# MICROFLORA CHANGES IN MISTED AND NONMISTED BROCCOLI AT REFRIGERATED STORAGE TEMPERATURES

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## ABTRACT

*Plate counts of three groups of microorganisms [aerobic plate count (APC), coliforms (C), and yeast and mold (Y/M)] were determined in misted (M), nonmisted in the same cold room (SNM), and nonmisted in a different cold room (DNM) broccoli stored at 4 ± 1C for five days. Relative humidity of the cold rooms, moisture content of the broccoli, and microbial quality of the misting water were also determined. Two-way ANOVA indicated significant differences ($p \leq 0.05$) between M, SNM and DNM treatments. Misting was found to minimize the increase in plate counts of the three microorganism groups monitored. Higher relative humidity in the misted samples compared with the nonmisted samples, the washing effect of the misting water and possible residual chlorine effects due to the use of chlorinated tap water may explain the reduced viable counts observed in the misted samples.*

## INTRODUCTION

Fresh vegetables have increased in popularity in recent years, partly because of the increase in consumer demand for foods perceived to be more nutritious than their processed counterparts. Vegetables are an important source

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of vitamins in the human diet, especially vitamin C. The quality of fruits and vegetables are affected by field and harvested conditions. Microorganisms are introduced to the vegetables by the soil, water, air, as well as machinery and humans involved in the harvesting operation. The most common vegetable spoilage due to microorganisms is caused by the saprophytic fungi, yeast and bacteria. Microorganisms, from the sources listed above, enter the plant tissue through the stomata or openings due to wounds on the plant, such as cuts and bruises.

Temperature, relative humidity, and composition of the atmosphere influence the postharvest quality of fruits and vegetables. These factors also influence the growth of microorganisms present on the fruits and vegetables. When conditions are favorable, microbes will grow and cause spoilage of the produce. Thus, it is very important to control the microbial population in fresh produce during storage. Refrigeration, modified atmosphere storage and packaging, and irradiation have been used to control microbial populations in fresh produce during storage (Cooper and Salunkhe 1963; Deak *et al.* 1987; Zagory and Kader 1988; Kader *et al.* 1989; James and Bailey 1990).

To date, no microbial study has been conducted on the use of washing treatments during storage of fresh produce. In most studies washing was used to clean fruits, vegetables or meat prior to, but not during, storage. Some of the washing agents used were water (Anderson *et al.* 1977), organic acids such as lactic acid (Smulders and Woolthuis 1985) and acetic acid (Anderson *et al.* 1988), or chlorinated water. In some methods, the water was recycled. The use of water as a washing agent to reduce microbial contamination has been practiced extensively for beef (Allen *et al.* 1987; Crouse *et al.* 1988), pork (Reynolds and Carpenter 1974), poultry (Lillard *et al.* 1984) and also for potatoes (Bartz and Kelman 1984a,b). For example, Crouse *et al.* (1988) reported that automated water sprayed on meat surfaces reduced aerobic plate counts by 0.87 $\log_{10}$ cfu/200cm$^2$ and coliform counts by 1.5 $\log_{10}$ cfu/200cm$^2$. In addition to the washing effects, water may also function as a humidifying agent and thus help maintain the initial moisture content of the product.

Automatic misting is a type of humidification technology developed to prevent dehydration, extend shelf-life and promote better appearance of fresh produce in retail display cases. During misting, water in the form of a mist is sprayed on the produce at a constant time interval. Misting is considered a humidification treatment even though some washing may occur during misting. In addition, depending on the source of the misting water, endogenous or added water treatment chemicals may also affect the quality attributes of the misted produce. Barth *et al.* (1990, 1992) found that misting was significantly effective in retaining color, texture, and ascorbic acid content of broccoli. The effect of misting on microflora changes in fresh produce, however have thus far not been reported.

The objective of this work was to determine the effect of misting on microflora changes in broccoli stored at 4 ± 1C for five days. In addition, data were collected to determine the effect of prewashing on the initial microbial load of the broccoli. Relative humidity in the cold rooms and moisture content of the broccoli were also determined.

## MATERIALS AND METHODS

### Broccoli

Broccoli was obtained approximately three days postharvest from a local wholesale distributor located in Urbana, IL. The broccoli was harvested in Salinas, CA, hydrocooled and packed in ice for transportation.

### Experimental Design

Upon delivery, the broccoli bunches were sorted for integrity, compactness, color, and freedom from defects. Minor defects were corrected by trimming the bunches with a sharp knife. The broccoli was washed under cold running tap water and randomly distributed into three groups: misted (M), nonmisted stored in the same cold room as the misted (SNM), and nonmisted stored in a different cold room (DNM). Cold rooms were used to minimize evaporative cooling effects of misting observed in a previous study using retail display cases (Barth *et al.* 1992). Two nonmisted control treatments were used to help assess humidity versus washing affects on the growth of microorganisms on the broccoli samples. For each treatment, the broccoli bunches were placed on a 45-degree slope wire rack supported by a metal frame. The wire rack was covered with rubber netting (Sure Grip Liner, Hubert Company, Harrison, OH) and had a 4500 $cm^2$ surface area. A Plexiglas tray was placed underneath the frame to collect the misting water. The cold rooms and equipment were sanitized prior to use with a concentrated water-Lysol (L&F Products, Montvale, NJ) solution or 500 ppm XY-12 Liquid sanitizer (Klenzade, St. Paul, MN) with 8.4% sodium hypochlorite as the active ingredient.

An automatic PVC misting system with a snap-fit design manufactured by the Corrigan Corporation (Northbrook, IL) was installed in an ordinary cold storage room which was equipped with temperature and humidity control and fluorescent 40 watt cool-white lights (average 50 $\mu$mole $s^{-1}$ $m^{-2}$ PPF at produce level). Fluorescent lights were used because they are commonly used in retail

stores. Lights were kept on during the entire experimental period, in both cold rooms. The source of the misting water was tap water, which contained 2.5 mg/l standard end point residual chlorine (Northern Illinois Water Company, Champaign, IL). Misting intervals were 4 s every 4 min, providing a total of 20 $\pm$ 0.3 ml water each interval. Temperature of the cold rooms and the misting water was 4 $\pm$ 1.0C. The water that drained from the system (referred to as rinsate) was collected for microbial analysis or discarded. A prefilter was fixed to the water supply system to trap any large particles and a microfilter (Versaflow capsule, 0.45 $\mu$m pore size, Gelman Sciences Inc., Ann Arbor, MI) was placed in the misting line to filter any small particles. A piece of plastic sheath was hung to separate the misted (M) from nonmisted (SNM) treatments. The plastic sheath was cut into strips to provide air flow and prevent condensation.

The misting experiment was done in duplicate.

## Sampling Procedure and Microbiological Analysis

At each time interval, 0, 1, 2, 3, 4 and 5 days, duplicate samples of broccoli were taken from the misted (M), nonmisted stored in the same cold room as the misted (SNM), and nonmisted stored in a different cold room (DNM) experimental conditions. For each sample, 25 g of broccoli were placed into a sterile stomacher bag. The stomacher bags were put into a container containing crushed ice and held for not more than 1 h until microbiological analysis. At the time of analysis, approximately half the volume of 225 ml of 0.1% peptone water was added to the sterile stomacher bag, after which the contents were homogenized for 1 min with a stomacher (Dynatech Laboratories, Alexandria, VA). The remainder of the peptone water was then added to the homogenate and mixed by shaking the bag. The homogenate was placed in a container of crushed ice for 10 min to allow the plant fibers to settle. Serial dilutions were made from the homogenate. From three appropriate dilutions 1 ml each was pour-plated according to the International Commission of Microbiological Specification for Food method (ICMSF 1978). Media of Plate Count agar (PCA), Violet Red Bile agar (VRBA) and acidified Potato Dextrose agar (PDA) (Difco Laboratories, Detroit, MI) were used respectively for enumeration of aerobic plate count, coliforms, and yeast and mold. Plates were incubated at room temperature (about 25C) for two days for PCA and VRBA and three days for PDA. Viable counts were determined by counting the number of colonies formed and reported as $\log_{10}$ of colony forming units per gram ($\log_{10}$ cfu/g). Each microbial analysis was performed in duplicate at each dilution.

Since the initial viable counts of the broccoli used in experimental trials one and two were different, viable count values were normalized by dividing by the

initial viable count for each microorganism group (aerobic plate count, coliforms, and yeast and mold) and for each experimental trial:

$$\text{Normalized Value (\%)} = \frac{\text{viable count at a particular time}}{\text{initial viable count}} \times 100$$

## Effect of Prewashing on Initial Microbial Load

Unwashed fresh broccoli and broccoli (25 g) washed under running cold tap water were weighed, homogenized, and pour-plated using Plate Count Agar as the growth media. Plate counts were conducted as previously described.

## Water Quality and Rinsate Microbial Load

At the start of the experiment, duplicate samples of water from the misting nozzles were taken for microbiological analysis. Each day of the experiment, duplicate rinsate samples were taken for microbiological analysis. Samples (1 ml of the original, without dilution, and 1/10 and 1/100 diluted) were pour-plated using Plate Count Agar as the growth media. Plate counts were conducted as described previously.

## Determination of Relative Humidity

The percent relative humidity (RH) of each cold room was measured each day for the five day storage period. Wet bulb and dry bulb values of both cold rooms were recorded using a psychrometer (Psychrodyne, Industrial Instruments and Suppliers, Southampton, PA) and the RH values were obtained using a psychrometric chart.

## Determination of Moisture Content

Broccoli florets with approximately 1 cm stems were cut from the spears, wiped with an adsorbent paper towel and then ground into small pieces. The ground broccoli was weighed and triplicate moisture contents were determined for the misted (M), nonmisted SNM and DNM broccoli samples (AOAC 1980). Samples were placed in a drying oven overnight at 55C and then transferred to

a vacuum oven (Equatherm, Curtin Matheson Scientific Inc., Houston, TX) for no less than 48 h at 60C, under 76.2 cm Hg until constant weights were attained. Samples were cooled in a desiccator for 30 min before weighing. Percent moisture content was determined on a wet weight basis (g water/100 g sample).

### Statistical Analysis

Statistical significance was assessed for the three different viable counts (aerobic plate count, coliforms, and yeast and mold) using a two-way ANOVA at the 95% confidence level. The sources of variations were time (0, 1, 2, 3, 4 and 5 days) and treatment condition (misted M, nonmisted SNM, and nonmisted DNM). A 95% confidence interval for the difference between means was developed for each microbiological analysis to determine the location of the significance differences obtained. Statistical significance was also assessed by ANOVA at the 95% confidence level for the effect of prewashing on initial microbial load, relative humidity difference in the cold rooms and moisture content of the broccoli under the three treatment conditions.

## RESULTS AND DISCUSSION

### Misting Experiment

To ensure the tap water used for misting was free from microbial contamination, microflora counts (APC) of the water from the misting nozzles was determined. No viable counts were detected.

### Aerobic Plate Count (APC)

The initial APC values for the two trials were 5.62 and 5.23 $\log_{10}$ cfu/g. Over the five day period, all treatments showed an increase in APC values (Fig. 1). APC values were found to be lowest in the M samples and highest in the SNM samples at all time periods studied. Two-way ANOVA indicated significant difference ($p \leq 0.05$) in APC values between M, SNM, and DNM treatments. The 95% confidence interval for the difference between means, calculated for the normalized APC values, was $\pm$ 6.93% $\log_{10}$ cfu/g. Significant differences were found at days four and five between M vs. SNM treatments.

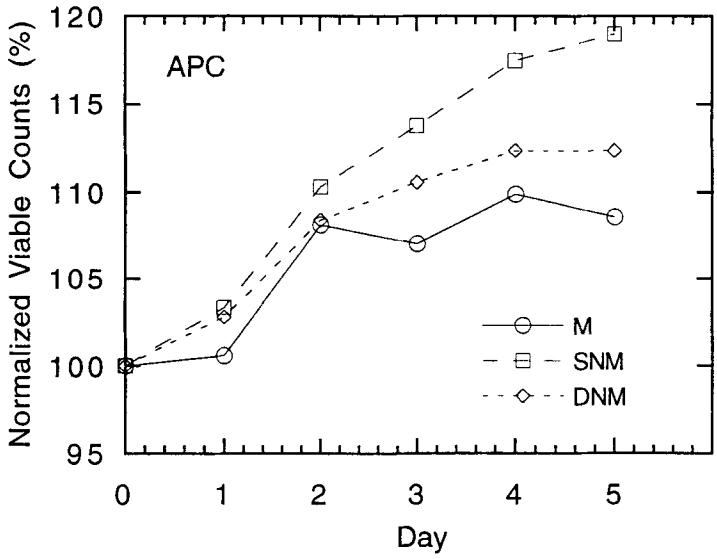


FIG. 1. NORMALIZED APC VALUES FOR BROCCOLI STORED FIVE DAYS AT 4 ± 1C UNDER THREE EXPERIMENTAL TREATMENT CONDITIONS, MISTED (M), NONMISTED STORED IN THE SAME COLD ROOM AS THE MISTED (SNM), AND NONMISTED STORED IN A DIFFERENT COLD ROOM (DNM)

## Coliforms

The initial coliforms counts from the two trials were 5.20, and 4.37 $\log_{10}$ cfu/g. No specific growth trends were exhibited by the coliforms in M, SNM, and DNM treatments, although in most cases, coliforms counts were highest in the SNM samples and lowest in the M samples (Fig. 2) . Two-way ANOVA indicated significant difference ($p \leq 0.05$) between the three treatments. The 95% confidence interval for the difference between means, calculated for the normalized coliform values, was ± 8.30% $\log_{10}$ cfu/g. Significant differences ($p \leq 0.05$) were found at days two, three and five between M vs. SNM treatments and at days four and five between M vs. DNM treatments.

## Yeast and Mold

Initial yeast and mold counts from the two trials were 3.52, and 3.60 $\log_{10}$ cfu/g. The counts in the DNM samples were highest at days one and three but

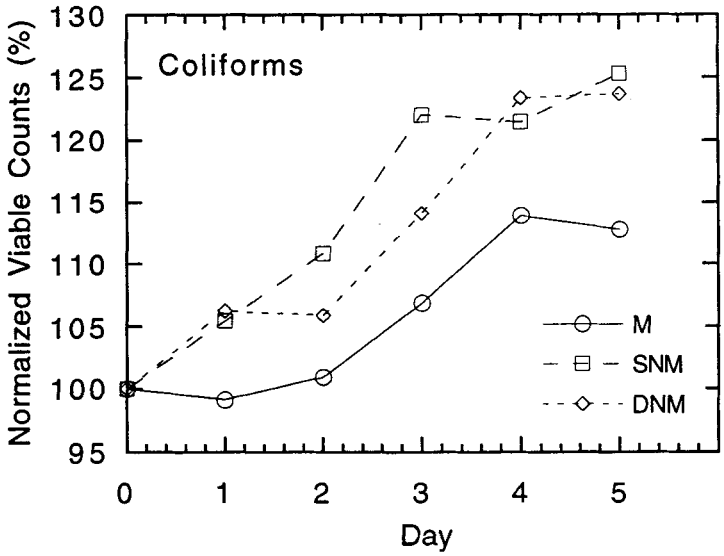


FIG. 2. NORMALIZED COLIFORM VALUES FOR BROCCOLI STORED FIVE DAYS AT 4 ± 1C UNDER THREE EXPERIMENTAL TREATMENT CONDITIONS, MISTED (M), NONMISTED STORED IN THE SAME COLD ROOM AS THE MISTED (SNM), AND NONMISTED STORED IN A DIFFERENT COLD ROOM (DNM)

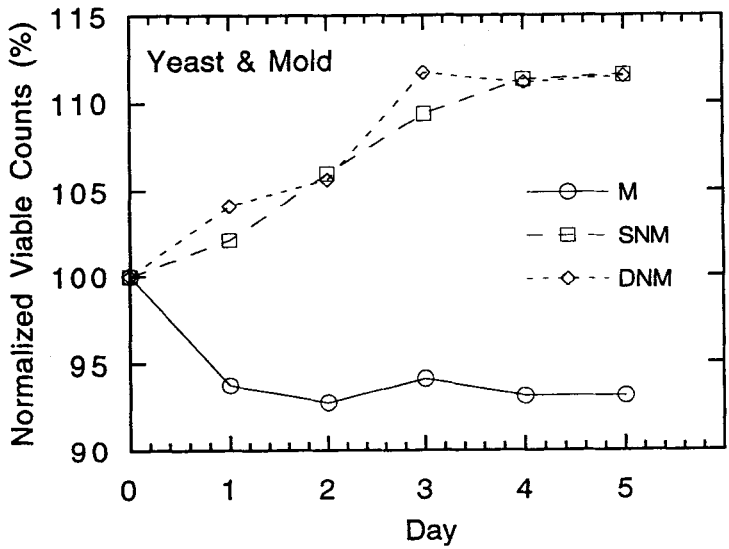


FIG. 3. NORMALIZED YEAST AND MOLD VALUES FOR BROCCOLI STORED FIVE DAYS AT 4 ± 1C UNDER THREE EXPERIMENTAL TREATMENT CONDITIONS, MISTED (M), NONMISTED STORED IN THE SAME COLD ROOM AS THE MISTED (SNM), AND NONMISTED STORED IN A DIFFERENT COLD ROOM (DNM)

the counts were nearly the same as the SNM samples at other days. Yeast and mold counts were consistently lowest in the M samples (Fig. 3). In the case of misting, on all five days mold and yeast counts were lower than ($> 100\%$) day zero counts. Two-way ANOVA indicated a significant difference ($p \leq 0.05$) between the three treatments. The 95% confidence interval for the difference between the means, calculated for the normalized yeast and mold values, was $\pm$ 8.65% $\log_{10}$ cfu/g. Significant difference between M vs. SNM treatments were found at days two, three, four, and five. Significant differences were observed between M and DNM treatments from days one through five.

## Effect of Prewashing on Initial Microbial Load

Viable counts of the three types of microorganism studied were found to be reduced by approximately one log cycle after washing the fresh broccoli with tap water (Table 1). There were significant differences ($p \leq 0.05$) in viable counts between broccoli before and after washing. Since the wash water used was tap water which contained approximately 2.5 mg/L residual chlorine some of the reduction in counts may be due to the effect of the chlorine.

TABLE 1.
EFFECT OF PREWASHING ON INITIAL MICROBIAL LOAD OF FRESH BROCCOLI

| Microorganism | Plate Count ($\log_{10}$ cfu/g)* | |
|---|---|---|
| | Before washing | After washing |
| Aerobic plate count | 6.15 $\pm$ 0.02[a] | 5.63 $\pm$ 0.05[b] |
| Coliforms | 6.22 $\pm$ 0.05[a] | 5.49 $\pm$ 0.06[b] |
| Yeast and mold | 3.60 $\pm$ 0.06[a] | 2.92 $\pm$ 0.07[b] |

*Values represent mean $\pm$ standard deviation. Different lower case letter superscripts between columns within a row indicate a significant difference at the 95% confidence level.

Prewashing is very important in removing soils and microorganisms which contaminated the produce during harvesting and handling. If not removed, the produce will not only look unattractive, but may also be more susceptible to spoilage problems during storage.

## Washing Effect of Misting

After three hours of misting (day zero), the microbial count of the rinsate was 3.48 + 0.01 $\log_{10}$ cfu/g. This value dropped after the first day of misting and remained relatively constant for the remainder of the study, at an average value of 2.87 $\pm$ 0.05 $\log_{10}$ cfu/g (Table 2). The presence of microorganisms in the rinsate suggests that washing occurred during misting, where the water from the misting system washed off microorganisms on the broccoli into the rinse water. Anderson *et al.* (1977) reported that washing reduced the bacterial counts on meat surfaces. However, significant difference were only found when the largest volume of water was used (i.e., 25.4 L per min). The rate of microorganisms being washed off from a surface was found to be affected by the nature of the ionic environment (Appl and Marshall 1984). KCl was observed to removed the highest average number of *Pseudomonas fluorescens* from meat surfaces compared to $NH_4Cl$ and $MgCl_2$. Compared to water, 0.1M KCl rinsed three times as many bacteria from the surface of the inoculated meat. As mentioned previously, some of the reduction in counts observed for the misting treatment may also be due to the effect of the residual chlorine in the tap water used for misting.

TABLE 2.
CHANGES IN MICROBIAL COUNT IN RINSATE DURING MISTING OF BROCCOLI AT 4C FOR FIVE DAYS

| Misting Period (Day) | Aerobic Plate Count ($\log_{10}$ cfu/g) |
|---|---|
| 0 | 3.48 $\pm$ 0.01 |
| 1 | 2.98 $\pm$ 0.05 |
| 2 | 2.76 $\pm$ 0.03 |
| 3 | 2.82 $\pm$ 0.06 |
| 4 | 2.97 $\pm$ 0.07 |
| 5 | 2.81 $\pm$ 0.03 |

## Relative Humidity

Despite using an identical relative humidity set point of 90% RH at the start of the study, the relative humidity (RH) was significantly higher ($p \leq 0.05$) in the cold room which housed the misting system than in the cold room

without the misting system throughout the five day storage period (Table 3). The RH for the cold room with misting continually increased over the five day storage period due to the effects of misting. Whereas, the cold room without misting continuously decreased over the same time period due to removal of humidity by condensation on the cooling system heat exchanger. The RH of the atmosphere outside the cold room was on the average 54.0%.

TABLE 3.
CHANGES IN THE RELATIVE HUMIDITY IN COLD ROOMS WITH AND WITHOUT MISTING DURING STORAGE OF BROCCOLI AT 4C FOR FIVE DAYS

| Storage (Day) | Relative Humidity (%)* | |
|---|---|---|
| | Cold Room with misting | Cold Room without misting |
| 0 | 90.0 ± 1.41[a] | 87.0 ± 1.41[b] |
| 1 | 91.0 ± 1.41[a] | 85.2 ± 0.35[b] |
| 2 | 94.5 ± 0.71[a] | 83.5 ± 0.71[b] |
| 3 | 95.8 ± 0.35[a] | 83.0 ± 1.41[a] |
| 4 | 96.3 ± 1.06[a] | 77.3 ± 1.06[a] |
| 5 | 95.3 ± 1.06[a] | 77.0 ± 0.71[a] |

*Values represent mean ± standard deviation. Different lower case letter superscripts between columns within a row indicate a significant difference at the 95% confidence level.

Several studies have reported that high moisture and/or RH will increase the growth rate and thus increase the viable count of the particular organism studied (Scott 1957; Cook and Papendick 1978; Troller 1980). In this study, microbial growth rates under the three conditions (M, SNM, DNM) varied depending on the microorganism type. In the case of the APC, the SNM values were larger than the M and DNM treatments. This indicates that the higher RH may have resulted in higher APC values (SNM compared to DNM), but that misting, through a washing effect, resulted in the lowest APC values, even at the same RH as the SNM treatment condition. In the case of coliforms and yeast and mold, the SNM and DNM treatments were both greater at all time periods than the M treatment. The difference in the RH (SNM vs. DNM) did not seem to cause a large difference in the growth rates of these microorganisms, but misting, again through a washing effect, had the lowest microbial activity.

The low microbial count in fresh produce stored in a high RH environment has been explained by a few workers. Cook and Dunniway (1980) suggested that some microbes, especially fungus with thick walls, are generally resistant to desiccation. Lewis *et al.* (1981) observed that the treatment of carrot roots at 25C or 15C in a humidity approaching saturation accelerated wound repair and thus diminished subsequent infection by *Mycocentrospora acerina*. The same observation was found by Wigginton (1974) who noted that the healing of wounds in potato tubers was most rapid at 10C and above 80% RH. He also noted that at lower RH (about 52%), the rate of drying of the surface cells was great enough to inhibit suberization and periderm formation, which are important in wound healing.

Thorne (1972) found that invasion of stored carrots at several temperatures, air-flows, humidities, and time periods by *Rhizopus stolonifer* only took place when the carrots had lost greater than a critical percentage of their weight (8%). Effect of RH on the production of an extracellular pectolytic enzyme was studied by Van den Berg and Yang (1969). They found that the fungi *B. cinerea* and *S. sclerotium* produced significantly more extracellular pectolytic enzyme when the carrots were exposed to 94.96% RH than when exposed to 98.10% RH.

TABLE 4.
CHANGES IN MOISTURE CONTENT IN MISTED AND NONMISTED BROCCOLI STORED AT 4C FOR FIVE DAYS

| Storage (Day) | Moisture Content (% wet wt*) | | |
|---|---|---|---|
| | M | SNM | DNM |
| 0** | | 88.6 ± 0.76 | |
| 2 | 88.4 ± 0.16[a] | 87.4 ± 0.46[b] | 86.3 ± 0.21[c] |
| 4 | 88.3 ± 0.16[a] | 87.4 ± 0.05[b] | 85.6 ± 0.16[c] |

*Values represent mean ± standard deviation. Different lower case letter superscripts between columns within a row indicate a significant difference at the 95% confidence level.
**Zero day moisture content value was the same for all three treatment conditions.

## Moisture Content

The mean initial moisture content of the fresh broccoli was 88.6% ± 0.76. Throughout the storage period, the moisture content of the misted (M) broccoli remained close to the initial value, only decreasing by 0.3% (wb). The SNM

treatment showed a slight decrease in moisture content while the nonmisted DNM showed a pronounced decrease in moisture content value (Table 4). A significant difference ($p \leq 0.05$) between all three treatment conditions was observed.

Moisture content decreased in the nonmisted DNM samples due to dehydration caused by the low RH in the cold room without misting. On the other hand, the nonmisted SNM had a higher moisture content than the DNM because the SNM sample was located in the cold room with the misting unit. However, the SNM had a lower moisture content than misted M because the SNM did not receive the direct misting treatment.

## CONCLUSION

This work showed that misting of broccoli stored at refrigerated temperatures reduced aerobic plate counts and coliform counts and reduced yeast and mold counts compared to nonmisted controls. Prewashing of broccoli was found to be important to reduce the initial microbial load before storage. A washing effect was shown to take place during misting. This washing effect may have been enhanced by the use of chlorine containing tap water as the misting water source. Misting was also found to affect the RH of the cold room and resulted in relatively constant moisture content values in the broccoli. Further work should be conducted to study the effects of contamination of the misting water on the microflora changes in misted produce. It is also important to identify the type of microbes present on the produce being misted compared with nonmisted produce. Another area which needs to be studied is the significance of misting water purification treatments (i.e., pH, ionization/distillation, ozonation or chlorination treatment) on microflora changes.

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