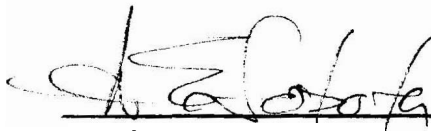THE EFFECTS OF CRANBERRY JUICE AND ITS COMPONENTS

ON BACTERIAL ADHERENCE AND THEIR POTENTIAL USE

IN THE PROPHYLAXIS OF URINARY TRACT INFECTIONS

by

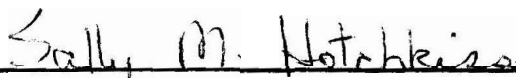Robert M. Felden

Submitted in Partial Fulfillment of the Requirements

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ABSTRACT

THE EFFECTS OF CRANBERRY JUICE AND ITS COMPONENTS

ON BACTERIAL ADHERENCE AND THEIR POTENTIAL USE

IN THE PROPHYLAXIS OF URINARY TRACT INFECTIONS

Robert M. Felden

Master of Science

Youngstown State University, 1987

It is widely believed that ingestion of cranberry juice can be used in the treatment and prophylaxis of urinary tract infections. Experiments to determine the mode of action of cranberry juice have revealed that ingestion of the juice can result in urine which will inhibit adhesion of bacteria to uroepithelial cells. The present study was conducted to determine the **factor(s)** within the cranberry juice responsible for antiadherence. Fractionation of the cranberry juice by an amberlite ion exchange column and dialysis through a **1000** molecular weight cut off membrane resulted in an active component that was water soluble and had a molecular weight of less than 1000. This component was able to prevent binding of Escherichia coli to Saccharomyces cerevisiae, an inter-action not unlike the one which occurs between bacteria and uroepithelial cells. Futhermore, the major organic acids found in cranberry juice ‑ citric, malic, and quinic acids ‑ were **tested and found to** successfully block adhesion in

the test system. It is concluded that a factor or factors tested, either alone or in combination with other factors, may be beneficial in the prophylaxis of urinary tract infections.

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CHAPTER I

INTRODUCTION

Acute urinary tract infections (UTIs), which occur most commonly in women, account for greater than 6 million physician visits yearly in the United States (Braunwald, 1987). The occurrence of UTIs in male subjects is greatest in the newborn, the only period in life in which the incidence is greater for males than females (Roberts, 1986). It is estimated that 1 to 3 percent of schoolgirls develop UTIs which markedly increases as sexual activity begins in adolescence (Braunwald, 1987). However, Braunwald (1987) contends that the majority of acute symptomatic infections occur in young women. Adult males rarely incur UTIs until after the age of 60, when the incidence increases sharply due to prostatic disease and medical instrumentation (Bell, 1983). Women also show a sharp rise in frequency of UTIs after 60 which may be due to a decrease in local estrogen levels, incomplete bladder emptying, and associated medical conditions such as diabetes mellitus (Bell, 1983).

Anatomically, acute UTIs can be subdivided into two categories: upper tract infections (acute pyelonephritis) and lower tract infections (urethritis, cystitis, and prostatitis). Froom (1980) infers that less than 10% of UTIs involve the upper tract. These can be determined by a

significant rise in serum IgG antibodies directed against the O antigen of the infecting strain. Additionally, there is temporary impairment in the concentrating ability of the kidney which may be associated with leukocyte casts in the urine. The other **90%** of UTIs, which involve the lower tract, infrequently result in increased antibody titers, concentrating defects, or white cell casts (Braunwald, **1987).**

The Merck Manual of Diagnosis and Therapy (Berkow, **1982)** states that individuals with UTIs may experience dysuria, urgency, frequency, and costovertebral angle tenderness accompanied by chills, fever or flank pain. An infection exists when pathogenic organisms are detected in the urine, urethra, prostate or kidneys. In most cases, growth of greater than 10^5 organisms/ml from a "clean catch" midstream urine is indicative of an infection. Yet there are instances where a patient is symptomatic and less than 10^5 organisms/ml can be recovered.

The gram-negative bacilli are the most common micro-organisms infecting the normal urinary tract, with Escherichia coli causing about **85** percent of the uncompli-cated infections, followed by Klebsiella, Proteus, and Enterobacter (Berkow, **1982).** Pseudomonas, staphylococci, and Group D streptococci comprise **5-10%** of the infections, but assume an increasing role in recurrent infections and **infections associated with urologic manipulation, calculi,** or obstruction (Berkow, **1982** and Braunwald, **1987).**

Sobel (1985) asserts that the ascending route of infection is the cause of most of the UTIs with colonization of the periurethral region by uropathogens preceeding the development of UTIs. Little is understood about the factors which contribute to colonization of the urethra with uropathogens in otherwise healthy women. The female urethra is short (approximately 4 cm), which, due to its proximity to the vulvar and perianal regions and termination beneath the labia, make it susceptible to contamination. In young women, urethral massage, as occurs during sexual intercourse may introduce bacteria into the bladder. Also, the contraceptive diaphragm appears to be associated with a twofold rise in UTIs (Braunwald, 1987). Prostatitis or urethral obstruction due to protatitis or urethral obstruction due to prostatic hypertrophy are important entities leading to bacturia in males (Berkow, 1982).

Despite the factors mentioned above, multiplication of bacteria is largely inhibited in the normal urethra (Sobel, 1985). The natural defense mechanisms still remain unclear. The flushing action of micturition and emptying of the bladder has been touted to be a defense mechanism (Mulholland, 1979). Yet Sobel (1985) asserts that, although the bulk of infected urine may be removed, a film of urine containing the uropathogen will remain and coat **the bladder mucosa. This film of urine is usually** sufficient to maintain colonization. The urine itself,

according to Kaye (1968), may also exert a protective effect against UTIs. The most important factors appear to be a very high or low osmolality, a high urea concentration, a high organic acid concentration and a low pH.

Other investigators have stressed the importance of bacterial adherence to bladder mucosa, in addition to colonization of the urine, as the cause of infections (Sobota, 1984; Aronson, 1979; Svanborg-Eden, 1982). Both animal and human studies demonstrate that E. coli type 1 pili attach to mannose residues present on surfaces of the bladder epithelial cells (Schaeffer, 1981 and Aronson, 1979). Aronson (1979) went on to demonstrate that addition of mannose will block E. coli adherence to receptor sites for type 1 pili by competitive inhibition. Immunoglobulins A and G also significantly reduce E. coli attachment to uroepithelial cells (Svanborg-Eden, 1982).

Another anti-adherence substance that has gained importance is the Tamm-Horsfall protein, or uromucoid. It appears that this is a glycoprotein secreted by the tubular cells of the kidney's ascending loop of Henle (Orskov, 1980). The mannose-rich oligosaccharide residues of the Tamm-Horsfall protein serve as an attachment site for the E. coli with type 1 pili. In this way, bacteria attached to the protein can be cleared from the bladder during micturition.

Even though the human body attempts to prevent bacterial adhesion, colonization and adhesion do frequently occur in the general population. To aid the body's own bacterial defense mechanisms, antimicrobial therapy should be initiated as soon as the diagnosis of UTI is made and a urine culture and sensitivity is performed. In uncomplicated infections, a **7-10** day course of antibiotics should be started and a urine culture repeated after completion of therapy. This is done to ensure complete eradication of the uropathogen (Braunwald, **1987).** Commonly employed antibiotics used to treat UTIs include amoxicillin, trimethoprim-sulfamethoxazole and nitrofurantoin. A problem arises with long term therapy for the patient who experiences recurrent UTIs. Prophylaxis has been recommended with single daily oral doses of trimethoprim-sulfamethoxazole, trimethoprim alone or nitrofurantoin in females having more than two infections in 6 months (Braunwald, **1987).** However, there are certain drawbacks which must be considered: drug side effects, toxicity, overgrowth of normal vaginal flora, and development of resistant bacterial strains. The ideal prophylactic agent for UTIs should be one which minimizes these side effects of conventional therapy. This suggests the potential use of cranberry juice as a long term prophylactic agent for the treatment of recurrent UTIs.

It has been demonstrated by Sobota (1984) that ingestion of cranberry juice cocktail resulted in urine capable of preventing the adhesion of E. *coli* to uroepithelial cells. Prodromos (1968) has shown that cranberry juice ingestion had no clinical side effects and was well tolerated by patients. Thus, cranberry juice cocktail would appear to be an ideal substance capable of being used for long term prophylaxis of UTIs.

The current study was constructed to further investigate and characterize the **factor(s)** present within the cranberry juice which enables it to prevent attachment of E. *coli* to uroepithelial cells. Both Ocean Spray cranberry juice cocktail and freshly prepared juice were tested as well as the major organic acids contained in the cranberry juice - quinic, malic, and citric acids.

CHAPTER II

MATERIALS AND METHODS

Cranberry Juice

Two preparations of cranberry juice were used in
these experiments: 1) Ocean Spray cranberry juice cock-
tail and 2) freshly prepared juice. Freshly prepared juice
was extracted from whole cranberries using the following
procedure. The berries were mixed with an equal volume of
glass distilled water (GDW) and crushed in a Warring
Blender for 10 minutes. The resulting mixture was filtered
twice with suction through Whatman No. 5 qualitative paper,
and centrifuged at 10,000 g for 30 minutes to express the
juice. The juice was used at its native pH of 2.6 or at an
adjusted pH where indicated.. Concentrated NaOH solution
was used to vary the pH and all pH determinations were per-
formed with a Sargent model DR pH meter.

Bacteria

The bacterium used in this study was Escherichia
coli strain 1876, a K-12 derivative (supplied by A.E.
Sobota, Youngstown State University, Youngstown, Ohio).
The E. coli stock culture was stored in a 125 ml. flask
containing 50 ml. of BBL #11777 trypticase soy broth (TSB)
held at $4^{O}C$. For each experiment requiring bacteria, the
organism was transferred aseptically via a wire loop into a

Pyrex test tube containing 10 ml. of sterile TSB. The tube
was incubated for 48 hours at 37^OC in a Lab-Line #400
incubator. The resulting culture contained approximately
10^8-10^9 bacteria/ml. The culture was then vortexed for
5 seconds on a Vortex Junior Mixer, and 1.0 ml. was
transferred to a conical centrifuge tube via a 1.0 ml.
serological pipette. The bacteria was then centrifuged at
top speed (3,000 rpm) in a clinical centrifuge for 10
minutes. The supernatant was decanted and the resulting
pellet was resuspended in 1.0 ml. of 0.01 M phosphate
buffered saline (PBS) at a pH of 7.2 (Sobota, 1984).

Bacterial Adherence Test

The bacterial adherence test is a modification
(Sobota, 1984) of the procedure outlined by Eisenstein, B.
I., et al. The yeast, Saccharomyces cerevisiae, used to
demonstrate bacterial adhesion, was maintained on a tryp-
ticase soy agar slant stored at 4^OC. To initiate the
test procedure, a sample of the stock culture was trans-
ferred from the slant to 50 ml. of sterile TSB and
incubated for 48 hours at 37^OC. The yeast was harvested
by centrifugation for 1 minute at top speed in a clinical
centrifuge. The pellet was washed with GDW and fixed with
5.0 ml. of gluteraldehyde (1 mg/ml) for 60 minutes at room
temperature (22^OC - 25^OC). The cells were then
centrifuged, supernatant decanted, pellet washed twice in
GDW, and incubated in 5.0 ml of glycine (10 mg/ml) for 30

minutes at room temperature. The cells were centrifuged, washed twice with GDW, and stained with **1%**Safranin for 24 hours. The stained yeast preparation was washed twice in GDW to remove excess Safranin and brought to a concentration of 10^7 cells/ml via the use of a hemocytometer.

A 96-well, flat bottomed microtiter plate with rows lettered A through H and columns numbered **1** through 12 was employed in the microtiter procedure. Using an eight-tipped microdispenser, 25 ul. of GDW was placed in wells A_1 through H_1, 75 ul. in A_2 through H_2, and 25 ul. in the remaining wells. Ten microliters of E. coli were placed in wells A_1 through G_1 and A_2 through G_2. Serial dilution was performed using alcohol sterilized 50 ul. tulip-tipped microdiluters from column 2 to 12 in all rows, with the final 50 ul. being disposed of in 95% ethanol. The tulip-tipped diluters were twirled 30 times to ensure adequate mixing. Thus, a plate was set up such that wells A_1 through G_1 had the highest concentration of bacteria and wells A_{12} through G_{12} contained a titer of 2048.

Rows B through G served as experimental wells to which 10 ul. of test substance was added proceeding from well 12 to **1** in an effort to limit dilutional errors due to cross mixing. Row A served as the E. coli control to which 10 ul. GDW was added. Row H served as the yeast control to

which 10 ul. of GDW was also added to maintain proper dilu-
tion.   The plate was placed on an Eberbach shaker and mixed
for 10 minutes at room temperature to allow the E. coli to
interact with the various test substances.   Ten microliters
of yeast preparation was then added to all 96 wells, pro-
ceeding from columns 12 to 1.   The plate was shaken for an
additional 10 minutes then allowed to incubate at room
.temperature for 60 .minutes to permit the E. coli and yeast
cells to adhere.   Examination was conducted at 10X magni-
fication with an inverted microscope to score for aggluti-
nation of the yeast cells by the E. coli.

Amberlite Ion Exchange Chromatography

Ten grams of dry amberlite ion exchange resin
(CG-50, Sigma) was hydrated and washed five times with a
total of 750 ml. GDW.   Following each wash, the fines were
removed by decanting after the bulk of the course particles
had settled.   GDW was added to the hydrated resin (2:1)
forming a slurry which was poured into a 1 cm. (internal
diameter) X 24 cm. column with a fritted glass disc at the
bottom.   The column was washed with GDW until no turbidity
was observed in the eluate.   Once hydrated, the column was
never permitted to dry.

The assay was initiated by adding 10 ml. of Ocean
Spray cranberry juice cocktail to the top of the column and
allowing it to settle.   One milliliter fractions were col-

lected using an ISCO model 1850 fraction collector. The individual fractions were eluted from the column with 40 ml. of GDW followed by 50 ml. of a solution containing 100% methanol and concentrated HC1 (100 ml. : 0.25 ml.). All fractions were stored at 0°C until assayed.

The above procedure is a modification of the work conducted by Chiriboga, C. and Francis, F.J. (1970) in which the amberlite CG-50 resin was used to recover the anthocyanin portion of cranberry pomace. In the present work, an effort was made to recover the water soluble con-stituents as well as the pigmented portion and test both for antiadherence properties. Where Chiriboga, C. et al. (1970) used 15 ml. GDW and 30 ml. of methanol-acid solution, 40 ml. GDW and 50 ml. methanol-acid solution was substituted.

Dialysis Procedure

Twenty-five centimeters of 1000 molecular weight cut off (MWCO) spectrapore (Fisher) membrane tubing was soaked in 100 ml. GDW for two hours. This procedure was necessary to eliminate the 1% sodium benzoate in which the membrane was stored. An aliquot of this soaking solution was tested to determine if it had any antiadherence effect; no effect was observed. Ten milliliters of Ocean Spray cranberry juice cocktail or cranberry juice extracted from the berries was placed in the tubing. Both ends were clamped

with Spectrum Closures and the resulting bag was covered with 50 ml. of GDW and refrigerated at $4^{\circ}C$ for approximately 10 hours. The dialysate and retentate were assayed using the bacterial adherence test.

Organic Acids

The following organic acids, obtained from Sigma, were tested using the bacterial adherence test: citric, malic, and quinic acids. Stock solutions of 1% and 5% were prepared for each acid by dissolving the powder in GDW. The stock solutions were further diluted with GDW to attain the desired dilution. The diluted stock solution was used at its native pH, or at an adjusted pH where indicated. Concentrated NaOH and HCl were used to alter the pH.

CHAPTER III

RESULTS

Ocean Spray cranberry juice cocktail was fractionated via amberlite column chromatography and the individual fractions were tested for antiadherence activity. The results .appear in Table 1 (Appendix A). **Antiadherence** activity was tested at **five .milliliter** intervals, beginning with the seventh milliliter. It can be observed from the Table that fractions 7, **12,** 17, 22, 27, 32, and 37 showed antiadherence activity as compared with the **E. coli** control with a titer of 2048. Fractions 42, 47, **52**, and 57 did not exhibit antiadherence activity, yielding titers equal to the **E. coli** control. It should be noted that the group of fractions demonstrating antiadherence activity were eluted from the column with GDW. The last four fractions tested showing no antiadherence activity were eluted with the **methanol/HC1** mixture. Fractions 47, 52, and 57 were red in color and presumably continued the anthocyanin pigments.

For the bacterial adherence assay, the 48 hour culture of **E. coli** was initially diluted so agglutination could still be observed at the highest dilution in the microtiter plate **i.e.** a titer of 2048. Titers of greater than 2048 showed no adherence. This dilution procedure permitted maximum sensitivity for the bacterial adherence test,

To characterize the approximate size of the "anti-adherence factor", a 1000 MWCO membrane was used. The results are displayed in Tables 2 and 3 for Ocean Spray cranberry juice cocktail and the juice extracted from the berry, respectively. The dialysate was the fluid which passed through the 1000 MWCO membrane and the retentate was that which remained within the bag. In three trials with the Ocean Spray cranberry juice cocktail (Table 2), it can be noted that both the dialysate and retentate yielded titers of zero as compared to an E. coli control titer of 1024. A titer with a value of zero indicates a total lack of adherence between the bacteria and the yeast, i.e., no agglutination of yeast was observed in any of the test wells on the microtiter plate.

Trials involving double dialysis, a redialysis of the dialysate and retentate, were conducted. This resulted in titers of 32 or 64, respectively. Also, some of the singly dialyzed dialysates and retentates were subjected to temperatures of 4°C and -15°C with a variable effect. The frozen (-15°C) dialysate and retentate yielded titers of 16 and 2 where the refrigerated (4°C) samples had higher titers of 128 and 8, respectively.

Cranberry juice contains about 1.08% citric acid, 0.92% malic acid, and 1.32% quinic acid (Coppola et al., 1978). Each of these organic acid was tested for its anti-adherence properties at different concentrations and pH's.

Tables 4, 5, and 6 contain the results of the antiadherence activity of citric acid. The highest concentration of citric acid tested was 0.02% at a pH of 2.60 (Table 4). The titer at this concentration of citric acid was zero, indicating complete inhibition of adherence. A GDW control (pH = 2.60) yielded a titer of 256. A citric acid concentration of 0.002% (pH = 3.00) produced a titer of 256 as did the GDW control with a pH of 3.04 (Table 5). Finally, a 0.0002% citric acid solution with a pH of 3.85 yielded a titer of 512 (Table 6). The GDW control with pH = 3.88 also gave a 512 titer. In all of these trials, the pH of the 48 hour *E. coli* culture control was measured with Color **pHast** (E. Merck, Darmstadt) indicator sticks and gave a value of 6.0.

The results of the antiadherence activity of malic acid appear in Tables 7, 8, 9, and 10. Concentrations of **5%, 1.25%,** and 0.31% malic acid were tested (Tables 7, 8, and 9). In all cases, with three trials at each concentration, adhesion was completely inhibited as indicated by a titer of zero. However, at a concentration of 0.01% malic acid (Table 10), a titer of 16 was attained which indicates a reduction in antiadherence activity. In all malic acid trials, the *E. coli* control was at pH = 6.0 and the GDW controls with pH's ranging from 1.90 to 3.33 yielded titers of 256 or 512.

The quinic acid antiadherence activity results appear in Table 11. Concentrations between 5% and 0.05% produced titers of zero compared with a 512 titer for the GDW controls. A titer of 128 is observed for the quinic acid concentration of 0.005% (pH = 2.78).

To test the effects of changing the pH on the three acids, trials were constructed with pH's starting at the native pH of the acid solution; then the pH was increased to 5.00 and 7.00 and finally returned to 2.60. The results appear in Tables 12, 13, and 14 for citric, malic, and quinic acids, respectively. To alter the pH, concentrated solutions of NaOH and HCl were added dropwise to reduce a possible dilutional effect. It can be observed in Tables 12, 13, and 14 that the antiadherence activity of each acid was lost at pH's of 5.00 and 7.00 and was recovered when the pH was lowered to 2.60.

Table 15 reflects the data collected from varying the pH of Ocean Spray cranberry juice cocktail. The pH range used was 2.45 through 6.00. It can be observed that at a pH of 3.10 the antiadherence activity began to decrease, yielding a titer of 16. This trend continued through pH 6.00 where the titer was 256. It can, however, be observed that this titer of 256 is still considerably lower than the controls which had a titer of 2048 at the same pH.

CHAPTER IV

DISCUSSION

Folklore medicine has attributed to cranberry juice the properties of urine acidification and bacteriostasis (Moen, 1962) and hence the potential use for the juice in the prophylaxis and/or treatment of urinary tract infections. There have been numerous attempts to explain the mode of action by which cranberry juice exerts its effects, but these have met with limited success (Bodel, 1959 and Eckman, 1984).

It is generally believed that bacterial colonization of a mucosal surface is dependent upon the bacteria's ability to adhere to the individual host epithelial cells (Ofek, I, et al., 1978). Recently, it has been reported that ingestion of cranberry juice results in the production of urine that possesses the ability to inhibit bacterial colonization of uroepithelial cells by preventing adherence (Sobota, 1984).

The present study assumes that cranberry juice contains an antiadherence factor capable of preventing the necessary receptor interaction between the bacteria and the uroepithelial cells. Based on this assumption, an attempt was made to characterize this factor.

Chiriboga and Francis (1970) report that cranberry juice can be separated into a water soluble and methanol/

HC1 soluble portion using an amberlite CG-50 column. In this study, the ion exchange resin was employed to separate Ocean Spray cranberry juice cocktail into fractions which could be tested for antiadherence properties. Among the compounds the resin absorbs are anthocyanin pigments, while many other components of the juice can be eluted from the column with glass distilled water (GDW). When assayed via the bacterial adherence test, it was demonstrated that the group of fractions eluting with the GDW possessed anti-adherence activity with titers ranging from 64 to 1024 compared to the control titer of 2048. Fractions eluting with the methanol/HC1 mixture had titers of 2048 which were equivalent to the control titer. A titer less than the control titer reflects inhibition of bacterial adhesion, where a titer equal to the control titer indicates a lack of inhibition. Additionally, it was observed that the fractions eluted from the column by the methanol/HC1 mixture were pink to red in color which indicated they contained anthocyanin pigments (Chiriboga and Francis, 1970 and 1973). Thus, it was concluded that the anthocyanin pigments do not possess antiadherence qualities and some other compound(s) eluting in the water soluble fraction is responsible for the observed antiadherence effect of the cranberry juice. Therefore, the remainder of this study concentrated on the water soluble fractions of the juice.

Next, a 1000 molecular weight cut off (MWCO)
dialysis membrane was used to determine the approximate
size of the antiadherence factor. The dialysis procedure
was performed on both the Ocean Spray cranberry juice cock-
tail and juice extracted from the berries. In three
separate trials involving the cocktail, both the dialysate
and retentate produced titers of zero, indicating total
inhibition of adherence. Since the cocktail was dialyzed
against GDW, which itself does not inhibit adhesion, it can
be assumed that the antiadherence factor(s) passed freely
through the membrane. This would indicate that the anti-
adherence factor(s) has a molecular weight of 1000 or less.
Similar results were obtained for the juice extracted from
the berries. In six trials involving the extracted juice,
both the dialysate and retentate yielded zero titers
indicating that the factor(s) freely moved through the
membrane. It should be noted that the usual dialysis
procedure was not employed in these experiments. The
cranberry juice and cocktail were dialyzed against 50 ml of
GDW – a volume twice that contained within the dialysis bag
– instead of the standard 3.0 liters.

Since both the dialysate and retentate possessed
activity, an additional dialysis procedure was conducted to
determine if antiadherence activity could again be observed
in both fractions. The titers for both the dialysate and
retentate increased slightly due to a dilutional effect.

The dilutional effect was demonstrated by diluting the dialysate and retentate proportionally to its doubly-dialyzed counterpart. This resulted in titers equivalent to the dialysate and retentate which underwent double dialysis.

Finally, the dialysates and retentates from the single dialysis procedure were subjected to freezing $(-15^{O}C)$ and refrigeration $(4^{O}C)$. As noted previously, both dialysates and retentates yielded titers of zero. The frozen dialysate scored a 16 and the refrigerated sample a 128. The retentate titers also increased to 2 for the frozen sample and 8 for the refrigerated aliquot. Thus, both the dialysate and retentate lose activity in response to a decrease in temperature.

Coppola et al. (1978) has demonstrated that the cranberry juice contains at least three major organic acids - citric, malic, and quinic acid. These three acids are water soluble and less than 1000 MW which fit the criteria for the antiadherence factor described above, and thus were tested for antiadherence activity. The activity was first determined for each organic acid at selected concentrations. Since the pH of the individual acids is much lower than the pH of urine, antiadherence activity was first tested using GDW within the range of pH exhibited by the three acids. No effect on adherence was observed. All three organic acids at concentrations ranging from 0.001%

to 0.1% were able to at least partially inhibit bacterial adherence. As the concentration was raised to approximately 0.5%, bacterial adherence to the yeast cells was completely eliminated. Mentioned above was the fact that the organic acid pH's were lower than normal urine pH, so the acids were retested at pH's of 5.0 and 7.0. According to Ames (1982), the normal urine pH ranges from 4.5 to 8.0 with a mean value of 6.0. At the higher pH's of 5.0 and 7.0, the antiadherence activity of all the organic acids was reduced from a zero titer to titers ranging from 256 to 1024. However, when decreased to a pH of 2.60, full activity returned. The E. coli control titers in these trials were 512 and 1024 at a pH of 6.0.

The pH of Ocean Spray cranberry juice cocktail was altered to determine if it possessed antiadherence activity within the pH range of normal urine. As with the individual acids, the increase in pH reduces the anti-adherence properties of the cranberry juice cocktail, but to a lesser extent. The Ocean Spray cranberry juice cock-tail possesses titers of 64 and 256 within the range of urine pH, four to six times below the control titer of 2048. Thus, it appears that the individual organic acids and the Ocean Spray cranberry juice cocktail are most effective in suppressing adherence at pH's below that of normal urine. Even though increasing the pH of the juice and the acids decreased their effectiveness at preventing

bacterial adhesion, antiadherence activity was still observed at pH's of normal urine.

It is well established that citric and malic acids, along with other intermediate organic substrates of the citric acid cycle, have been identified in human urine (Stryer, 1981 and Gamble, W. et al., 1961). Also, the kidney tubules appear to have the ability to synthesize malate from citric acid cycle precursors and secreting it into the tubular lumen (Gamble, W. et al., 1961). Quinic acid, not a component of the citric acid cycle, is aromatized by the gut flora in humans (Adamson, 1970) and excreted in the urine as hippuric acid (Adamson, 1969).

It is important from the medical standpoint to draw parallels to the effects that ingestion of cranberry juice may have on the inhibition of bacterial adherence ~~in vivo~~. The present investigation has demonstrated that each of the organic acids ‾ citric, malic and quinic ‾ possess the ability to inhibit bacterial adhesion in vitro. They are maximally active at PH's below that of normal urine, yet their combination in the cranberry juice cocktail does have antiadherence activity within the pH of normal urine, It is hypothesized that an effect may be present between the acids themselves, possibly accentuated by a factor(s), as yet unidentified, within the cranberry juice. It remains to be determined if oral ingestion of Ocean Spray cranberry juice cocktail does increase the urinary excretion of these

organic acids and, if so, does this increase correlate with the production of a bacteriostatic or bacteriocidal urine. To make this determination, a sensitive quantative analysis technique, as high performance liquid chromatography (HPLC), could be performed on urine samples before and after ingestion of cranberry juice cocktail. This type of analysis may also reveal other substances which may be contributing to the antiadherence properties of cranberry juice. It is hoped that further research may answer the questions surrounding the mechanism of action of cranberry juice and possibly lead to a new form of prophylaxis and/or treatment for urinary tract infections.

APPENDIX A

<u>Tables</u>

TABLE 1

BACTERIAL ADHERENCE TEST RESULTS OF FRACTIONS COLLECTED
FROM AMBERLITE ION EXCHANGE CHROMATOGRAPHY
OF OCEAN SPRAY CRANBERRY JUICE COCKTAIL

FRACTION NUMBER[a]	TITER
E. coli control	2048
7	128
12	64
17	64
22	256
27	1024
32	1024
37	1024
42	2048
47	2048
52	2048
57	2048

[a]Fraction Number = # milliliters eluted to that point

TABLE 2

BACTERIAL ADHERENCE TEST RESULTS OF THE DIALYSATE
AND RETENTATE OF OCEAN SPRAY CRANBERRY JUICE
COCKTAIL PLACED IN 1000 MWCO SPECTRAPORE MEMBRANE

TRIAL NUMBER	DIALYSATE TITER	RETENTATE TITER
1	O	O
2	O	O
3	O	O

E. coli control titer = 1024

TABLE 3

BACTERIAL ADHERENCE TEST RESULTS OF THE DIALYSATE
AND RETENTATE OF CRANBERRY JUICE (EXTRACTED)
PLACED IN 1000 MWCO SPECTRAPORE MEMBRANE

TRIAL NUMBER	DIALYSATE TITER	RETENTATE TITER
1	O	O
2	O	O
3	O	O
4	O	O
5	O	O
6	O	O

E. coli control titer = 2048

TABLE 4

RESULTS OF 0.1% CITRIC ACID ANTIADHERENCE ACTIVITY

TRIAL NUMBER	pH	FINAL CITRIC ACID CONCENTRATON	TITER
E. coli control	6.0		1024
GDW control	2.60+0.05		256
1	2.60+0.05	0.02%	0
2	2.60+0.05	0.02%	0
3	2.60+0.05	0.02%	0
4	2.60±0.05	0.02%	0

TABLE 5

RESULTS OF 0.01% CITRIC ACID ANTIADHERENCE ACTIVITY

TRIAL NUMBER	pH	FINAL CITRIC ACID CONCENTRATION	TITER
E. coli control	6.0		1024
GDW control	3.04+0.05		256
1	3.00+0.05	0.002%	256
2	3.00+0.05	0.002%	256
3	3.00+0.05	0.002%	256
4	3.00±0.05	0.002%	256

TABLE 6

RESULTS OF 0.001% CITRIC ACID ANTIADHERENCE ACITIVITY

TRIAL NUMBER	pH	FINAL CITRIC ACID CONCENTRATION	TITER
E. coli control	6.0		1024
GDW control	3.88+0.05		512
1	3.85+0.05	0.0002%	512
2	3.85+0.05	0.0002%	512
3	3.85+0.05	0.0002%	512
4	3.85±0.05	0.0002%	512

TABLE 7

RESULTS OF 5% MALIC ACID ANTIADHERENCE ACTIVITY

TRIAL NUMBER	pH	FINAL MALIC ACID CONCENTRATION	TITER
E. coli control	6.0		1024
GDW control	1.90±0.05		256
1	1.86+0.05	1%	0
2	1.86+0.05	1%	0
3	1.86+0.05	1%	0

TABLE 8

RESULTS OF 1.25% MALIC ACID ANTIADHERENCE ACTIVITY

TRIAL NUMBER	pH	FINAL MALIC ACID CONCENTRATION	TITER
E. coli control	6.0		1024
GDW control	2.13+0.05		512
1	2.13+0.05	0.25%	0
2	2.13+0.05	0.25%	0
3	2.13±0.05	0.25%	0

TABLE 9

RESULTS OF 0.31% MALIC ACID ANTIADHERENCE ACTIVITY

TRIAL NUMBER	pH	FINAL MALIC ACID CONCENTRATION	TITER
E. coli control	6.0		1024
GDW control	2.40+0.05		512
1	2.42+0.05	0.06%	0
2	2.42+0.05	0.06%	0
3	2.36+0.05	0.06%	0

TABLE 10

RESULTS OF 0.5%, 0.05%, and 0.005%
MALIC ACID ANTIADHERENCE ACTIVITY

TRIAL NUMBER	pH	FINAL MALIC ACID CONCENTRATION	TITER
E. coli control	6.0		1024
GDW control	2.28±0.05		512
GDW control	2.75±0.05		512
GDW control	3.33±0.05		512
1	2.27±0.05	0.1%	0
2	2.76±0.05	0.01%	16
3	3.35±0.05	0.001%	256

TABLE 11

RESULTS OF VARIOUS QUINIC ACID CONCENTRATIONS
ON ANTIADHERENCE ACTIVITY

TRIAL NUMBER	pH	FINAL QUINIC ACID CONCENTRATION	TITER
<u>E. coli</u> control	*6.0*		512
GDW control	2.00±0.05 thru 3.00±0.05		512
1	2.00±0.05	1%	0
2	2.15±0.05	0.5%	0
3	2.30±0.05	0.25%	0
4	2.46±0.05	0.125%	0
5	2.48±0.05	0.1%	0
6	2.63±0.05	0.06%	0
7	2.69±0.05	0.01%	0
8	2.78±0.05	0.001%	128

TABLE 12

RESULTS OF VARYING pH OF 0.5% CITRIC ACID[a]
ON ANTIADHERENCE ACTIVITY

	INITIAL pH=2.30±0.05	NEW pH=5.00±0.05	FINAL pH=2.60±0.05
Trial 1 Titer	0	512	0
Trial 2 Titer	0	512	0
Trial 3 Titer	0	512	0
	INITIAL pH=2.30±0.05	NEW pH=7.00±0.05	FINAL pH=2.60±0.05
Trial 1 Titer	0	1024	0
Trial 2 Titer	0	1024	0
Trial 3 Titer	0	1024	0

E.coli control titer = 1024, pH = 6.0.

[a]Final citric acid concentration = 0.1%.

TABLE 13

RESULTS OF VARYING pH OF 0.5% MALIC ACID [a]
ON ANTIADHERENCE ACTIVITY

	INITIAL pH=2.31±0.05	NEW pH=5.00±0.05	FINAL pH=2.60±0.05
Trial 1 Titer	0	256	0
Trial 2 Titer	0	256	0
Trial 3 Titer	0	256	0

	INITIAL pH=2.31±0.05	NEW pH=7.00±0.05	FINAL pH=2.60±0.05
Trial 1 Titer	0	512	0
Trial 2 Titer	0	512	0
Trial 3 Titer	0	512	0

E. coli control titer = 512, pH = 6.0

[a] Final malic acid concentration = 0.1%.

TABLE 14

RESULTS OF VARYING pH OF 0.5% QUINIC ACID[a]
ON ANTIADHERENCE ACTIVITY

	INITIAL pH=2.45±0.05	NEW pH=5.00±0.05	FINAL pH=2.60±0.05
Trial 1 Titer	0	512	0
Trial 2 Titer	0	512	0
Trial 3 Titer	0	512	0
	INITIAL pH=2.45±0.05	NEW pH=7.00±0.05	FINAL pH=2.60±0.05
Trial 1 Titer	0	512	0
Trial 2 Titer	0	512	0
Trial 3 Titer	0	512	0

E. coli control titer = 1024, pH = 6.0

[a]Final quinic acid concentration = 0.1%.

TABLE 15

RESULTS OF VARYING pH OF OCEANSPRAY CRANBERRY JUICE COCKTAIL ON ANTIADHERENCE ACTIVITY

pH	TITER
2.45 ± 0.05	0
3.00 ± 0.05	0
3.10 ± 0.05	16
3.20 ± 0.05	32
3.50 ± 0.05	64
4.00 ± 0.05	64
5.00 ± 0.05	64
6.00 ± 0.05	256

E. coli control titer = 2048, pH = 6.0

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