

# **INACTIVATION OF BACTERIA, YEASTS AND MOLDS ON PALLETIZED Highbush BLUEBERRIES USING CHLORINE DIOXIDE SACHETS**

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# Picture of moldy pint of blueberries



# INTRODUCTION

- **Blueberry marketing chain improvements needed:**
  - **Quality - improved microbial standards for bacteria, yeasts and molds**
  - **Safety - low or “zero” tolerance for human pathogens**
    - **Suspected listeriosis outbreak - Connecticut 1984**
    - **Hepatitis A outbreak – New Zealand 2003**
    - **Food safety is a legitimate concern**

## Number of processed blueberry samples from 2003-2004 survey (Popa et al. 2005 IAFP P3-09 ) meeting microbial limits (CFU/g) set by purchasers (n = 18)

Test	Company A	Company B	Company C	Company D
Bacteria	150,000 (14/18)	50,000 (12/18)	50,000 (12/18)	10,000 (9/18)
Yeast	20,000 (13/18)	10,000 (13/18)	5,000 (6/18)	1,000 (3/18)
Mold	5,000 (8/18)	10,000 (8/18)	5,000 (10/18)	1,000 (3/18)
Coliforms	100 (13/18)	100 (13/18)	<1,000 (18/18)	10 (3/18)
<i>E.coli</i>	Absent (10/18)	<10 (11/18)	Absent (10/18)	Absent (3/18)
Overall passed	(5/18)	(4/18)	(4/18)	(0/18)



# INTRODUCTION

- **Current sanitizer treatments (sodium hypochlorite 20-200 ppm) inadequately reduce microbial populations on blueberries during processing**
- **New microbial reduction strategies for blueberries are needed**



# **OBJECTIVE**

- **Assess the efficacy of chlorine dioxide gas sachets for decreasing microbial levels on blueberries**

A photograph of a blueberry bush with several clusters of ripe, blue berries. The leaves are green and glossy. The background is a blurred outdoor setting with a wooden fence and a green lawn.

# **MATERIALS AND METHODS**

# EXPERIMENTAL DESIGN

- **Pilot study: buckets containing 500 g of fruit**
- **Palletized fruit: pallets containing 273 kg (600 lb) of fruit**



# BACTERIAL PATHOGEN COCKTAILS

- ***Escherichia coli* O157:H7 - AR,  
AD 305, AD 317**
- ***Listeria monocytogenes* - CWD 95,  
CWD 102, CWD 184**
- ***Salmonella* -**
  - ***S. Typhimurium* H3380**
  - ***S. Heidelberg* F5038 BG1**
  - ***S. Enteritidis* H3502**

# SPOILAGE MICROORGANISMS

## ➤ **Mold Cocktail**

- *Colletotrichum*
- *Epicoccum*
- *Cladosporium*
- *Phoma*
- *Alternaria*

## ➤ **Yeast Cocktail**

- *Aureobasidium*
- *Bullera*
- *Cryptococcus*
- *Sporidiobolus*
- *Filobasidium*

# INOCULATION OF FRUIT

- **Fresh blueberry samples (100 g each) separately inoculated with cocktails of *L. monocytogenes*, *Salmonella*, *E. coli* O157:H7, yeasts or molds by submersion for 20 min**

# INOCULATION OF FRUIT

- **Inoculated fruit dried in a laminar flow hood for 2 h and then stored overnight at 4°C**
- **Fruit re-dried in a laminar flow hood for 2 h before use**

# CHLORINE DIOXIDE GAS

- **Generated in a 20 L bucket at 99.9% R.H. / 22°C by a commercial dry chemical sachet (ICA TriNova, LLC Forest Park, GA)**
- **Inoculated fruit exposed to 0.16 mg ClO<sub>2</sub>/g of fruit for 12 h**

# PILOT STUDY (n = 3)



# MICROBIAL ANALYSES

- **Fruit (25 g) added to 100 ml of neutralizing buffer and plated for:**
  - ***Listeria monocytogenes* – Modified Oxford Agar**
  - ***Salmonella* – McConkey Agar**
  - ***E.coli* O157: H7 – Sorbitol McConkey Agar**
  - **Yeasts and Molds – Potato Dextrose Agar containing streptomycin (20 ppm) and ampicillin (50 ppm)**

# **EXPERIMENTAL DESIGN – PALLETIZED FRUIT**

- **30 lugs of blueberries (20 lb / lug) stacked on 4 x 4 ft pallets (5 lugs / level, 6 levels / pallet)**
- **Experiment replicated 6 times**
- **Each replicate: one gassed pallet (tarped) and one ungassed pallet (control)**
- **Fruit pallets held at 12-14°C during gassing**



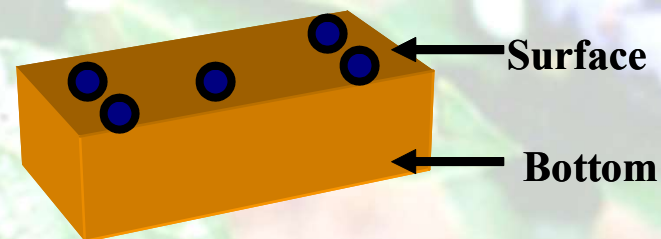
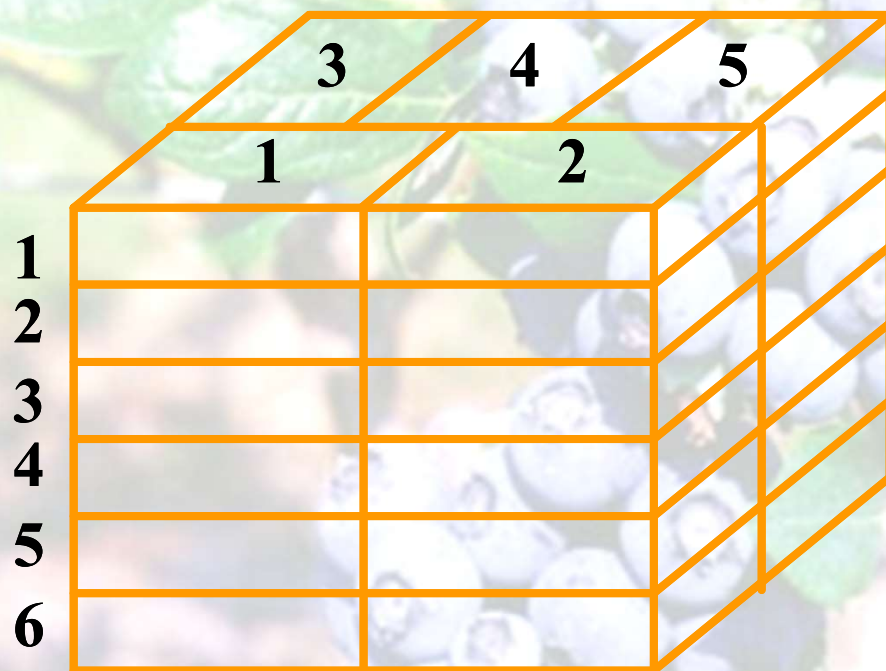
# **GASSING OF PALLETIZED FRUIT**

- **ClO<sub>2</sub> gas for each gassed pallet generated by three 3-kg commercial chemical sachets**
- **ClO<sub>2</sub> circulation system: 2 fans with tubing**
- **Pallets (273 kg, 600 lb fruit) tarped, exposed to 0.13mg ClO<sub>2</sub>/g fruit for 12 h**

# GASSED FRUIT



# Sample location for blueberry pallets and lugs



# **PALLETIZED FRUIT SAMPLES**

- **Top of lug: 1 composite sample from each of 5 lugs at 6 pallet levels = 6 samples**
- **Bottom of lug: 1 composite sample from each of 5 lugs at 6 pallet levels = 6 samples**
- **Above samples from gassed and ungassed pallets taken initially (0 h) and after 12 h**

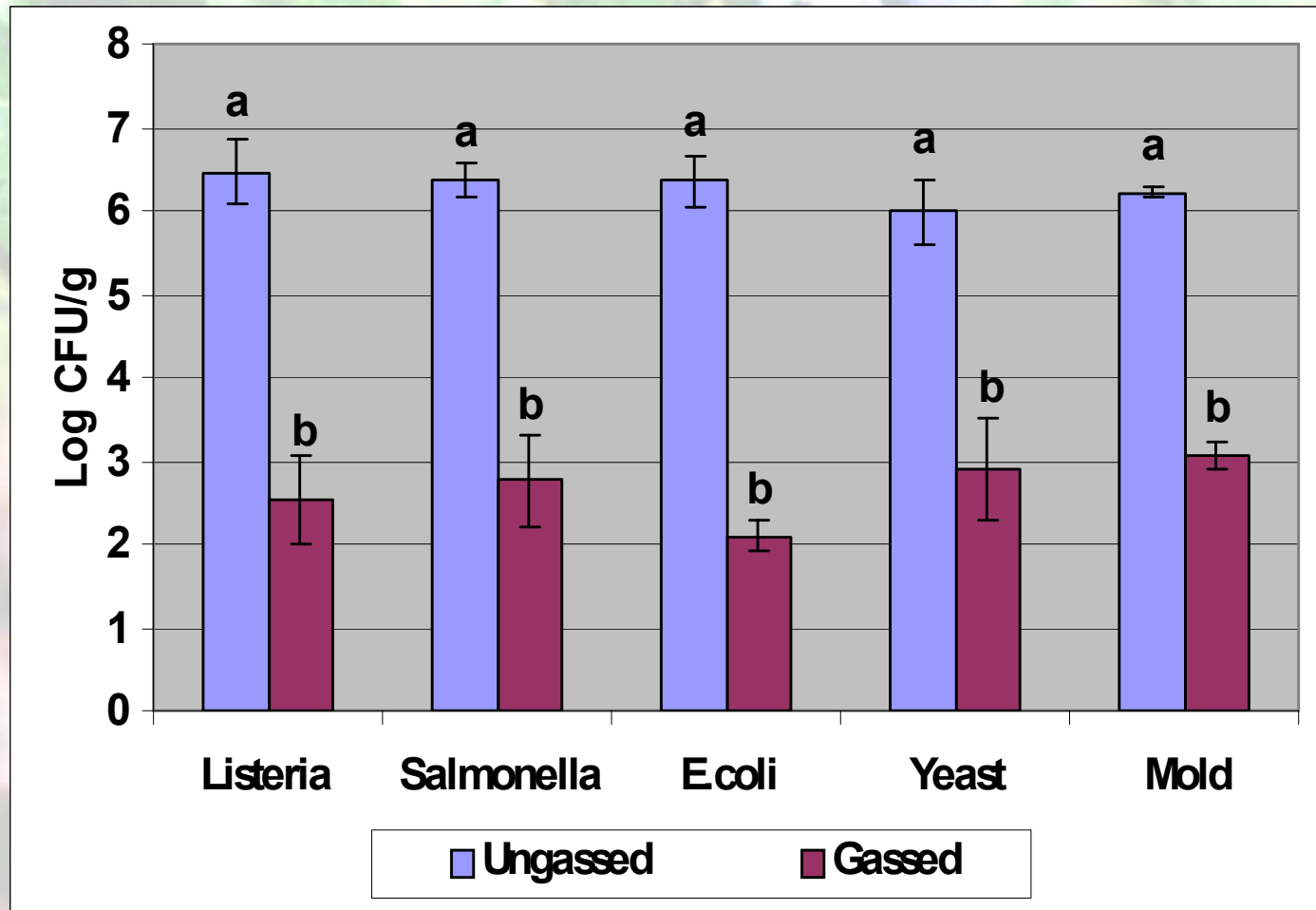
# MICROBIOLOGICAL ANALYSES

- **Blueberry samples (100 g) diluted in 200 ml neutralizing buffer and plated:**
  - **Bacteria – Trypticase soy agar containing 0.6% yeast extract and 100 ppm cyclohexamide**
  - **Coliforms and *E. coli* – 3M Petrifilm™**
  - **Yeasts and molds – Potato dextrose agar containing 20 ppm streptomycin and 50 ppm ampicillin**

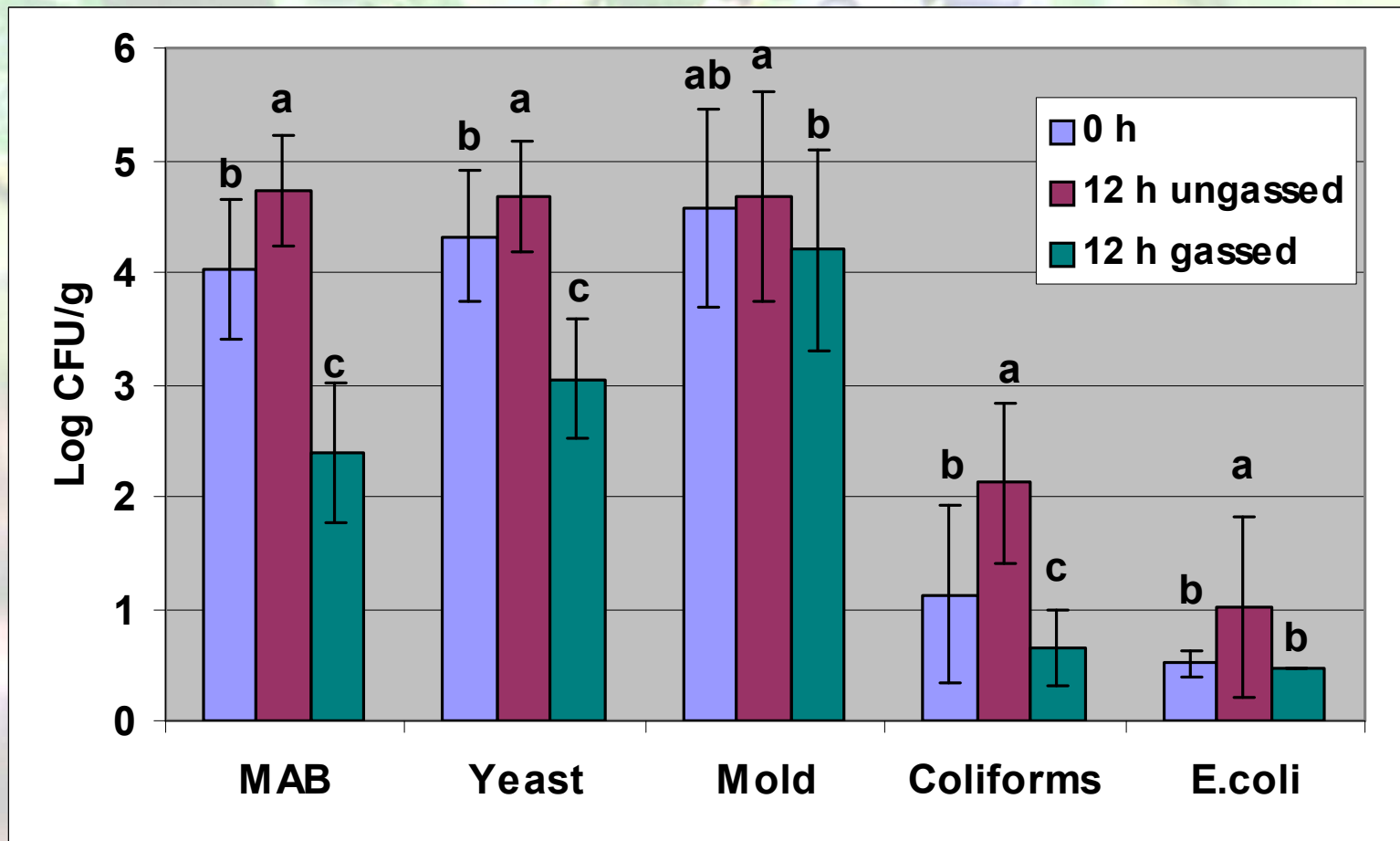


# RESULTS

# Pilot Study: Microbial levels on ungassed and gassed (0.16 mg ClO<sub>2</sub> / g) fruit after 12 h

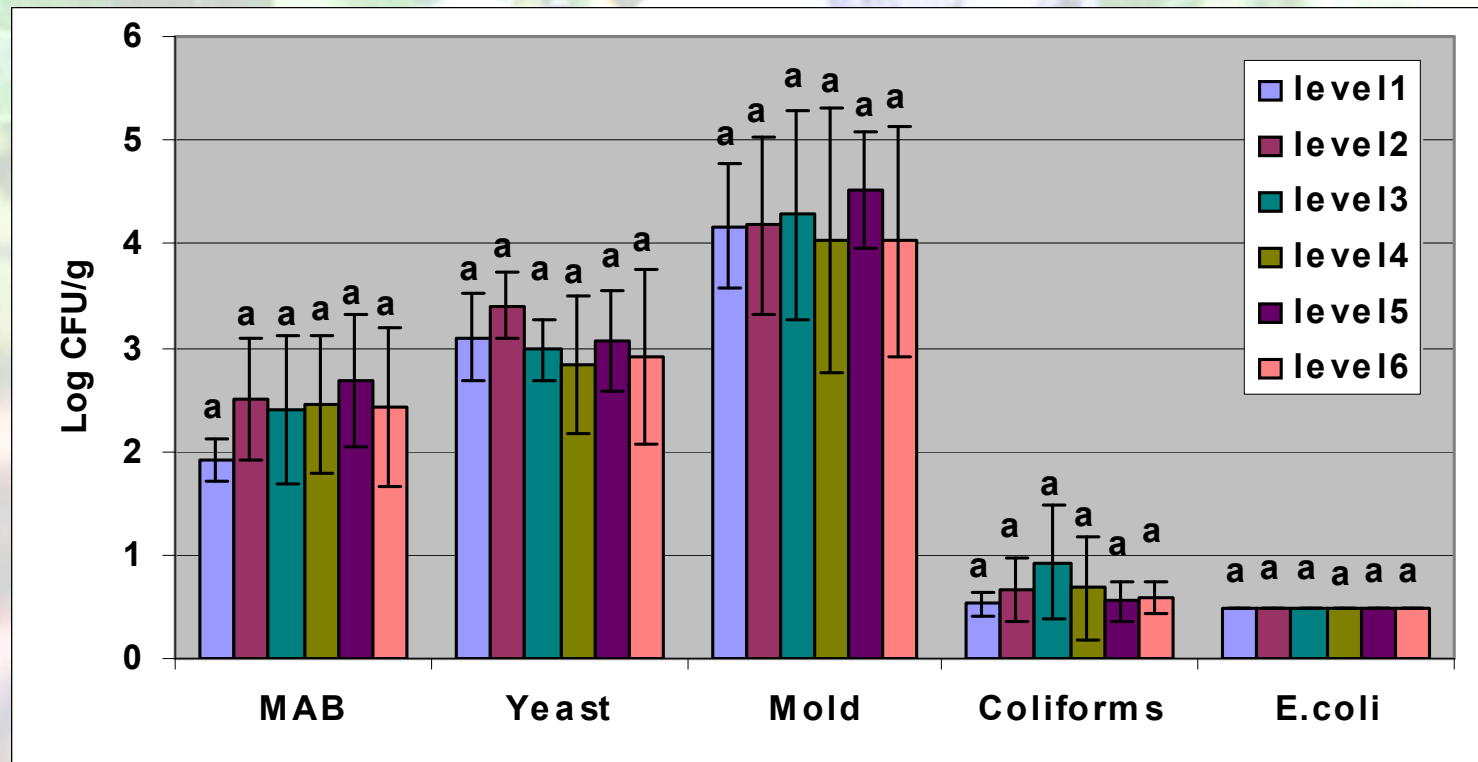


# Palletized Fruit: Microbial populations on blueberries before and after gassing



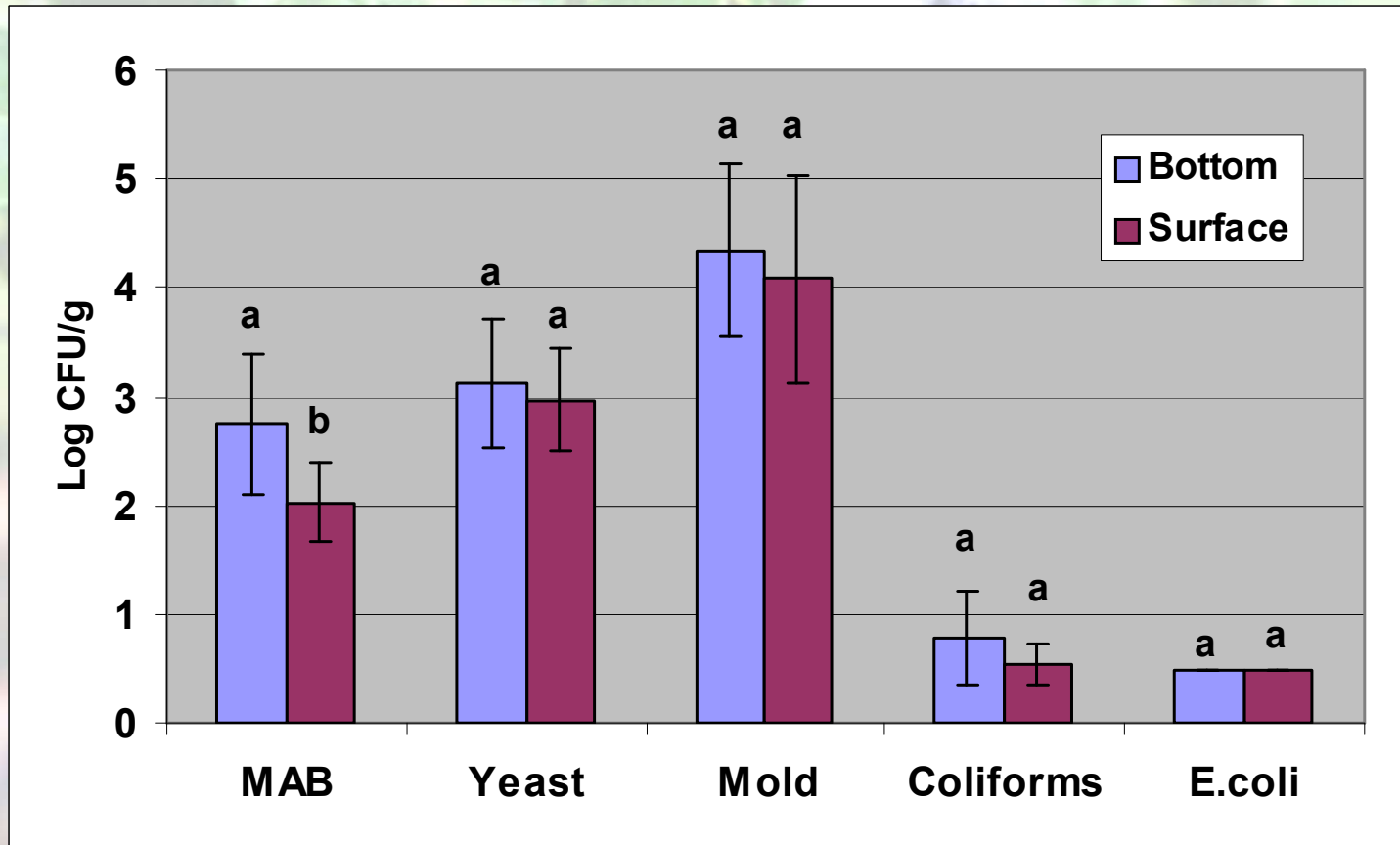


# Average microbial populations recovered from different pallet levels after gassing



No significant difference ( $P > 0.05$ ) in microbial inactivation between levels 1 to 6 for gassed pallets

# Average microbial populations recovered from the top and bottom of gassed lugs

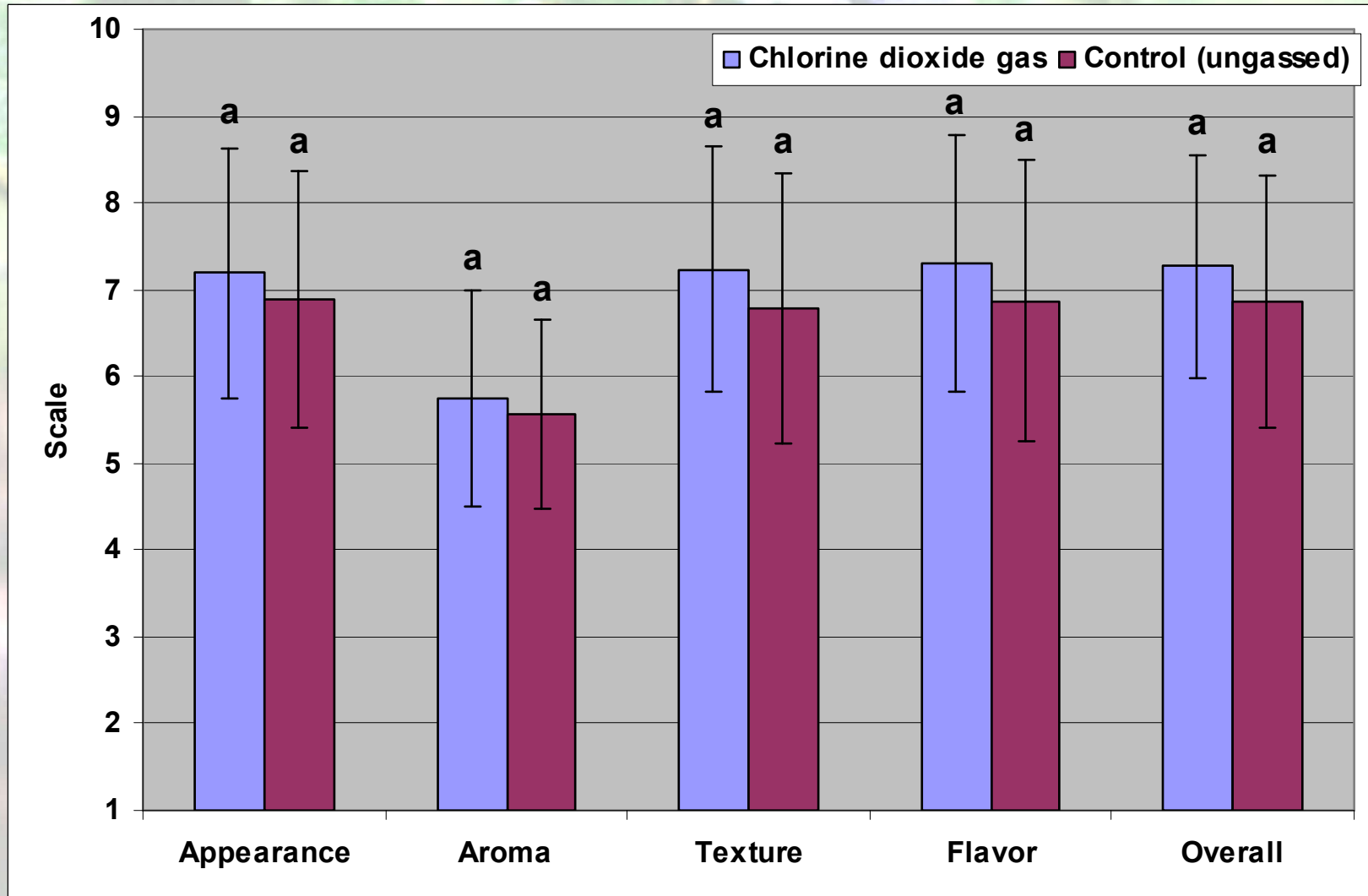


No significant difference ( $P > 0.05$ ) in microbial inactivation between the top and bottom surface samples except for MAB gassed pallets

# SENSORY ANALYSES

- Gassed (12 h) and ungassed samples evaluated for sensory attributes
- Appearance, aroma, texture, flavor and overall acceptability
- Consumer panel (n=110)
- Hedonic scale -“like extremely” (9) to “dislike extremely”(1)

# Average consumer acceptability for gassed (12h) and ungassed blueberry samples



# CONCLUSIONS

- **ClO<sub>2</sub> gas sachets:**
  - **Provide a simple, economical and effective means for meeting microbial standards set by buyers**
  - **No significant difference ( $P > 0.05$ ) in sensory attributes after fruit gassing**
  - **A viable option for blueberry growers and processors with a water tank sanitizing time shorter than 5 minutes**

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