dataset	Untitled
Response	control3,commercial3,probiotics3

Procedure Input Settings

Autosaved Template File
C:\Users\Sunghoo\Documents\NCSS 12\Procedure Templates\Autosave\One-Way Analysis of Variance - Autosaved 2019_2_17-16_9_23.t5

Variables Tab

Input Type	Response Variable(s) and a Factor (Grouping) Variable

-- Variables ----------

Response Variable(s)	control3, commercial3, probiotics3
Factor Variable	<Empty>

-- Comparisons ----------

Planned Comparisons	None

Reports Tab

-- Select Reports ----------

Assumptions (Normality and Equal Variance) Reports	Checked
EMS Report	Checked
ANOVA Report	Checked
Welch's Test	Checked
Kruskal-Wallis / Van der Waerden / Terry-Hoeffding Tests	Checked
Means Report	Checked

·· Alpha

Test Alpha	0.05
Assumption Alpha	0.20

-- Multiple Comparison Tests ----------

Bonferroni Test (All Pairs)	Unchecked
Bonferroni Test (Versus Control)	Unchecked
Dwass-Steel-Critchlow-Fligner Test	Unchecked
Duncan's Test	Unchecked
Dunnett's 2-Sided (Versus Control)	Unchecked
Dunnett's Lower 1-Sided (Versus Control)	Unchecked
Dunnett's Upper 1-Sided (Versus Control)	Unchecked
Dunnett's Confidence Intervals	Unchecked
Fisher's LSD Test	Unchecked
Hsu's M.C. with Best	Unchecked
Kruskal-Wallis Z Test (Dunn's Test)	Checked
Newman-Keuls Test	Unchecked
Scheffe's Test	Checked
Tukey-Kramer Test	Checked
Tukey-Kramer Confidence Intervals and P-Values	Unchecked

·· Multiple Comparison Alpha and Decimals

MC Alpha	0.05
MC Decimals	All

Dataset	Untitled
Response	control3,commercial3,probiotics3

Procedure Input Settings (Continued)

Report Options Tab

-- Report Options ----------	
Variable Names	Names
Value Labels	Data Values

-- Decimal Places ----------	
Means and C.I. Limits	Auto (Up to 7)
Std Deviations and Std Errors	Auto (Up to 7)
P-Values	5
Test Statistics	4
Rank Statistics	2
Fractional DF	2
α in Titles	2

Plots Tab

-- Select Plots ----------	
Means Plot	Checked
Box Plot	Checked

Storage Tab

-- Data Storage Options ----------	
Storage Option	Do not store data

Appendix B

Repeated Measures ANOVA Report

Dataset C:\Users\Sunghoo\Desktop\RESEARCH\RM_report.NCSS
Response pH

Expected Mean Squares Section

Source Term	DF	Term Fixed?	Denominator Term	Expected Mean Square
A: Groups	2	Yes	B(A)	S+csB+bcsA
B(A): sample	24	No	S(ABC)	S+csB
C: time	9	Yes	BC(A)	S+sBC+absC
AC	18	Yes	BC(A)	S+sBC+bsAC
BC(A)	216	No	S(ABC)	S+sBC
S(ABC)	0	No		S

Note: Expected Mean Squares are for the balanced cell-frequency case.

Analysis of Variance Table

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Groups	2	65.80453	32.90226	2716.08	0.000000*	1.000000
B(A): sample	24	0.2907333	0.01211389			
C: time	9	80.10532	8.90059	2177.32	0.000000*	1.000000
AC	18	38.30251	2.127917	520.55	0.000000*	1.000000
BC(A)	216	0.8829778	0.00408786			
S	0					
Total (Adjusted)	269	185.3861				
Total	270					

* Term significant at alpha = 0.05

Probability Levels for F-Tests with Geisser-Greenhouse Adjustments

Source Term	DF	F-Ratio	Regular Prob Level	Lower Bound Epsilon Prob Level	Geisser Greenhouse Epsilon Prob Level	Huynh Feldt Epsilon Prob Level
A: Groups	2	2716.08	0.000000*			
B(A): sample	24					
C: time	9	2177.32	0.000000*	0.000000*	0.000000*	0.000000*
AC	18	520.55	0.000000*	0.000000*	0.000000*	0.000000*
BC(A)	216					
S	0					

Power Values for F-Tests with Geisser-Greenhouse Adjustments Section

	Lower Bound	Geisser Greenhouse	Huynh Feldt

Source Term	DF	F-Ratio	Regular Power (Alpha=0.05)	Epsilon Power (Alpha=0.05)	Epsilon Power (Alpha=0.05)	Epsilon Power (Alpha=0.05)
A: Groups	2	2716.08	1.000000			
B(A): sample	24					
C: time	9	2177.32	1.000000	1.000000	1.000000	1.000000
AC	18	520.55	1.000000	1.000000	1.000000	1.000000
BC(A)	216					
S	0					

Repeated Measures ANOVA Report

Dataset C:\Users\Sunghoo\Desktop\RESEARCH\RM_report.NCSS
Response pH

Box's M Test for Equality of Between-Group Covariance Matrices Section

Source Term	Box's M	DF1	DF2	F Value	Prob Level	Chi2 Value	Prob Level	Covariance Matrices Equal?
BC(A)								No Test

Covariance Matrix Circularity Section

Source Term	Lower Bound Epsilon	Geisser Greenhouse Epsilon	Huynh Feldt Epsilon	Mauchly Test Statistic	Chi2 Value	DF	Prob Level	Covariance Matrix Circularity?
BC(A)	0.111111	0.412505	0.538038	0.000693	151.3	44.0	0.000000	Violated

Note: Mauchly's statistic actually tests the more restrictive assumption that the pooled covariance matrix has compound symmetry.

Means and Standard Error Section

Term	Count	Mean	Standard Error
All	270	5.806815	
A: Groups			
commercial	90	5.434222	0.01160167
control	90	6.504445	0.01160167
probiotics	90	5.481778	0.01160167
C: time			
0	27	6.625926	0.01230456
45	27	6.425926	0.01230456
90	27	6.303704	0.01230456
135	27	6.158148	0.01230456
180	27	5.997037	0.01230456
225	27	5.691482	0.01230456
270	27	5.448889	0.01230456
315	27	5.264074	0.01230456
360	27	5.117407	0.01230456
405	27	5.035555	0.01230456

AC: Groups,time			
commercial,0	9	6.616667	0.02131213
commercial,45	9	6.415555	0.02131213
commercial,90	9	6.346667	0.02131213
commercial,135	9	6.182222	0.02131213
commercial,180	9	6.004445	0.02131213
commercial,225	9	5.294445	0.02131213
commercial,270	9	4.746666	0.02131213
commercial,315	9	4.436666	0.02131213
commercial,360	9	4.196667	0.02131213
commercial,405	9	4.102222	0.02131213
control,0	9	6.636667	0.02131213
control,45	9	6.584445	0.02131213
control,90	9	6.54	0.02131213
control,135	9	6.506667	0.02131213
control,180	9	6.484445	0.02131213
control,225	9	6.483333	0.02131213
control,270	9	6.475555	0.02131213

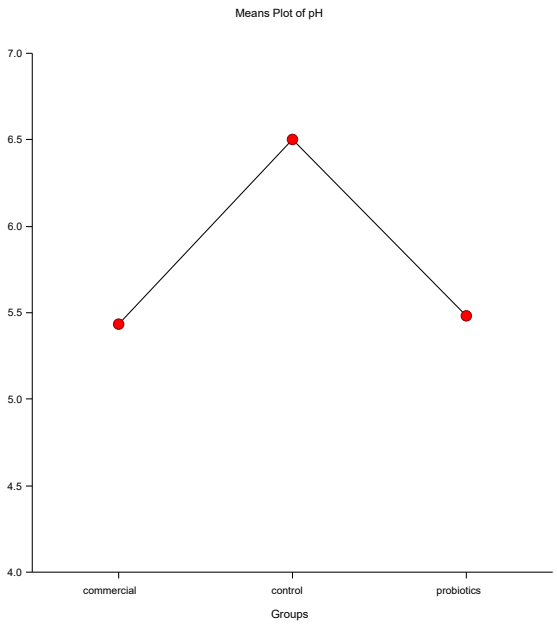
Repeated Measures ANOVA Report

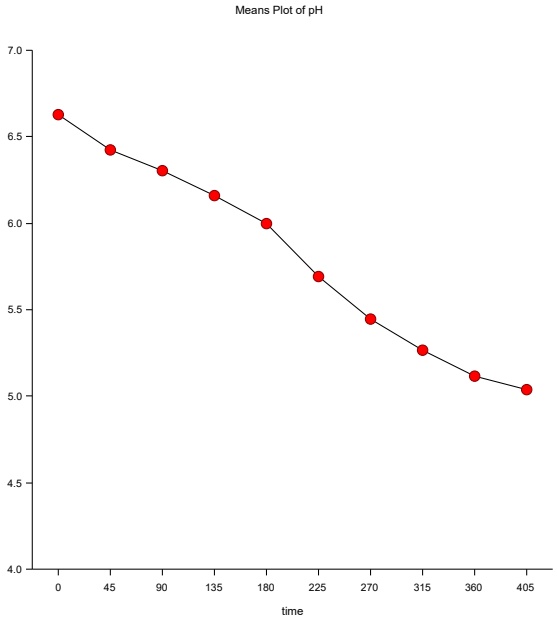
Dataset	C:\Users\Sunghoo\Desktop\RESEARCH\RM_report.NCSS
Response	pH

Means and Standard Error Section

Term	Count	Mean	Standard Error
AC: Groups,time			
control,315	9	6.46	0.02131213
control,360	9	6.446667	0.02131213
control,405	9	6.426667	0.02131213
probiotics,0	9	6.624444	0.02131213
probiotics,45	9	6.277778	0.02131213
probiotics,90	9	6.024445	0.02131213
probiotics,135	9	5.785555	0.02131213
probiotics,180	9	5.502222	0.02131213
probiotics,225	9	5.296667	0.02131213
probiotics,270	9	5.124444	0.02131213
probiotics,315	9	4.895555	0.02131213
probiotics,360	9	4.708889	0.02131213
probiotics,405	9	4.577778	0.02131213

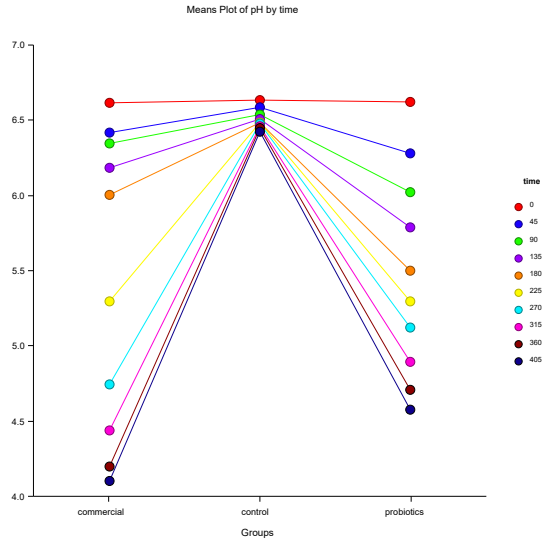
Plots Section

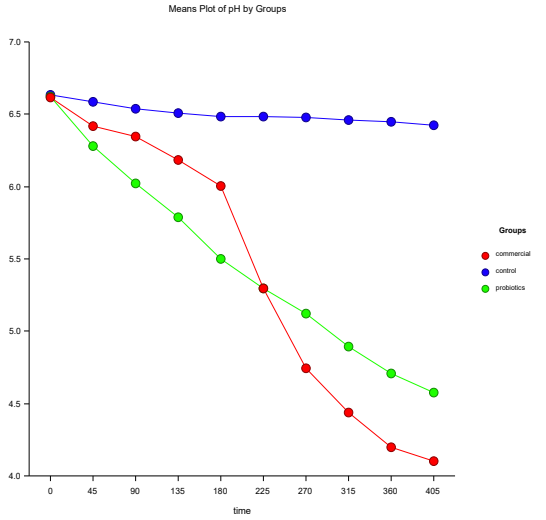


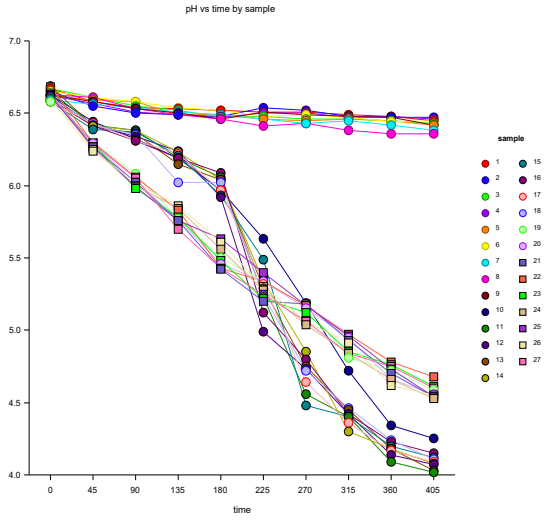


Repeated Measures ANOVA Report

Dataset	C:\Users\Sunghoo\Desktop\RESEARCH\RM_report.NCSS
Response	pH


Means Plot of pH by time
7.0
6.5
6.0
5.5
5.0
4.5
4.0
commercial
control
probiotics
Groups
time
0
45
90
135
180
225
270
315
360
405


Means Plot of pH by Groups
7.0
6.5
6.0
5.5
5.0
4.5
4.0
0
45
90
135
180
225
270
315
360
405
time
Groups
commercial
control
probiotics


pH vs time by sample
7.0
6.5
6.0
5.5
5.0
4.5
4.0
0
45
90
135
180
225
270
315
360
405
time
sample
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27

Repeated Measures ANOVA Report

Dataset C:\Users\Sunghoo\Desktop\RESEARCH\RM_report.NCSS
Response pH

Tukey-Kramer Multiple-Comparison Test

Response: pH
Term A: Groups

Alpha=0.050 Error Term=B(A) DF=24 MSE=0.01211389 Critical Value=3.5390

Group	Count	Mean	Different From Groups
commercial	90	5.434222	control, probiotics
control	90	6.504445	commercial, probiotics
probiotics	90	5.481778	commercial, control

Notes:
This report provides multiple comparison tests for all pairwise differences between the means.

Planned Comparison: A: commercial vs. control

Response: pH
Term A: Groups

Alpha=0.050 Error Term=B(A) DF=24 MSE=0.01211389

Comparison Value=1.070222 T-Value=65.2287 Prob>|T|=0.000000 Decision(0.05)=Reject
Comparison Std Error = 0.01640724 Comparison Confidence Interval = 1.036359 to 1.104085

Group	Comparison Coefficient	Count	Mean
commercial	-1	90	5.434222
control	1	90	6.504445
probiotics	0	90	5.481778

Planned Comparison: A: commercial vs. probiotics

Response: pH
Term A: Groups

Alpha=0.050 Error Term=B(A) DF=24 MSE=0.01211389

Comparison Value=0.04755555 T-Value=2.8984 Prob>|T|=0.007889 Decision(0.05)=Reject
Comparison Std Error = 0.01640724 Comparison Confidence Interval = 0.01369268 to 0.08141843

Group	Comparison Coefficient	Count	Mean

commercial	-1	90	5.434222
control	0	90	6.504445
probiotics	1	90	5.481778

Notes:
This section presents the planned comparison testing whether the each group is significantly different from the first group. This would be useful when the first group is a control group.

Repeated Measures ANOVA Report

Dataset	C:\Users\Sunghoo\Desktop\RESEARCH\RM_report.NCSS
Response	pH

Tukey-Kramer Multiple-Comparison Test

Response: pH
Term C: time

Alpha=0.050 Error Term=BC(A) DF=216 MSE=0.00408786 Critical Value=4.5250

Group	Count	Mean	Different From Groups
0	27	6.625926	45, 90, 135, 180, 225, 270, 315, 360, 405
45	27	6.425926	0, 90, 135, 180, 225, 270, 315, 360, 405
90	27	6.303704	0, 45, 135, 180, 225, 270, 315, 360, 405
135	27	6.158148	0, 45, 90, 180, 225, 270, 315, 360, 405
180	27	5.997037	0, 45, 90, 135, 225, 270, 315, 360, 405
225	27	5.691482	0, 45, 90, 135, 180, 270, 315, 360, 405
270	27	5.448889	0, 45, 90, 135, 180, 225, 315, 360, 405
315	27	5.264074	0, 45, 90, 135, 180, 225, 270, 360, 405
360	27	5.117407	0, 45, 90, 135, 180, 225, 270, 315, 405
405	27	5.035555	0, 45, 90, 135, 180, 225, 270, 315, 360

Notes:
This report provides multiple comparison tests for all pairwise differences between the means.

Procedure Input Settings

Autosaved Template File
C:\Users\Sunghoo\Documents\NCSS 12\Procedure Templates\Autosave\Repeated Measures Analysis of Variance - Autosaved 2019_3_31-19_7_2.t108

Variables Tab
-- Response Variables ----------
Response Variable(s) pH

-- Subject Variable ----------
Subject Variable sample

-- Between Factors ----------

Between Factor Variable 1	Groups
Type	Fixed
Comparisons	Each with First
Between Factor Variable 2	<Empty>
Between Factor Variable 3	<Empty>

-- Within Factors ----------

Within Factor Variable 1	time
Type	Fixed
Comparisons	None
Within Factor Variable 2	<Empty>
Within Factor Variable 3	<Empty>

Repeated Measures ANOVA Report

Dataset	C:\Users\Sunghoo\Desktop\RESEARCH\RM_report.NCSS
Response	pH

Procedure Input Settings (Continued)

Variables Tab (Continued)

-- Model Specification ----------

Which Model Terms	Full model. Use all terms.
Custom Model	<Empty>
Write model in 'Custom Model' field. Do not process data.	Unchecked

Reports Tab

-- Select Reports ----------

EMS Report	Checked
ANOVA Report	Checked
G G Prob Report	Checked
Power Report	Checked
Box's M Report	Checked
Circularity Report	Checked
Means Report	Checked

·· Alpha

F-Test Alpha	0.05
Assumptions Alpha	0.10

-- Multiple Comparison Tests (For Fixed Factors Only) ----------

Bonferroni Test (All Pairs)	Unchecked
Bonferroni Test (Versus Control)	Unchecked
Duncan's Test	Unchecked
Dunnett's 2-Sided (Vs Control)	Unchecked

Dunnett's Lower 1-Sided (Vs Control)	Unchecked
Dunnett's Upper 1-Sided (Vs Control)	Unchecked
Dunnett's Confidence Intervals	Unchecked
Fisher's LSD Test	Unchecked
Hsu's M.C. with Best	Unchecked
Newman-Keuls Test	Unchecked
Scheffe's Test	Unchecked
Tukey-Kramer Test	Checked
Tukey-Kramer Confidence Intervals and P-Values	Unchecked
Tests for Two-Factor Interactions	Unchecked

·· MC Options
……

MC Alpha	0.05
MC Decimals	All

-- Report Options ----------

Precision	Single
Variable Names	Names
Value Labels	Data Values

Plots Tab

-- Select Plots ----------

Means Plot(s)	Checked
Y-Axis Scaling	Uniform
Subject Plot	Checked