Antimicrobial Protection of Moisturized Deglet Noor Dates

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The growth of Saccharomyces rouxii and Saccharomyces mellis, which are two of the main spoilage organisms of dates, can be inhibited by various treatments. The most effective treatment found in this study that did not affect flavor consisted of a predip of the dates in 2% potassium sorbate solution followed by injection of methyl bromide into the sealed package.

Dates have long been recognized as an energy-rich food throughout many countries of the world. About 22,000 tons of dates are harvested annually in the United States, giving a return to the grower of more than $3,000,000. Normally, these dates are quite dry when harvested and seldom create a microbial spoilage problem. However, if they are marketed at this moisture level, which is usually around 21%, the consumer complains about their hardness. The texture of dates can be softened by hydrating them to 24% moisture or higher, depending upon the amount of sugar inversion that has occurred. At this moisture level, yeast spoilage of the dates can be a problem (10).

Microbial spoilage in dates, as well as prunes and figs, has generally been controlled in the past by an epoxide treatment. The epoxides have a high degree of killing power and produce essentially a sterile product (3, 9, 12). However, in the past few years the use of epoxides on dried fruits has been prohibited. Since then, the major prune and fig processors have used potassium sorbate for treating high-moisture fruits (7). The present study was undertaken to determine the effectiveness of potassium sorbate and its derivatives (11) as well as other potential antimicrobial agents in retarding microbial spoilage of processed dates.

MATERIALS AND METHODS

Untreated Deglet Noor dates, pitted and unpitted, packaged in 9-kg bulk cartons, were obtained from Indio, Calif. The dates were divided into two batches and their moisture levels were adjusted in a steam chamber to 22% and 27 to 30%, respectively. The 22% moisture dates were used in dip treatments, which resulted in a final moisture after treating of 28 to 30%. The dates were hydrated to a high moisture level for this study to provide conditions in which spoilage was certain to occur without treatment.

Dates were inoculated after hydration with approximately equal numbers of the osmophilic yeasts, Saccharomyces rouxii (Zygossaccharomyces japonicus) and Saccharomyces mellis, which are two of the main spoilage organisms of dates (5). The yeasts were washed from 48-hr potato-dextrose-agar slants with 0.1% peptone water, and the optical density of the resulting suspension was adjusted by dilution. A calculated inoculum of 3 × 10⁵ yeast cells/g was added to the dates. The dates with yeast inoculum were then incubated for 24 hr at 25 C before antimicrobial treatment.

The 22% moisture dates were dipped into the following solutions for 30 sec: 2 and 5% potassium sorbate; 1 and 2% propyl-p-hydroxy benzoate; 2% heptyl-2-hydroxybenzoate; 2% 3-acetyl-4-hydroxy coumarin; 2% octylgallate; 2% obtusastrene (p-cinnamylphenol); and 2% calcium propionate. These inoculated and treated dates were drained for 1 min, after which 160-g portions were sealed in polyester-polyethylene laminated pouches (Scotchpak). Duplicates were prepared of samples from each treatment. The inoculated 27 to 30% moisture dates were packaged as above, and the respective antimicrobial agents, peracetic acid, ethyl formate, diethyl pyrocarbonate, propylene oxide, and ethyl sorbate, were added before sealing. The peracetic acid was dispersed over the inside surface of the pouch before the dates were added. The bags of dates treated with ethyl sorbate were heated for 2 min in a 93 C oven to vaporize the material. For isomaltol vapor treatment, a 20% solution of isomaltol in methanol was prepared. Three milliliters of this solution was absorbed on absorbent paper (3 by 12 cm). Alcohol was allowed to evaporate and the paper was put into the bag of inoculated dates. For gaseous treatment, the bags of dates were sealed and the gas was injected through a needle into the bag; then the hole was sealed over.

These packages of treated dates were all stored in a 25 C incubator. Samples were removed periodi-
cally during the 90-day testing period to determine whether any microbial growth had occurred. Microbial growth was determined in two ways: by visual examination and by measuring the changes in bag volume by water displacement.

Potassium sorbate was determined by the method of Nury and Bolin (6) and moisture was determined by the AOAC (Association of Official Agricultural Chemists) method (1).

RESULTS AND DISCUSSION

Propylene oxide provided good microbial protection at the 0.3% level (v/w). However, an objectionable odor was noticed when the packages treated with this chemical were opened. This off-odor was present regardless of the purity of the commercial propylene oxide. Taste was not affected and the odor disappeared when the dates were aerated.

Potassium sorbate has been used successfully and extensively for treating other high-moisture dried fruits, such as prunes and figs, by spraying or dipping in a 2% solution (7). However, this same dip did not keep yeast from growing on dates during extended storage (Table 1). This difference in effectiveness is probably because dates have a higher pH than either prunes or figs. Sorbic acid imparts antimicrobial activity in the undissociated form, and dissociation becomes greater at higher pH. In prunes and figs having a pH between 4 and 5, 50 to 80% of the sorbic acid is present in the undissociated form. Dates have a pH of about 5.9, so that only a small percentage of the sorbate is present as undissociated sorbic acid. Therefore, a greater amount of sorbate is required in dates to provide microbial protection.

Taste threshold for sorbate varies with the dried fruit to which it is added. In prunes about 600 to 700 μg/g can be detected. A taste panel evaluation of date paste indicated that a significant percentage of panel members could not detect potassium sorbate added at 4,800 μg/g. However, sensitivity to sorbate varied considerably, with a few panel members consistently able to detect sorbate in dates at 1,000 μg/g. At higher levels, those who could taste it indicated that it produced a slight burning effect or a reduction in flavor. Moreover, the sorbate was not distributed uniformly throughout dates. Pitted dates, dipped in 2% potassium sorbate solution at 23 C and analyzed after 2 weeks, contained 900 μg/g sorbate in the whole date, but concentration in the skin was 1,250 μg/g while the flesh contained 890 μg/g. Therefore, sorbate can be used to give antimicrobial control for dates if the concentration is kept in the range where no taste difference occurs and yet the needed protection is obtained. This would be applicable only to dates having a low moisture level and a low level of contamination.

Methyl and propyl p-hydroxybenzoates are members of a group of antimicrobial agents known to retard mold and yeast growth in certain commodities (4). Dates were dipped in propyl p-hydroxybenzoate solutions of various concentrations (Table 2). Even a 0.5% dip provided protection for about a month, and a 2% dip inhibited microbial growth for the full 90-day storage period. However, off-odor and flavor were imparted to the dates at this higher treatment level. Therefore, it appears that these hydroxybenzoates cannot by themselves be useful in inhibiting microbial growth on high-moisture dates.

Numerous other treatments were tried with varying success (Table 3). When 20 ml of nitrous oxide and 4 ml of diethyl pyrocarbonate were added to 160 g of dates, no protection was obtained; also, 2% dips in 3-acetyl-4-hydroxy coumarin and octylgallate were ineffective. However, dips in 2% solutions of obtusastyrene or calcium propionate, or isomaltol vapor treatment extended storage life up to a maximum of 2 weeks. Heating was also investigated, but excessive heat caused the dates to darken noticeably. Darkening also seemed to occur more readily as the moisture level of the dates increased.

Ethyl sorbate, a derivative of sorbic acid, when added to the bags of packaged dates, did not provide microbial protection. However, if the sealed packages were heated for a few minutes to vaporize the ethyl sorbate, protection was provided depending upon concentration (Fig. 1). Full protection was realized at a 0.3% treatment level; however, dates treated at this level also had a possibly objectionable odor which would limit the usefulness of this treatment.

Methyl bromide gas is widely used for the

| Whole or pitted | Potassium sorbate dip (%) | 5 days | 10 days | 15 days | 25 days | 40 days | 60 days | 90 days |
|-----------------|--------------------------|--------|---------|---------|---------|---------|---------|---------|
| W               | 0                        | ++     | -       | -       | +       | ++      | ++      | ++      |
| W               | 2                        |        |         |         |         |         |         |         |
| W               | 5                        |        |         |         |         |         |         |         |
| P               | 0                        | ++     | -       | -       | +       | ++      | ++      | ++      |
| P               | 2                        |        |         |         |         |         |         |         |
| P               | 5                        |        |         |         |         |         |         |         |

* - = No spoilage; +, ++, +++ = %, %, and all spoiled.
Therefore, spoiled.

Nitrous Diethylpyrocarbonate detectable (Table 3-Acetyl, Calciumpropionate Octylgallate)

When sorbate-treated cals was obtained with as little as 0.5 ml of gas (Table 4). At this low treatment level, taste panel evaluation indicated that there were no detectable odor or flavor changes in the fruit. Therefore, this appears to be a practical method of microbial control for high-moisture dates. Methyl bromide has been cleared by the Food and Drug Administration for use on dates if the residual bromide is below 100 ppm, calculated as inorganic bromide. If all of the methyl bromide injected (using 1 ml of the gas) were absorbed by the dates, the maximum possible would be 21 ppm. However, methyl

| Propyl-p-hydroxybenzoate (%) | Spoilage after storage* | 5 days | 10 days | 15 days | 25 days | 40 days | 60 days | 90 days |
|-----------------------------|-------------------------|--------|--------|--------|--------|--------|--------|--------|
| 0                           | +++                    |        |        |        |        |        |        |        |
| 0.5                         | ++-                    |        |        |        |        |        |        |        |
| 1.0                         | -=                     |        |        |        |        |        |        |        |
| 2.0                         | -=                     |        |        |        |        |        |        |        |

*— = No spoilage; ++, +++ = %, % and all spoiled.

| Treatment                | Spoilage after storage* | 5 days | 10 days | 15 days | 25 days |
|--------------------------|-------------------------|--------|--------|--------|--------|
| Calcium propionate       | +                       | +++    |        |        |        |
| Diethylpyrocarbonate     | + +                    |        |        |        |        |
| Isomaltol (vapor)        | —                       | —      | +      | +++    |        |
| Nitrous oxide            | + +                    |        |        |        |        |
| 3-Acetyl, 4-hydroxy      | —                       | —      | +      | +++    |        |
| coumarin                 | —                       | —      | +      | +++    |        |
| Obtusasyrene             | —                       | —      | +      | +++    |        |
| Octylgallate             | —                       | —      | +      | +++    |        |

*— = No spoilage; ++, +++ = %, % and all spoiled.

Fumigation of dried fruits to prevent insect infestation. This gas was investigated for its effectiveness against microorganisms on fruits. When dates were treated at levels of 1 to 10 ml of gas per package, varying degrees of protection were obtained (Fig. 2). Only the 10-ml gas injection gave complete protection. Yet, this effective treatment imparted an off-odor to the dates.

At this point, studies were undertaken to determine whether combination treatments would result in an increased antimicrobial activity at chemical concentrations below the odor and flavor thresholds. Potassium sorbate (2% dip) was used as the first primary treatment, because it did not impart a noticeable flavor change at this level, and various other chemicals were added as secondary treatments. When methyl bromide was added to inoculated sorbate-treated dates, microbial protection was obtained with as little as 0.5 ml of gas (Table 4). At this low treatment level, taste panel evaluation indicated that there were no detectable odor or flavor changes in the fruit. Therefore, this appears to be a practical method of microbial control for high-moisture dates. Methyl bromide has been cleared by the Food and Drug Administration for use on dates if the residual bromide is below 100 ppm, calculated as inorganic bromide. If all of the methyl bromide injected (using 1 ml of the gas) were absorbed by the dates, the maximum possible would be 21 ppm. However, methyl
bromide passes readily through packaging materials, so the amount absorbed by the dates should only be a fraction of the amount injected into the headspace (8). No methyl bromide could be detected by gas chromatographic analysis when bag headspace was analyzed 2 weeks after addition of 2 ml of methyl bromide. This loss of methyl bromide also illustrates the advantage of using a combination treatment. The methyl bromide reduces the original microbial count and is then dissipated while the potassium sorbate remains to provide lasting protection.

Peracetic acid alone also exhibited antimicrobial properties, but its effectiveness was greatly increased when the dates were first dipped in a 2% potassium sorbate solution (Table 5). However, even at the 0.2% peracetic acid treatment level, a certain tartness and vinegar odor were given to the dates.

Ethyl formate was found to inhibit microbial growth when added to dates at 3 ml per lb (ca. 453.6 g) (Table 6). However, at this concentration it also imparted an objectionable odor. When the dates were first dipped in a sorbate solution, ethyl formate treatment could be reduced to 1.5 ml per lb, but even at this level it could be detected in the treated dates. The usefulness of this combination treatment is questionable. Possibly the effectiveness of the formate could be increased by a short heating in the sealed package to vaporize it quickly.

The best overall method for prevention of yeast spoilage in this series of experiments was the combination sorbate-methyl bromide treatment. Other procedures provided protection but imparted a flavor or odor change to the moisturized dates. The sorbate or sorbate-ethyl formate treatment could have some application possibilities if either the microbial level or the moisture level of the dates is sufficiently low.

The effectiveness of antimicrobial agents is increased as the moisture content of the fruit is decreased (2). This knowledge, plus the information on the effectiveness of various chemical or chemical treatment combinations, can be used to produce packaged succulent dates free from microbial spoilage.

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