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RESEARCH ARTICLE

Varying concentrations of sucralose and the acid-producing capability of *Lactobacillus acidophilus*

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ABSTRACT

Background: Commercially prepared food products containing sucralose are becoming increasingly popular among consumers. **Aims and Objectives:** This study determined the effects of varying concentrations of sucralose on the acid-producing capability of *Lactobacillus acidophilus*. **Materials and Methods:** *L. acidophilus* was inoculated in de Man, Rogosa, and Sharpe broth selective medium containing varying concentrations of sucralose per kg broth powder. The change in pH was measured after 48 h of culture using a pH meter. The data were analyzed using one-way analysis of variance and Bonferroni pairwise comparison test at 5% level of significance. **Results:** The largest mean pH change of 0.120 was observed in 250 mg/kg, followed by 0.070 in the 350 mg/kg treatment group. The control and 500 mg/kg treatment groups had the least observed mean pH changes of −0.010 and −0.008, respectively. These mean pH changes varied significantly among all treatment groups. **Conclusion:** There was a decrease in the change in pH as the concentration of sucralose was increased suggesting that the addition of certain concentrations of sucralose influenced the acid production of *L. acidophilus*.

KEY WORDS: Sucralose; *Lactobacillus acidophilus*; pH Change; Acid-producing Capability

INTRODUCTION

Non-nutritive sweeteners (NNS) serve as an alternative to sucrose in various food items, most especially for those with known high-caloric value. Commercially available goods that contain this type of sugar substitute or those often labeled as diet, low calorie, and sugar-free, are becoming increasingly popular among consumers. The higher sweetening ability and lower caloric content of a variety of NNS as compared to table sugar make them an appealing option for those who are undergoing weight control and special diet maintenance.[1] However, promising they may be, many controversies regarding the detrimental health and metabolic effects of NNS's breakdown products have yet to be resolved.[2]

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One of the NNS that has been approved by the food and drug administration (FDA) of the Philippines is sucralose.[3] Sucralose contains strong carbon-chlorine bonds, thus remaining stable at high temperatures and low pH levels, and is not hydrolyzed even upon digestion and metabolism.[4] It is 500-750 times sweeter compared

to sugar and is marketed for household use mainly as SPLENDA®, a low-calorie sweetener composed of 1.1% sucralose and maltodextrin and glucose as fillers.[5] As any other NNS, SPLENDA® low-calorie sweetener underwent scrutiny from numerous researches. SPLENDA® lowered fecal acidity, triggered histopathological changes in the colon, increased body weight, and suppressed good gut bacteria.[5]

Gut flora, the microbial community inhabiting the gastrointestinal (GI) tract, performs metabolic activities which aid in the process of digestion and absorption of food.[6,7] Although yet to be thoroughly investigated, the understanding of the compositional and functional components of this microbiota holds the key to the intricacies of the digestive physiology and the pathogenesis of several disorders. A great diversity and variation among gut microbiota of individuals exists as they may be mutated by exogenous and endogenous factors,[8] but there also exists a baseline for healthy human gut microbiota[9] One of the most famous microbes present in the GI tract is *Lactobacillus* as it is used in commercial drinks. Several species of *Lactobacillus* exist but its most distinctive characteristic is its production of lactic acid as an end-product after glucose metabolism.[10] *Lactobacillus acidophilus*, a probiotic assisting in digestion, offers a lower pH environment for the growth of other gut microbiota and end-products suppressing the development of harmful pathogens.[11] In the lack or excess of these conditions, the viability of gut microbiota may deteriorate and possibly cause disorders within the GI tract.

Previous researches have shown the different effects of NNS to the human body, specifically the digestive system. Particular interest is given to investigations regarding the suppression of gut flora due to the breakdown products of this kind of sweetener. Hence, this study determined the effect of SPLENDA® low-calorie sweetener on the acid-producing capability of *L. acidophilus* as the change in the acidity of media containing various concentrations of SPLENDA® 48 h after the inoculation of *L. acidophilus* were measured and compared. The study focused on the effect of granulated SPLENDA® low-calorie sweetener on the acid-producing capability of *L. acidophilus* which refers to the presence of acid produced by the *L. acidophilus* in its fermentative process as it undergoes anaerobic respiration.[10] Findings obtained from this research will contribute to the ever-growing literature involving the most efficient conditions for the use of *L. acidophilus* and subsequently suggest possible harmful or beneficial effects of artificial sweeteners on microbes residing in the human intestines.

MATERIALS AND METHODS

Research Design

A completely randomized design was employed in this study as standardized *L. acidophilus* inoculums were assigned at random without restriction to the following treatment groups: (1) Control contains de Man, Rogosa, and Sharpe (MRS) broth only, (2) MRS broth supplemented with 250 mg, (3) 350 mg, and (4) 500 mg granulated SPLENDA® low-calorie sweetener per kg of broth powder. Each treatment group contains 25 replicates. The chosen amounts of granulated SPLENDA® low-calorie sweetener for each of the treatments were based from the permitted amount of additives in different food categories.[3]

Acquisition of *L. acidophilus* Strain

An ampoule of *L. acidophilus* Biotech 1900 was obtained from the Biotech Philippine National Collection of Microorganisms, National Institute of Molecular Biology and Biotechnology, University of the Philippines Los Baños, Laguna, Philippines.

Preparation of MRS Broth

For the control treatment, 52.5 g of MRS broth powder was suspended in 1 L of purified water, mixed thoroughly, heated with agitation, and boiled for 1 min for complete dissolution of powder.[12] For the other three treatments, concentrations within the recommended values set by the FDA, 242.91 mg, 340.08 mg, and 485.83 mg SPLENDA® low-calorie sweetener per kg broth powder were placed in prepared media before sterilization. Subsequently, the prepared MRS broth was autoclaved at 115°C psi for 15 min.

Preparation of *Lactobacillus* Inoculum

To obtain the inoculum needed for the experimental units, the *L. acidophilus* Biotech 1900 ampoule was subcultured. Under the laminar flow hood, the ampoule of lyophilized (l-dried) *L. acidophilus* culture was cracked open using a pair of pliers. Using sterile pasteur pipettes, the contents of the ampoule were suspended in 10 ml of MRS broth. The tubes containing the subculture solution were placed inside the candle jars and incubated for 24 h at 37°C. After the incubation period, the inoculum concentration was standardized to 0.5 McFarland standard to set an approximate cell density of 1.5×10^8 colony forming units per ml for use in inoculation to all the treatments.

Inoculation of *L. acidophilus*

Under the laminar flow hood, using stereological pipettes and a pipet aid, 5 ml of MRS broth from all treatments was

transferred into 15-ml test tubes with caps. Subsequently, 0.5 ml of the prepared *Lactobacillus* inoculum was pipetted into the test tubes containing 5 ml of media. All equipment and materials used were sterile as aseptic techniques including flaming of the pipettes and the mouths of test tubes, flasks, and beakers before and after each transfer of media and inoculum were observed during the entire process.[13]

Cultivation of *L. acidophilus*

After inoculation, the broth tubes were placed in candle jars to provide an environment with increased CO_2 concentrations needed for the growth of bacteria. Glass mason jars were cleaned and disinfected using 70% ethyl alcohol. A lighted matchstick was used to exhaust the residual alcohol. Twenty sterile inoculated test tubes were placed inside each jar. A lighted candle was also inserted before the jar was sealed to consume the gaseous oxygen trapped inside. This environment provides a CO_2 atmosphere of about 3%. After the flame of the lighted candle went out, the glass jars were incubated for 48 h at 37°C.

Measurement of pH after Bacterial Growth

After 48 h of cultivation, the acidity of the culture solution was measured using pH meter (Denver Instrument Ultra Basic). The pH meter was calibrated using buffer solutions of pH 4.00, 7.00, and 10.00. The pH measurements were done by directly immersing the probe of the pH meter in the culture solution. Before and after every reading, the pH meter probe was washed with distilled water and dried using paper towels.

Handling and Disposal of *Lactobacillus* Cultures

L. acidophilus bacteria are under Biosafety Level 1 as organisms not known to cause diseases in healthy adult humans, presenting a low risk to laboratory personnel and the environment.[14] Containment Level 1 facilities and equipment were used in the entire working procedure. Protective laboratory clothing and gloves were used in handling infected materials. In the event of skin contact or accidental spillage, 70% ethanol was used as a disinfectant.[15] For disposal, cultures were decontaminated through autoclaving, and then, stored in labeled leak-proof containers for proper disposal.[16]

Statistical Analysis

The pH change values across the different treatment groups were reported as means ± standard deviations. Differences on the mean pH change values were identified using one-way analysis of variance and pairwise comparison was assessed using Bonferroni test. All statistical analyses were performed using STATA/SE V12.0 at 5% level of significance.

RESULTS

The largest mean change in pH of 0.124 was observed among samples suspended in 250 mg/kg (Treatment 1), followed by the samples suspended in 350 mg/kg (Treatment 2) with a mean of 0.066 (Table 1). The positive value change suggests that, on the average, *L. acidophilus* when suspended in MRS broth containing 250-350 mg/kg concentrations of SPLENDA® low-calorie sweetener increased the pH making the organism's environment less acidic. The control and the 500 mg/kg treatment groups had mean pH changes of −0.010 and −0.008, respectively (Table 1). This negative mean pH change value suggests an increase in the acidity.

Furthermore, the means of pH change across the different treatment groups differed ($P < 0.000$) suggesting that there is at least one pair of treatment means that vary. The changes in pH varied significantly between the 250 mg/kg treatment group and the control group ($P = 0.000$), the 350 mg/kg treatment group and the control group ($P = 0.004$), the 350 mg/kg and 500 mg/kg treatment groups ($P = 0.006$), and the 250 mg/kg and 500 mg/kg treatment groups ($P = 0.000$). However, there is no difference identified between the 250 mg/kg and 350 mg/kg treatment groups ($P = 0.056$). Likewise, there is no difference identified between the 500 mg/kg treatment group and the control group ($P > 0.05$) which suggests that the negative change in pH of MRS broth supplemented with 500 mg/kg SPLENDA® is comparable to the pH change in MRS broth without SPLENDA®. Results showed that there was a decrease in the change in pH as the concentration of SPLENDA® was increased.

DISCUSSION

Most sugar substitutes, such as sucralose and rebaudioside A, despite being derived from naturally occurring substances such as sucrose and stevia plant, are still considered synthetic and high-intensity since they can be 13,000 times sweeter than sugar and the number of these sweeteners innovated through deriving a present resource has multiplied noticeably.[17,18] The demand for sucralose continues to increase not only because of its non-caloric characteristic but also because of its stability at high temperatures, acidic environments, and in media containing ethanol due to the strong carbon-chlorine bonds comprising this NNS.[19] Consequently, its sweetness

Table 1: Means and variability of the pH changes across the different treatment groups

Treatment	pH change (mean±SD)	*n*
Control	−0.010±0.071	25
1 (250 mg/kg)	0.124±0.050	25
2 (350 mg/kg)	0.066±0.121	25
3 (500 mg/kg)	−0.008±0.037	25

SD: Standard deviation

level stays the same after cooking, baking, and pasteurization and is not absorbed by the human body.[4] The FDA approved its limited use in 1998 and consent as a NNS in 1999 after the assessment of several studies at that time that sucralose did not cause carcinogenic, reproductive, or neurological risks.[20] At present, it can be found in a variety of products such as desserts and non-alcoholic beverages. It is commonly manufactured and marketed as SPLENDA® low-calorie sweetener, a sugar substitute formed from minimal amounts of sucralose, dextrose, and maltodextrin. One of the major issues regarding its use is the possible consequences that the chlorine component in sucralose may pose to the human body, as it is considered as a carcinogenic agent. In addition, dextrose and malt dextrin are not precisely calorie-free.[21] Researches on the effects and risks presented by sucralose on humans after long-term consumption are still insufficient, especially on the gut flora.

Gut flora, also known as gut microbiota or intestinal flora, is the population of an assortment of bacteria, viruses, and eukaryotes inhabiting the GI tract. At least 100 trillion microbial cells[22] act as a community, a virtual organ within an organ, performing metabolic activities with underlying mechanisms yet to be thoroughly investigated. Each distinctive species may be beneficial or harmful depending on factors such as quantity, life stage, and location as these are susceptible to mutations caused by either exogenous or endogenous agents.[7] The complex interplays of the gut microbiota play an integral role in human physiology, from metabolism, nutrition, to immune function.[6] One of the "friendly" bacteria in our body that helps in the maintenance of healthy intestinal flora is the *Lactobacillus*.

Lactobacilli are rod-shaped, Gram-positive coccobacilli fermentative organotrophs commonly associated with the human GI tract as well as the human mouth and the vagina. Occurring singly or in small chains, they are usually 0.5-0.8 μm across by 2-9 μm long.[10] Rich media is required for this species to thrive and they are generally characterized by their ability to exist in both aerobic and anaerobic environments, earning the classification microaerophilic, and their ability to manufacture lactic acid.[23] There are variations among the amount of lactic acid produced by different *Lactobacillus* species leading to their popularity in different commercial industries. Several species of *Lactobacillus* are utilized in food production of sour milks, cheeses, and yogurt. Others are also used in fermented vegetables and sourdough breads, among others.[10]

L. acidophilus, known as a probiotic commonly found in the human mouth, GI tract, and genitalia, gains stability in acidic conditions due to its high cytoplasmic buffering activity of pH 3.72-7.74 with optimum viability occurs at pH 6[24] and 35°C–40°C but it can tolerate temperatures up to 45°C[25]. Through fermentation, it produces lactic acid and other substances such as hydrogen peroxide, aiding in the process of the digestion of food as it provides an unfriendly environment for pathogens, suppressing their growth.[11] More recent studies have shown that *L. acidophilus* does not affect human gut microbial diversity but increases abundance of other microbes such as *Lactobacillus* spp., *Bifidobacterium*, and *Eubacterium*.[26] Certain strains of *L. acidophilus* are beneficial not only to GI digestion and absorption but also to the immune system. At present, *L. acidophilus* is used to treat diarrhea, chronic constipation, symptoms of irritable bowel syndrome and inflammatory bowel disease, lactose intolerance, some pollen allergies, childhood eczema, and high cholesterol.[15] It may also be recommended to treat some vaginal yeast infections.[27] However, small colonies of *L. acidophilus* in the GI tract may cause disorders as the intestines will be unable to fully absorb nutrients. Hence, it is of paramount importance to assess the effects of sugar substitutes on *Lactobacillus*.

Only recently have clinical intervention researches on the consequences of the intake of sugar substitutes on gut microbiota becoming more prevalent. One of the latest studies is how NNS contributes to the development of glucose intolerance by altering the compositional and functional components of intestinal flora.[28] SPLENDA® low-calorie sweetener also altered gut microflora by increasing intestinal p-glycoprotein and cytochrome p-450, decreasing anaerobes, *Bifidobacteria*, *Lactobacilli*, *Bacteriodes*, clostridia, and total aerobic bacteria resulting to increased fecal pH but had no significant effect on enterobacteria.[5] Regular consumption of NNS altered microbial profile and resulted to poorer glucose tolerance and glycemic response.[28,29] However, the number of *L. acidophilus* in media added with saccharides including prebiotics significantly increased reflecting the acid-creating capability of the bacterium.[30] Moreover, the effect of prebiotics on *L. acidophilus* in skim milk and a model peptone system displayed beneficial outcomes in the growth of *L. acidophilus*.[31] However, the viability of *Lactobacillus* casei decreased in a fermented milk drink during refrigerated storage.[32] In the present study, there was a decrease in the change in pH as the concentration of SPLENDA® was increased. This decrease in pH was associated with the increase in the number of *L. acidophilus* bacteria.[30] The pH change may also be attributed to some possible underlying mechanisms behind interactions among SPLENDA®, MRS broth, and *L. acidophilus*.

CONCLUSION

The changes in pH differed significantly across varying concentrations of sucralose when introduced to a culture medium containing *L. acidophilus*. The decrease in pH may be due to an increase in the number of *L. acidophilus* and possible interactions with SPLENDA® and MRS broth. The addition of certain concentrations of SPLENDA® in *L. acidophilus* culture affects the acid-production of the bacteria. To further explore on the effects of of SPLENDA® low-calorie sweetener on gut microbiota, it is recommended

that the incubation time be extended while the growth of *L. acidophilus* in varying concentrations of NNS is monitored.

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