

EFFECT OF BITING BEFORE DIPPING (DOUBLE-DIPPING) CHIPS ON THE BACTERIAL POPULATION OF THE DIPPING SOLUTION

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ABSTRACT

The effect of “double-dipping” crackers/chips on the transfer of bacteria from the mouth to the dipping solution was determined in three separate experiments. In experiment 1, eight subjects dipped crackers either three or six times into sterilized water either without biting or biting before each dip. The dipping solutions had higher ($P \leq 0.05$) bacterial populations when crackers were bitten before dipping compared with when no double-dipping occurred. The second experiment utilized sterile water dipping solutions with pHs of 4, 5 and 6, and tested the solutions at 0 and 2 h after dipping. There was again significant ($P \leq 0.05$) bacterial transfer due to biting then dipping; however, the pH 4 dipping solution had initially lower bacterial populations than the higher pH solutions and even lower populations after 2 h. In the third experiment, three dipping solutions (salsa, chocolate sauce and cheese) were tested, and higher initial populations ($P \leq 0.05$) were transferred to the salsa compared with chocolate and cheese; however, the salsa had lower levels of bacteria after 2 h of hold time at room temperature. Three experiments determined that the bacterial population of food dips increased due to the practice of “double-dipping,” and that dip type can influence the dip’s bacterial population.

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PRACTICAL APPLICATIONS

The practical application of these results to food safety will be similar to studies on hand washing. It is clear that foodborne disease can be spread by both practices (double-dipping and improper hand washing), and showing this is true using controlled studies may change the behavior of some people some of the time. But like hand washing, a no double-dip policy will not prevent the practice nor prevent the spread of disease. By determining that the bad practice of double-dipping does in fact transfer oral bacteria to a food dip, the practice may be reduced, subsequently reducing the spread of harmful microorganisms to some degree.

INTRODUCTION

While mingling at some social event, you notice someone performing the infamous “double-dip” (the redipping of a chip that has already been bitten). The type of chip and the type of dip are irrelevant to the act that many people consider impolite and unsanitary, yet others see no danger in the double-dip and feel no shame in dunking the same chip two, three or even four times in the dip. Whether the act is rude or not, the real question remains: Does the double-dip practice pose any concerns? How many bacteria are transferred from the perpetrator’s mouth to the dip?

Bacterial cells in the mouth attach to each other and mouth surfaces to form dynamic bacterial communities (Kolenbrander *et al.* 2002). Most of these oral bacteria communities reside on the teeth and eventually develop into plaque. Human oral bacteria are not naturally found anywhere else in the body and outside of the mouth; oral bacteria are considered pathogenic (Kolenbrander *et al.* 2002). Previous studies’ estimates of the number of oral bacterial species range from 500 types (Kolenbrander *et al.* 2002) to 700 types (Aas *et al.* 2005). According to Micik *et al.* (1969), coughing, sneezing, or even simply speaking can cause oral bacteria to become airborne. Particles that are small enough in size can become aerosolized and may be transmitted to other individuals. Bacterial aerosol counts for speaking and breathing were low; however, for coughing and sneezing, Micik *et al.* (1969) observed median bacterial aerosol counts of 36 (cough) and 530 cfu/min (sneeze), respectively, using an enclosed chamber to capture the patients’ aerosoled bacteria. For sneezing and coughing, Micik *et al.* (1969) reported values as high as 1,000 and 3,400 cfu/min, respectively. In fact, Harrel and Molinari (2004) cited five infectious diseases known to be spread by oral saliva droplets and aerosols, including pneumonic plague, tuberculosis, influenzas, Legionnaires’ disease and severe acute respiratory syndrome (SARS). If bacteria can be transferred

among individuals from the mouth through the air, then surely bacteria can also be transferred between humans by exposure to foods that have become contaminated with saliva. The Centers for Disease Control (<http://www.cdc.gov/flu/protect/covercough.htm>) recommends covering the mouth to prevent spreading “serious respiratory illnesses like influenza, respiratory syncytial virus (RSV), whooping cough, and severe acute respiratory syndrome (SARS).” While coughing, sneezing and touching contaminated surfaces are vectors for spreading disease, the disease agents originate from the mucus of infected persons (<http://www.health.state.ny.us/publications/7110/>). Due to the pathogenic microorganisms that may be found in the human mouth, some veterinarians warn against letting pets eat food being held in their owner’s mouth to protect the animal from disease (Wissman 2006). Thus, there may be health concerns due to the transfer of oral bacteria between humans from food sauces or dips contaminated by persons dipping bitten chips.

To analyze the impact of double-dipping, an undergraduate student research team conducted an experiment to determine the extent of bacterial transfer that occurs when a person bites a chip or cracker then dips the bitten chip into a solution. The inspiration of the experiment was a popular television episode of *Seinfeld*, written by Peter Mehlman, in which the character George Costanza is scolded for double-dipping. The objective of this study was to determine if biting a chip or cracker before dipping it in a solution compared with dipping an unbitten chip would result in different bacterial populations in the dipping solution or sauce. An additional objective was to determine the pH, holding time and dip type effects on the bacterial population of the dip.

MATERIALS AND METHODS

Prior to conducting the dipping experiment, total aerobic bacteria in the mouths of the subjects were measured by rinsing each subject’s mouth with 20 mL of sterile 0.1% peptone water. Subjects were asked to eat and drink as they do normally, but not within 2 h of sampling for both the oral cavity bacterial count and double-dipping experiments, and tests were conducted 2 h after normal meal times. Each subject rinsed their mouth for 20 s then expectorated into a sterile bag. One millimeter of the mouth rinse sample was directly pour plated and serially diluted and pour plated (petri dish, polystyrene sterile, 100 × 15 mm, VWR International, Suwanee, GA) in duplicate on nutrient agar (Difco, plate count agar, Becton, Dickinson & Company, Baltimore, MD), then after incubation for 48 h at 38C, plates from dilutions with between 25 and 250 cfu were counted and converted to log cfu per mL of the rinse solution.

For dip testing, three separate experiments were conducted to determine various factors affecting transfer and population of the dipping solutions. Low

sodium wheat thins (Nabisco, subsidiary of Kraft Foods, Northfield, IL) were used as the food product dipped for all three experiments.

Experiment 1

For experiment 1, 20 mL of sterile 0.1% peptone water acted as the dipping solution and was placed into sterile bacterial plates. Each of eight subjects carried out four treatments. For the dipping treatments, a cracker (held with sterile gloves) was bitten, dipped in the sterile water then discarded. Each cracker was dipped only to a depth that covered the bitten surface (~1 cm) and held in the dipping solution for 3 s. The control treatments consisted of dipping a cracker without biting. The four treatments were: three dips without biting, six dips without biting, three dips with biting and six dips with biting. One millimeter from each of the dipping solutions was serially diluted in 0.1% peptone water then pour plated in duplicate in nutrient agar (Difco, plate count agar, Becton, Dickinson & Company). Again, after incubation for 48 h at 38C, plates from dilutions with between 25 and 250 cfu were counted and converted to log cfu per mL of the dipping solution.

Experiment 2

Three popular types of commercial dips (salsa, chocolate and cheese) were chosen to test for pH. pH was determined using a probe (Triode pH electrode, Orion Inc., Boston, MA) attached to a pH meter (Model 420 A, Orion Inc.). The pH meter was calibrated using standardized solutions (Orion Inc.).

These dips had average pHs of 4.0 (salsa), 5.1 (chocolate) and 6.1 (cheese). Three batches of 0.1% sterile peptone water were adjusted to match each of these pH levels using 0.1 normal citric acid solutions. The pH-adjusted solutions were designed to determine the effect of pH on bacterial population in the dip without confounding the results with effects from other food dip components or properties. The dipping solutions were placed into sterile bacterial plates for testing. For dipping treatments, each of the nine subjects was allotted nine experimental plates: three (one for each pH) plates each for the no dip, control dip and bite dip treatments. For the bite dipping treatments, the subject bit and dipped a cracker for 3 s in 10 mL of each pH solution and repeated the dipping twice more (using a total of three wheat thins). For the control dipping, the subject dipped – without biting – three crackers in 10 mL of each pH solution. The “no dip” plates were left untouched. The sample was mixed by gently moving the petri dish in a figure eight motion for four cycles. A 1 mL sample of each of the experimental solutions was directly pour plated and serially diluted before pour plating in duplicate using nutrient agar (Difco, plate count agar, Becton, Dickinson & Company) at 0 and after the dipping solution was held covered for 2 h at room temperature ($25 \pm 4C$). After the plates were incubated

for 48 h at 37C, the colony forming units of bacteria were counted, recorded and converted to log cfu per mL of the dipping solution.

Experiment 3

Three commercial food dips were used as the dipping solution: All Natural Tostitos Chunky Hot Salsa (Tostitos brand, FritoLay, Dallas, TX), pH 4, Genuine Chocolate Flavor Hershey's Syrup (Hershey Corp., Hershey, PA), pH 5.3 and Fritos Mild Cheddar Flavor Cheese Dip (FritoLay), pH 6.0. The same procedure and treatments to test bite-dipping and time effects were used for experiment 3 as were used for experiment 2, with the three different dips being used instead of the three different pH levels, and six subjects were used in experiment 3. In addition, the viscosity of each dip was measured using a Brookfield viscometer with a cylindrical spindle (model LV, Brookfield Engineering Inc., Middleboro, MA). Three separate measurements of viscosity expressed in poise for each dip were taken at room temperature (25 ± 4 C) and averaged. The amount of dip that adhered to the chip was estimated by determining the weight loss from the initial 11 g by dipping three wheat thins into 11 g of dip, and allowing the dip to drain off the cracker for 5 s, then recording the weight of the remaining dip. The weight loss for the 11 g was recorded and expressed in g and as a percentage of lost weight.

Statistical Analysis

Subjects were used as experimental replicates, and experiment 1 was replicated eight times, experiment 2 was replicated nine times, experiment 3 was replicated six times and all plating was in duplicate. The dipping treatments, pH levels, hold time effects and dip types were subjected to an analysis of variance using SAS (2006) to determine if there was a significant ($P \leq 0.05$) overall effect due to these treatments. The model error term for each experiment was any treatment interaction that included the replication term. Since there was a significant effect, the means were separated using the pdiff command of SAS. Viscosity and dip weight loss due to dipping were expressed as simple averages, with variation given by the range of values.

RESULTS

Experiment 1

Oral cavity bacterial counts ranged from 5.8×10^5 to 4.8×10^6 cfu/mL (5.8 to 6.7 log cfu/mL) and averaged 1.8×10^6 cfu/mL with less than 1 log cfu/mL variation among the seven subjects (Table 1).

TABLE 1.
TOTAL ORAL AEROBIC BACTERIAL POPULATION FOR
SEVEN SUBJECTS IN EXPERIMENT 1

Subject	log cfu/mL
1	6.10
2	6.34
3	6.35
4	6.69
5	5.98
6	5.76
7	5.83
Mean (SD)	6.26 (.31)

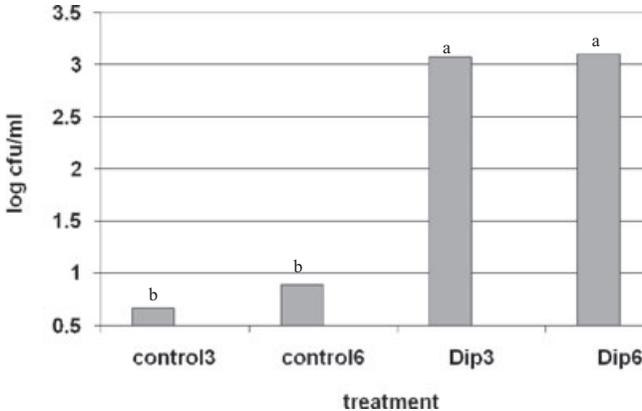


FIG. 1. LOG cfu/mL OF TOTAL AEROBIC BACTERIA RECOVERED FROM STERILE WATER, IN WHICH THREE OR SIX CRACKERS HAD BEEN DIPPED WITH OR WITHOUT BEING BITTEN BEFORE DIPPING (EXPERIMENT 1)

Standard error = 0.1, n (subjects) = 8. ^{a,b}Means with different superscripts are significantly different ($P \leq 0.05$). Control3, three different wheat crackers, each dipped once without biting; Control6, six different wheat crackers, each dipped once without biting; Dip3, three different wheat crackers, each bitten once before dipping; Dip6, six different wheat crackers, each dipped once without biting.

For experiment 1, a higher population of bacteria ($P \leq 0.05$) was found for solutions dipped with crackers after biting compared with solutions dipped without biting (Fig. 1). There was no difference between the three and six dips ($P > 0.05$) as far as bacteria transferred to the dipping solution. Bacterial populations found in the solution after crackers were dipped without biting were less than 10 cfu/mL of the dipping solution. A significant amount of bacterial transfer occurred that was attributable to “double dipping”. Bacterial

counts averaged 2.6×10^3 cfu/mL and 1.9×10^3 cfu/mL for three and six dips, respectively, when the chip/cracker was bitten once prior to dipping.

Experiment 2

All variables (time, pH, dipping treatment) and interactions were found to significantly affect the \log_{10} cfu/mL of the dipping solution ($P \leq 0.01$). Regardless of the variables of holding time and pH, two findings were constant: the bacterial population of the "bite dip" treated dip was always higher ($3\text{--}4 \log_{10}$ cfu/mL \sim 12,000 cfu/mL) than both the control and no dipping treatments ($<0.1 \log_{10}$ cfu/mL, <2 cfu/mL), and there were no significant difference between the bacterial populations of the control dip and no-dip solutions. The pH did, however, have a significant affect on the bacterial population of the bite-dipped solution, as seen by the incremental increase in \log_{10} cfu/mL from 2.5 at pH 4 to 3.5 at pH 5 and 3.8 at pH 6 (a, b and c, respectively in Table 2). The holding time's effect on bacterial population depended on the pH of the dipping solution. The pH 4 solution's population decreased from 3.19 \log_{10} cfu/mL at 0 h to 1.90 \log_{10} cfu/mL at 2 h. The mean bacterial population of the pH 5 solution also decreased from 3.67 to 3.35 \log_{10} cfu/mL after 2 h. The number of bacteria in the pH 6 solution, however, did not decline between 0 and 2 h, with a difference of only 0.01 \log cfu/mL (Fig. 2).

TABLE 2.
EFFECT OF DIPPING SOLUTION pH AND BITING BEFORE
DIPPING ON THE TOTAL AEROBIC BACTERIAL
POPULATION OF THE DIPPING SOLUTION
(EXPERIMENT 2)

pH	Treatment	Log ₁₀ cfu/mL
4	Bitedip	2.5 ^c
4	Nodip	0.1 ^d
4	Contdip	0.0 ^{d*}
5	Bitedip1	3.5 ^b
5	Nodip	0.0 ^d
5	Contdip	0.1 ^d
6	Bitedip1	3.8 ^a
6	Nodip	0.0 ^d
6	Contdip	0.3 ^d

^{a-d} Means with different superscripts are significantly different ($P \leq 0.05$). $n = 18$. Standard error = 0.08.

* Log₁₀ cfu/mL values of 0.0 represent levels below the detection level of approximately one cell per 10 mL, as the dipping solution was directly plated without dilution for treatments with low numbers of cells.

Bitedip, biting the chip before dipping; Nodip, no chip dipped in the solution; Contdip, dipping the chip without biting before dipping.

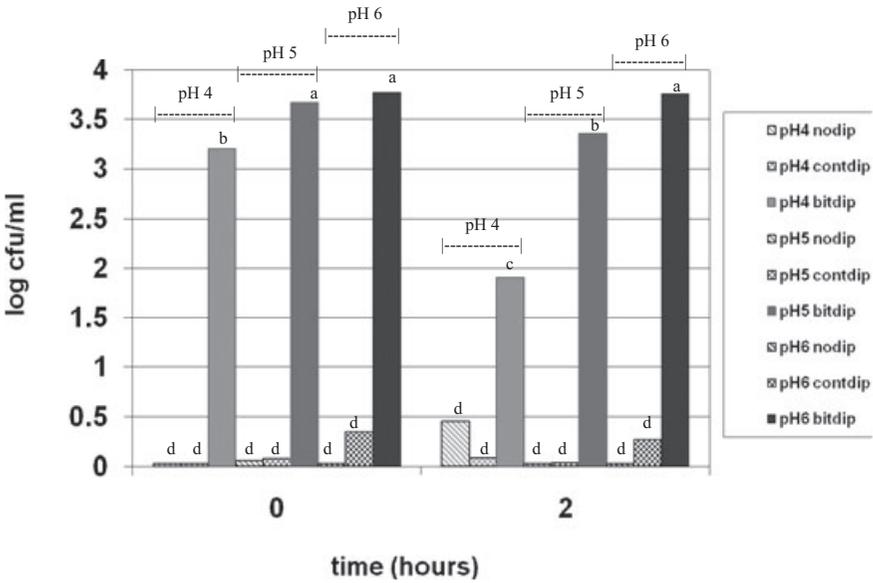


FIG. 2. THE EFFECT OF SOLUTION pH, SAMPLING TIME AND BITING BEFORE DIPPING ON THE TOTAL AEROBIC BACTERIAL POPULATION OF THE DIPPING SOLUTION (EXPERIMENT 2)

Means with different superscripts are significantly different ($P \leq 0.05$). n (subjects) = 9. Standard error = 0.1. Nodip, no cracker was dipped in the sauce; Contdip, the cracker was not bitten before dipping in the sauce; Bitdip, the cracker was bitten before dipping in the sauce.

Experiment 3

As with experiment 2, all variables (time, dip type, dipping treatment) were found to significantly affect ($P \leq 0.0001$) the aerobic bacterial population of the dip (Fig. 3). The bite dip treatment resulted in over 2 log cycles (~600 cfu/mL) higher bacterial populations, regardless of recovery time or dip type. The control and no-dip treatments had similar and very low bacterial populations (<10 cfu/mL) regardless of recovery time or dip type. Salsa that had been exposed to “double-dipping” had higher initial populations (time 0) compared with chocolate and cheese sauce. This was theorized to be due to the adherence of the dipping sauce to the cracker in that more of the sauce exposed to the bitten cracker was removed from the contaminated along with the cracker. To test this theory, the viscosity and amount of dipping solution that adhered to the cracker was measured gravimetrically (weighing the dip before and after dipping). It was discovered that less salsa was removed from the bulk dipping solution compared with the more viscous chocolate and cheese sauces. The viscosities of the dips were in descending order: cheese (939,000; 896,000

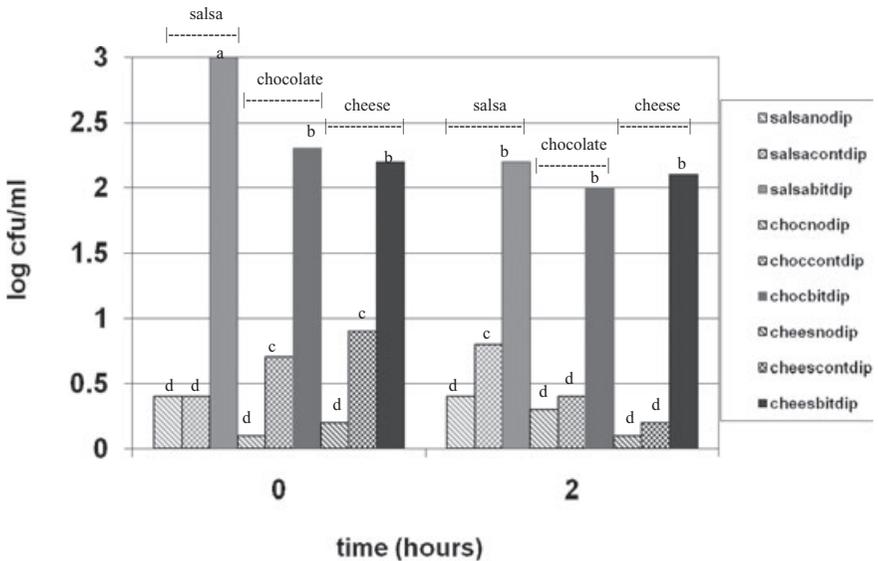


FIG. 3. THE EFFECT OF DIPPING SOLUTION, SAMPLING TIME AND BITING BEFORE DIPPING ON THE TOTAL AEROBIC BACTERIAL POPULATION OF THE DIPPING SOLUTION (EXPERIMENT 3)

Means with different superscripts are significantly different ($P \leq 0.05$). n (subjects) = 6. Standard error = 0.1. Nodip, no cracker was dipped in the sauce; Contdip, the cracker was not bitten before dipping in the sauce; Bitdip, the cracker was bitten before dipping in the sauce.

to 961,000 poise), chocolate (118; 108–132 poise) and salsa (71; 70–73 poise). The percent weight loss by the dipping sauce to the cracker after three dips also followed the same descending order: cheese (18.2, 18.1–18.4%), chocolate (7.9, 7.4–8.7%) and salsa (6.5, 5.3–7.3%). Thus, the viscosity of the dipping solution, and, more specifically, the adherence of the solution to the cracker were related to the transfer of bacteria to the dip.

The salsa exposed to biting before dipping decreased in bacterial population to levels not different from chocolate and cheese after 2 h. This was assumed to be due to the lower pH or other antimicrobial properties of the dip.

DISCUSSION

Thus, the practice of double-dipping does contaminate the dip. According to Kang *et al.* (2006), 14 genera were identified in human saliva. Reports vary in the number of different bacteria found in the human mouth, from 300 to over 1,000. *Streptococcus*, *Prevotella* and *Veillonella* were among the genera most

common to the human oral cavity, with *Streptococcus* being the predominant genus. *Staphylococcus epidermis*, *Streptococcus mitis*, *Streptococcus salivarius*, *Streptococcus mutans*, *Lactobacillus spp.* and *Spirochetes spp.* were reported as being found in nearly 100% of human oral cavities (Todar 2002).

Oral bacteria can include pathogenic strains that can be life threatening (Sumi *et al.* 2006). Therefore, the practice of “double-dipping” may have more relevance than just popular interest. This “bad habit” may also have negative health implications. In fact, very sophisticated statistical models have been developed to predict the spread of disease from a single individual to large populations (James *et al.* 2007). Disease can be spread from infected individuals through the mucus via sneezing, handshaking and coughing from infected individuals. People can harbor and spread serious disease, as did the infamous cook Mary Mallon “Typhoid Mary,” who spread typhoid to numerous families in the 19th century by handling their food. Therefore, individuals sharing a dip may be passing disease-causing agents to the dip yet not show outward signs of illness. Dips are often low-acid foods ($\text{pH} \leq 4.6$) that inhibit many types of bacteria. However, some dips also contain proteins, lipids and other food components that can act as buffers to protect microorganisms. In fact, probiotic bacteria have been shown to survive, and, in some cases, increase in population when added to an acidic cheese-based dip (Tharmaraja and Shah 2004). In the current study, the low-pH dip did have an inhibitory affect on bacterial populations when the dip was held for 2 h; however, these levels were still relatively high. Bacteria can also modify their environment to facilitate their survival and growth, and repeated dilution and inoculation of a dip via double-dipping may further compromise the safety of said dip. The concern over human saliva as an infection vector is not a new concept. Dental laboratories are a known source of bacterial infection for patients and dental workers (Kang *et al.* 2006). Gargle water and dental water lines were found to contain bacterial contamination originating from human saliva (Fitzgibbon *et al.* 1984; Peters and McGaw 1996; Pankhurst *et al.* 1998). The spread of contaminating microorganisms from saliva by any means is a potential health and food safety concern.

One interesting observation was that the viscosity and amount of dipping sauce adhering to the cracker had a greater effect on initial bacterial population transferred by “double-dipping” than did the pH of the sauce. In experiment 2, where the viscosities of all dipping solutions were equal, the low pH solution yielded the lowest initial bacterial population. However, in experiment 3, where viscosity and adhesion differed across dipping solutions, the dipping sauce with the lowest pH yielded the highest initial bacterial populations.

In conclusion, whether the type or amount of contamination is dangerous to a dippers’ health or not is debatable and depends upon multiple external factors, including the type of chip used, the dip composition and the relative

health/illness state of the person whose mouth contributed the bacteria. The physical properties of a dipping solution (i.e., viscosity, cohesion and adhesion) may have a significant effect on the transfer of bacteria from a contaminated chip to the dip. One conclusion that was clearly evident from this study is that biting before dipping had a significant impact on the bacterial population of the dipping solution.

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