Journal of Food Protection, Vol. 60, No. 10, 1997, Pages 1230–1234

Enhancement of Shelf Life of Grated Beetroots

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(MS# 96-127: Received 28 May 1996/Accepted 16 January 1997)

ABSTRACT

Microbiological quality in grated beetroots was studied. The vegetable was washed with cold water (8°C) or with chlorinated water (8°C and 252 mg active chlorine per liter water) and packaged in trays wrapped in high permeability film (PVC). Storage temperatures were 0, 4, and 20°C. Aerobic count colonies (ACC), fungi and yeasts, and lactose fermentative bacteria were determined. Representative colonies were identified. The results obtained showed that the treatment with chlorinated water caused significant decreases ($P < 0.05$) in the initial yeast counts and total aerobic counts. Treatment with cold chlorinated water significantly decreased the yeast counts and PCA counts after 3 days at 0 and 4°C. The change to 20°C caused significantly increased ($P < 0.05$) PCA counts in the grated beetroots (10^7 to 10^8 CFU/g). The microorganisms predominant among the isolates from fresh grated beetroots were gram negative bacteria (80%) and yeasts. None of the trays with grated beetroots contained molds. Some of the bacteria identified were *Micrococcus luteus, Acinetobacter* spp., *Corynebacterium* spp., and *Enterobacteriaceae.*

Key words: Microflora, grated beetroots, shelf life, ready-to-use, chlorinated water

Minimally processed vegetables are widely marketed in Europe and the USA, and their consumption is on the increase. This market growth has been stimulated by twin consumer demands for unadulterated freshness and maximum convenience *(10).* These products have been limited by their short shelf life, which is partly due to tissue disruption produced by processing of vegetables (peeling, cutting, grating, slicing) *(29).* These cut "ready-to-use" vegetables spoil faster than whole vegetables and are more easily affected by microorganisms *(24).* The processing-induced wounds cause exudation of cellular juices which favor microbial development that eventually leads to spoilage *(13, 18, 26).* Consequently, to retard quality deterioration and the multiplication of microorganisms, ready-to-use vegetables must be stored between 0 and 4°C *(6).* An average temperature between 0 and 1°C is advisable *(19).* Fortunately, the initial microbial contamination can be reduced through adequate hygiene during harvesting, handling, packing, and transportation *(16).*

The processing of ready-to-use vegetables starts with water washing. Chlorine is widely used in washing these products for more efficient disinfection. Mazollier *(22, 23)* has studied the effect of chlorine concentration on the microbial populations of vegetables and observed reductions in the total microbial counts. He suggested the product be washed with a 50 mg/liter chlorine solution. Chlorine is a powerful germicide, is not poisonous to humans at low concentrations, and is easy to handle *(11).* In sliced carrots a chlorinated water wash produces a significant reduction of coliforms, whereas the mesophilic aerobes are not affected *(31).* These results are identical to those obtained with shredded carrots *(17).* The effectiveness of this disinfection procedure is evidenced in salad mixtures by a reduction of one log cycle for the aerobic colony counts *(26).*

Microbiological quality has been studied in different minimally processed vegetables: sliced carrots, lettuce, shredded cabbage, and broccoli florets *(18, 24, 30, 31).* In Argentina there has been a rapid expansion in the sale of ready-to-use products, though no statistical data about total sales have been compiled yet. Among the marketed products raw grated beetroots are sold in trays, either individually or in combination with other vegetables. Grated beetroots are consumed as salad. These products are sold in plastic trays wrapped in high permeability film (PVC) and are sold in supermarkets and small retail shops. The latter present problems with the cold chain, since small shops often do not have adequate facilities to keep the product at refrigeration temperatures. The need for good handling practices such as proper temperature control, good sanitation, and awareness of the proper shelf life do not disappear simply because the product has reached its supposed final destination, but this is precisely the place where the breakdown in sanitation often occurs *(5).*

Owing to the absence of contributions on the microbiology of grated beetroots, and to the increased interest in the product, we decided to carry out a study on this subject. Thus, the aim of this work was to monitor and describe the microbiological flora (aerobic plate count, yeasts and molds) of grated beetroots (*Beta vulgaris L.*) stored at different temperatures and times and to determine their evolution

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during the experiment. The effect of thermal abuse (20°C) during storage was also analyzed. In addition, we evaluated the effectiveness of treatments with chlorinated water for reducing populations of microflora and extending shelf life.

MATERIALS AND METHODS

Beetroots

Table beetroots (*Beta vulgaris L.* var. *Detroit Dark Red*) (60 to 70 mm diameter) were purchased in the Regional Market of La Plata (Argentina). Immediately, after that, the vegetable was divided into two groups: one lot was washed only with cold (8°C) water and stored for 24 h at 4°C (untreated beetroots) and the other was first washed in cold (8°C) chlorinated water (252 mg active chlorine per liter water) and stored for 24 h at 4°C (treated beetroots). The active chlorine concentration used here was selected on the basis of previous research on grated or sliced carrots, diced onions, and shredded lettuce *(1, 2, 7, 14, 17).*

Sample preparation of treated beetroots consisted of the following steps: (a) hand-peeling, (b) a second washing in cold (8°C) chlorinated water for 10 minutes using a solution of NaOCl with 252 mg active chlorine per liter water, (c) grating in a food processor, (d) a last wash in cold (8°C) chlorinated water, and (e) draining. The other lot (untreated beetroots) was washed only with cold water in all stages of sample preparation. Utensils, containers, and work surfaces were sanitized with NaOCl (with 252 mg active chlorine per liter water) and gloves were used to reduce potential contamination.

Samples of 80 g of grated beetroots were then packaged in polypropylene trays and wrapped in PVC film RF-50 (permeability to O_2 and CO_2: 11,232 and 48,552 $cm^3/m^2/24$ h, respectively; permeability to water vapor: 40 $g/m^2/24$ h).

Experimental design

Two different ways of preparing of grated beetroots were compared: (1) beetroots washed only with cold water without NaOCl (untreated beetroots) and (2) beetroots washed with cold chlorinated water (treated beetroots). All experiments were duplicated. In each experiment, i.e., for each combination of temperature and storage time, a minimum of two trays were employed.

Storage

The trays so prepared were divided into two groups. One group was stored at 0°C and the other at 4°C, both for 7 days. On days 3 and 7 of the storage period two trays previously stored at 0 and 4°C were transferred to 20°C and left at that temperature for 24 h.

Microbial analysis

Beetroots were analyzed immediately after treatment with cold water (untreated beetroots) and chlorinated cold water (treated beetroots), after 3 and 7 days of storage, and after the change to 20°C.

Twenty grams of sample from each tray were placed in sterile bags, diluted with 180 ml of 0.1% (wt/vol) sterile peptone water and homogenized for three minutes in a Seward Stomacher (Model 400). From the homogenate, several dilutions (10^{-3} to 10^{-7}) were made in 0.1% peptone water and poured (0.1 ml) in duplicate plates containing appropriate media. Total aerobic plate counts were determined on plate count agar (PCA; tryptone, 5 g/liter; yeast extract, 2.5 g/liter; glucose, 1 g/liter; agar, 12 g/liter; pH 7) with incubation at 30°C for 48 h. Lactose fermentative bacteria were assessed on Man-Rogosa-Sharp (MRS) agar (Oxoid) and lactose agar (1% lactose, 1% tryptone, 1% yeast extract [wt/vol]). Colonies were counted after aerobic incubation at 30°C for 48 h.

Aliquots for assessment of yeasts and molds were incubated for 5 days at 25°C in yeast extract-glucose-chloramphenicol (YGC, Merck). Viable counts were determined and expressed as log CFU/g (CFU: colony forming units).

All samples were analyzed within an hour after they were removed from cold storage.

Identification

From each sample, 20 representative colonies with similar macroscopic morphology were selected and then isolated from the PCA plates. The microorganisms were purified by culture on PCA plates and characterized by Gram staining, an oxidase reaction *(20),* and a catalase test.

Gram negative rods were tested by oxidative-fermentative (O-F) glucose metabolism on Hugh and Leifson medium *(15). Enterobacteriaceae* were determined by incubating plates 16 to 18 h at 37°C employing violet red bile glucose agar (VRBGA, Merck).

The results obtained were analyzed from a Costin *(9)* and Mossel and Moreno García diagnosis table *(25).* Other biochemical tests used to identify gram negative rods were reduction of nitrate, production of acetic acid from ethanol, and mobility. Gram positive cocci and rods were identified using *Bergey's Manual (4).* Isolated yeasts were purified in YGC and characterized by fermentation of sugars (glucose, maltose, galactose, lactose, and sucrose) and growth in malt extract (ME) broth (Merck).

Statistical analysis

Data were subjected to analysis of variance (ANOVA). Differences among treatment means were evaluated by the least significant difference (LSD) test with $P < 0.05$. The sources of variation were time (0, 3, 7 days), temperature (0, 4, and 20°C), and treatment (with or without chlorinated water). All analyses were conducted using the System for Statistics (SYSTAT Inc., Evanston, IL).

RESULTS AND DISCUSSION

Storage of beetroots washed only with cold water

Total counts in grated beetroots not treated with NaOCl were determined immediately after processing (washing, grating, etc.) and during storage. The results obtained are shown in Table 1. The initial value of total aerobic count was 5.5 log CFU/g. At 0°C, total aerobic counts remained unchanged throughout the storage, whereas at 4°C there was a continued increase in the values. Total counts in trays stored at 4°C for 7 days were 7.3 log CFU/g, whereas in those trays stored at 0°C the total counts remained at the initial level (5.5 log CFU/g) ($LSD_{0.05} = 0.2$). The trays stored at 4°C for 7 days thus showed an increase of 1.8 log cycles with respect to the initial value. Storage temperatures caused significant differences ($P < 0.05$) in the counts of yeasts and molds; in the trays stored for 3 days at 4°C there was an increase of 2.9 log cycles in the counts with respect to the initial value.

Storage of beetroots washed with chlorinated cold water

The total aerobic counts, counts of lactose fermentative bacteria, and counts of yeasts and molds for grated beetroots washed with chlorinated cold water and stored at 0 and 4°C for 3 and 7 days are shown in Table 2. There was a significant increase ($P < 0.05$) in total counts due to the

TABLE 1. *Total aerobic counts and counts of yeasts and molds in grated beetroots treated with cold water, after storage at 0 or 4°C for 3 and 7 days and then after changing to 20°C for 24 h (results are expressed as log CFU/g)*

Storage temperature	Time	PCA n	PCA log N	PCA SD	YGC n	YGC log N	YGC SD
0°C	Initial	4	5.5	0.0	4	4.4	0.0
	3 days	4	5.5	0.1	4	5.4	0.1
	7 days	4	5.5	0.2	4	4.8	0.2
4°C	3 days	4	6.0	0.1	4	7.3	0.2
	7 days	4	7.3	0.1	4	ND	—
	Total counts after passage to 20°C for 24 h						
0°C	3 days	4	8.3	0.1	4	7.3	0.0
	7 days	4	8.5	0.2	4	8.3	0.1
4°C	3 days	4	8.4	0.1	4	7.5	0.2
	7 days	4	8.7	0.3	4	>7.0	0.0

PCA = plate count agar; YGC = yeast extract-glucose-chloramphenicol; n = number of samples; N = CFU/g; SD = standard deviation; ND = not determined.

effect of storage time and temperature ($LSD_{0.05} = 0.3$). After 7 days of storage values of 5.4 and 6.6 log CFU/g were obtained for storage at 0 and 4°C, respectively. An interaction between time and storage temperature was detected. The counts obtained in samples stored for 3 days at 4°C were comparable to those obtained after 7 days at 0°C, as no significant differences were observed ($P > 0.05$). This means that the microbiological levels reached in storage at 4°C were similar to those found at 0°C after a longer storage period.

Storage time, temperature, and interactions between temperature and storage time caused significant differences in the corresponding YGC counts ($LSD_{0.05} = 0.3$). The counts for trays stored for 7 days at 0°C (5.2 log CFU/g) were significantly lower than those for beets stored at 4°C (6.9 log CFU/g).

Furthermore, a residual effect of the treatment was observed during the first 3 days of storage at 0°C in counts of both bacteria and yeasts. After 7 days of storage this effect disappeared. This fact might be related to a probable decrease in the germicidal efficacy of NaOCl after 3 days, which allowed the number of microorganisms to increase. The effectiveness of chlorine treatment is limited to short-term storage *(28)*.

The counts of lactose fermentative bacteria for trays stored for 7 days at 4°C (5.7 log CFU/g) were significantly higher ($P < 0.05$) than for those at 0°C ($LSD_{0.05} = 0.3$). These levels were similar to those determined for grated carrots *(8)*.

Upon comparison, we can see that there were significant differences ($P < 0.05$) between the initial total counts for chlorinated-water-treated and untreated beetroots ($LSD_{0.05} = 0.3$). After 3 days of storage at 0°C the total aerobic counts for treated beetroots were significant lower ($P < 0.05$) than those for the untreated samples; at 4°C the PCA counts were comparable to those for untreated beetroots. After 7 days of storage at 0°C, total aerobic counts for treated beetroots were similar to those for untreated samples. At 4°C the counts for beets not treated with chlorinated water were higher (0.7 log cycle) than those for beetroots treated with NaOCl. Therefore, with identical temperature and storage time conditions, treatment with chlorinated water kept the microbial counts lower than those for untreated samples.

A significant decrease ($P < 0.05$) was observed in YGC counts due to the effect of chlorinated water washing except in the trays stored for 7 days at 0°C. Counts in the samples treated with chlorinated water and stored for 3 days at 0 and 4°C (Table 2) were about 1.4 and 3.2 log cycles lower, respectively, than those of the untreated samples (Table 1).

Effect of thermal abuse

In beetroots not treated with NaOCl (Table 1) the change to 20°C caused a significant ($P < 0.05$) increase in total aerobic counts. These counts were affected by previous storage temperature and time: when temperature abuse (change to 20°C) was applied after 3 days of storage, there were increases of 2.8 and 2.4 log cycles in the counts for the

TABLE 2. *Total aerobic counts, counts of yeasts and molds, and counts of lactose fermentative bacteria in grated beetroots treated with cold chlorinated water; after storage at 0 or 4°C for 3 and 7 days and then after changing to 20°C for 24 h (results are expressed as log CFU/g)*

Storage temperature	Time	PCA n	PCA log N	PCA SD	YGC n	YGC log N	YGC SD	Lactose agar n	Lactose agar log N	Lactose agar SD
0°C	initial	4	5.0	0.1	4	4.3	0.0	4	4.0	0.2
	3 days	8	4.7	0.3	8	4.0	0.3	8	4.5	0.1
	7 days	8	5.4	0.1	8	5.2	0.1	8	4.4	0.2
4°C	3 days	8	5.6	0.1	8	4.1	0.2	8	4.5	0.0
	7 days	8	6.6	—	8	6.9	0.1	8	5.7	0.2
	Total counts after passage to 20°C for 24 h									
0°C	3 days	8	7.8	0.1	8	7.0	0.1	8	6.3	0.5
	7 days	8	8.4	0.4	8	6.8	0.1	8	6.3	0.1
4°C	3 days	—	ND	—	8	6.8	0.0	8	6.6	0.0
	7 days	8	8.0	0.1	8	6.6	0.3	8	6.3	0.1

PCA = plate count agar; YGC = yeast extract-glucose-chloramphenicol; n = number of samples; N = CFU/g; SD = standard deviation; lactose agar = 1% yeast extract-1% lactose-1% tryptone.

trays previously stored at 0 and 4°C, respectively, whereas there were increases of 3.0 and 1.4 log cycles when the transferred samples had previously been stored for 7 days at 0 or 4°C, respectively. In the corresponding YGC counts there were increases of 1.9 and 3.5 log cycles in the counts for the trays previously stored for 3 and 7 days at 0°C, respectively (Table 1).

Results in Table 2 indicate that in beetroots treated with chlorinated water there are significant differences in the PCA counts obtained with different storage temperatures. For instance, when thermal abuse was applied after 7 days of storage at 0°C, total counts increased by 3 log cycles, whereas when the change to 20°C occurred after the same storage period at 4°C, the increase was of 1.4 log cycles. This means that after 7 days of storage at 4°C the counts were already high and the transfer to 20°C causes only a small increase in the microflora. In YGC the differences reached 3.0 log cycles when the change to 20°C was effected after 3 days at 0°C and 1.6 log cycles when the thermal "jump" was conducted after 7 days at 0°C. On lactose agar, there the counts for trays stored at 20°C did not differ significantly by temperature of prior storage except in the trays previously stored for 7 days at 4°C (Table 2).

In studies on sliced carrots the increase in aerobic microorganisms was found to be faster at 15°C than at 10 or 4°C *(7)*. Temperature abuse during storage is not recommended *(2)*. Refrigeration control is an important factor in obtaining products of good quality.

Isolation of microorganisms

The predominant microorganisms isolated from grated beetroots treated with chlorinated water were gram negative rods (80%). Other microorganisms isolated were gram positive cocci (18%), gram positive rods (2%), and yeasts. None of trays with grated beetroots contained molds. These percentages represent 100 isolations. No growth was observed in MRS even at dilutions of 10^{-2}.

Gram negative rods isolated in grated beetroots were tested with the O-F method (oxidative/fermentative metabolism). In a minority of cases (10%) nonfermentative rods such as *Acinetobacter* spp., catalase positive, oxidase negative bacteria found in soil and water *(3)*, were found. The rest of the groups (90%) were *Enterobacteriaceae*. Gram positive cocci occurring in tetrads, catalase positive, oxidase negative, and forming and yellow colonies, were identified as *Micrococcus luteus*. Gram positive rods, catalase positive and oxidase positive with curved ends, were found in the sample as *Corynebacterium* spp. In malt extract (ME) medium, most isolated yeasts were globose to ovoid cells. A sediment and a surface film or rings were present and a pseudomycelium was formed in some cultures. Yeasts isolated from grated beetroots were not fermentative.

Some strains with orange-red colonies on agar media did not ferment any of the substrates tested. They were identified as *Rhodotorula* spp.

Microorganisms found in beetroot are not only those of its own flora but also those of the soil and irrigation water. The normal microbial floras of fruits and vegetables consist mainly of organisms commonly found in soil: coryneforms and gram negative organisms *(21)*. Generally, yeasts and molds are less numerous than mesophilic bacteria *(6, 25)*.

Several authors have found that 80 to 90% of mesophilic bacteria in processed vegetables were gram negative rods *(8, 24, 27)*. For example, 90% of the flora initially isolated from sliced carrots was dominated by gram negative rods *(7)*. In sliced carrots about 4% of the microflora was identified as *M. luteus (31)*.

In our experiment none of the trays had fungi. Similar results were obtained by Nguyen-The and Carlin *(27)*.

After the thermal abuse (24 h at 20°C after 3 or 7 days of storage), a decrease in the number of gram negative bacteria was observed. When the temperature was changed after 3 days of storage at 0°C, the proportion of gram negative isolates diminished from an initial value of 80% to a final value of 65%. In trays taken to 20°C after 7 days of storage at 0°C, the final proportion of gram negative isolates was 55%. When the previous storage temperature had been 4°C for 7 days, the percentage of gram negative isolates decreased even further to 46%. The transfer to 20°C after 7 days of storage at 4°C caused the proportion of gram positive cocci to increase to 55% of the isolates.

In grated beetroots stored at 0 and 4°C the isolated bacteria were nonfermentative. After the change to 20°C, we observed that the nature of the metabolism changed to a fermentative type; this fact suggests that the development of such microorganisms was favored by the temperature increase. Temperature abuse during storage would accelerate the accumulation of CO_2, thus increasing the risk of spoilage by fermentative microorganisms *(18)*. Anaerobic conditions could arise more rapidly with temperature abuse, since the respiration rate increases with temperature. This risk is increased in many minimally processed vegetables because processing increases the respiration rate *(28)*. As previously mentioned, there was a decrease in the presence of gram negative isolates whereas the gram positive cocci increased. These results are in agreement with those of Farber *(12)*.

CONCLUSIONS

The microbiological results suggest that storage at 0°C yields a high quality product. At 0°C, the shelf life of grated beetroots was 7 days, whereas at 4°C the shelf life was 3 days only when the product was washed with chlorinated water. Chlorinated water caused significant decreases in the yeast and bacterial counts after 3 days at 0 and 4°C. According to the results obtained in this study, the microbial counts would depend on the temperature of the previous storage stage and on the storage time elapsed before transferring the samples to 20°C. The greater the thermal difference involved in the transition from the cold of the store outlet and the change to 20°C, the greater the increase in microbial counts will be.

ACKNOWLEDGMENT

The authors are grateful to the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) for financial support and to CIDCA microbiological laboratory

members for their suggestions, help, and technical assistance.

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