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EFFECT OF MODIFIED ATMOSPHERES ON MICROFLORA AND RESPIRATION OF CALIFORNIA PRUNES

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ABSTRACT

We investigated the possibility that California prunes stored in modified atmospheres (MAs) will tolerate higher water activity (a_w) levels than those required at normal atmospheres and that the naturally occurring microfloral infection level on the prunes may generate the MAs that inhibit mold activity in airtight conditions. Dry prunes were moisturized to a range from 16 to 38% moisture content (m.c.) to acquire samples with 0.575 to 0.858 a_w. In unsealed conditions at 35°C, there was a level of naturally occurring microorganisms (aerobic plate count, yeast and mold count) that generally increased above 0.70 a_w. However, incubating these samples in a sealed container for 35 days at 35°C indicated that levels of naturally occurring microorganisms remained unchanged throughout the range of water activities. Microfloral respiration as a function of temperature was determined by incubating similarly moisturized prune samples at 25, 30 and 35°C and then measuring the declining O_2 concentrations through time. Results showed that the higher both the water activity and temperature, the more intense the O_2 consumption by the product. A nearly linear relationship was observed between O_2 depletion and time. Anaerobic conditions were reached in less than 2 days at 25°C and 0.858 a_w, the highest water activity tested. At higher temperatures, anaerobic conditions were achieved at water activities of 0.824 and above. These results indicate that under aerobic conditions microorganisms can flourish on and cause deterioration of prunes if the water activity of the fruit is above 0.7 (m.c. of 24.6%). However, under sealed conditions, prunes can tolerate a higher water activity without microorganism growth or deterioration of the fruit.

INTRODUCTION

Low water activity (a_w) retards the growth of yeasts and molds on dried prunes (Pitt and Christian, 1968; Tanaka and Miller, 1961). However, a few fungi are capable of growth in environments with low water activity. *Eurotium amstelodami* was isolated at 0.77 a_w, (Kinderlerer, 1984) and 0.753 a_w (Wheeler and Hocking, 1988) and germinated at a_w as low as 0.738 (Pitt and Christian, 1968). Many

Aspergillus species are xerophilic and are usually capable of growing on media containing high concentrations of salt or sugar (Thom and Raper, 1945). In addition, *Xeromyces bisporus* has been shown to asexually sporulate at a water activity as low as 0.663 in 80 days at 25°C (Pitt and Christian, 1968).

Mold growth is dependent upon such factors as temperature, a_w, and atmospheric conditions. Many studies indicate that most storage fungi are inhibited by atmospheres containing less than 1% O_2 or greater than 80% CO_2 (Horner and Anagnostopoulos, 1973; Northolt and Bullerman, 1982; Ellis *et al*., 1994; El Halouat and Debevere, 1997). At moisture contents (m.c.) below the level permitting bacterial growth (a_w <0.87), most storage fungi are fully inhibited in atmospheres containing less than 1% O_2. This concept has not yet been addressed to the preservation of dried fruits such as raisins and prunes.

Thus, we hypothesized that prunes stored in modified atmospheres (MAs) will tolerate higher water activity levels than those stored at normal atmospheres, and that the prunes, based on their infection level by microorganisms, may generate the MAs that inhibit mold activity in airtight conditions. Therefore, the objective of this study was to investigate the respiration rate of moist prunes at various temperatures and water activities to determine if airtight conditions will be sufficient to create self-generated atmospheres to prevent microbial populations from causing damage to the fruit.

MATERIALS AND METHODS

Prune samples

Prunes were taken from the 1998 crop that had been commercially dried and stored four months at an initial m.c. of 16% (National Raisin Company, Fowler, CA) prior to sampling. Twelve 2.5 kg samples were artificially moisturized in 2% increments between 16 and 38% m.c., allowed to equilibrate in 3.785 L jars for at least 4 weeks at 0±2°C, and then were sealed in the jars and incubated at 25, 30, and 35°C. At regular intervals, water activity was determined using a Novasina ms1 Defensor® with an enMBRK-3 sensor and an r.h. equilibration chamber of 68 mm i.d. and 100 mm length (363 mL). A sample of 25-28 prunes weighing about 150 g was taken from the selected sub-sample and placed in the ERH apparatus. After equilibrating to the set temperature for 24 h, the samples were left in the apparatus for another 2 h to equilibrate before the relative humidity was determined.

Microbial count

The level of microorganisms on the prunes was determined by aerobic plate count (APC) on single 50 g samples taken at the start and at the end of the experiments (Maturin and Peeler, 1998). Also, the level of yeasts and molds was determined on the same 50 g samples as were the APC tests (Tournas *et al*., 1998).

Respiration of prunes

The respiration rate was based on O_2 consumption. The interstitial air space of about 500 g prunes was periodically measured in a respirometer that consisted of a 0.95 L Mason jar with two 1.6 mm i.d. copper tubes soldered to the lid. An oxygen monitor (electrolytic sensor type) (Oxychek-2, Bishop, UK), equipped

with an internal air pump, measured O_2 levels. The monitor was fitted to the two copper tubes soldered to the jar lid. The copper tubes, fitted with two-way valves outside the jar, extended inside the jar to about 180 mm below the lid (inlet) and to about 20 mm below the lid (outlet). Because pressurization of some respirometers occurred, particularly in those containing high moisture prunes, the pressure differential (up to 27 kPa) was measured using a manometer. Because the Oxychek-2 sensor was influenced by excessive pressure, the pressure was released before taking a measurement.

RESULTS AND DISCUSSION

Microfloral count

The aerobic plate count (APC) is intended to indicate the level of microorganisms in a product. In order to ensure that microorganismal growth did not take place during the equilibration period at the low temperature, we measured respiration before the prunes were sealed in the respirometers. These results (Fig. 1) showed that the microorganisms remained at acceptable levels, except at 0.83 a_w. The increase in microflora at 0.83 a_w is inexplicable but could be a result of contamination that occurred after the sample was removed from the cold chamber. Because these determinations were carried out without replicates, the increase in the APC count at 0.83 a_w will require a more detailed study. The samples sealed in the respirometer at 35°C for 35 days showed a low microfloral load without significant changes throughout the range of moisture contents (Fig.1). Microorganism growth was not detected at 25 or 30°C and is not reported.

Microscopic food-borne yeasts and molds have the ability to attack prunes due to their relatively broad environmental requirements. The majority of yeasts and molds are obligate aerobes, i.e. they require free oxygen for growth. Also, their temperature range is broad (between 10 and 35°C). Moisture requirements for molds are low; most species can grow at 0.85 a_w or less. However, yeasts generally require a higher water activity (Tournas *et al.*, 1998). Our yeast counts (Fig. 2) show that initial samples remained low, indicating that cold storage during the moisturizing period prevented development of yeasts. But on three samples kept in the respirometer, final yeast counts were markedly higher than initial samples. In one (0.84 a_w), counts were similar to those of the initial samples, indicating the presence of aerobic and anaerobic yeasts in the samples. In that instance, aerobic yeasts may have been inhibited due to the low O_2 content that prevailed in the respirometer; alternatively, some contamination could have occurred.

Mold count gave the most meaningful results (Fig. 3). Their development on initial samples was not intensive but a population survived. However, during sealed storage in the respirometer, counts were extremely low indicating that aerobic molds could not survive the modified atmospheres. El Halouat and Debevere (1997) studied the effect of water activity, MA packaging and storage temperature on spore germination of molds isolated from prunes. Their results showed that under anaerobic atmospheres, germination and growth of all tested

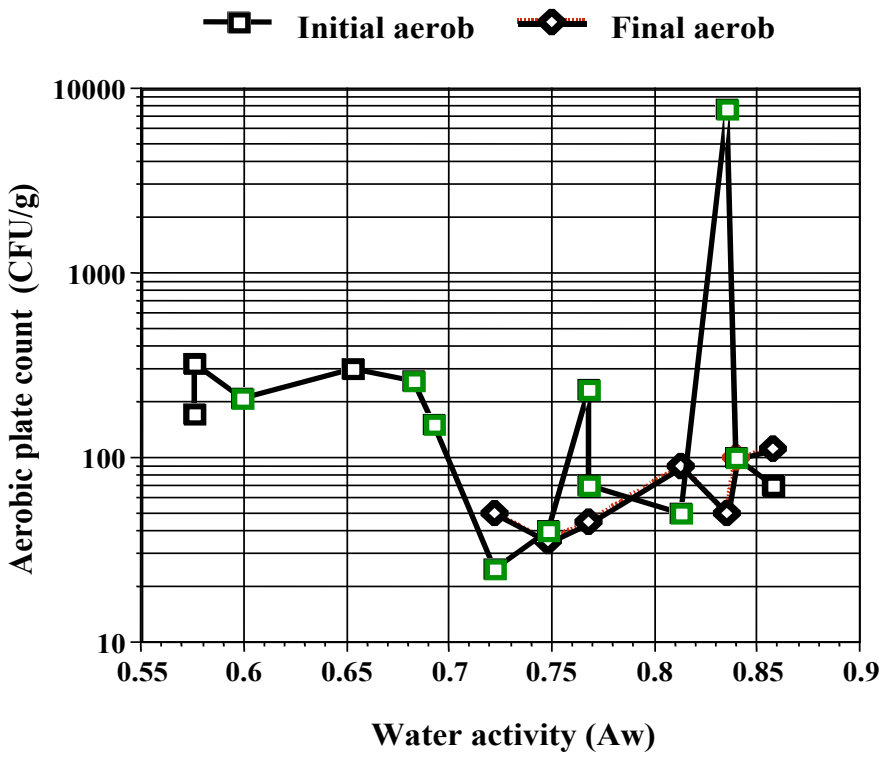

Fig. 1. Aerobic plate count (APC) in CFU/g in relation to water activity of moisturized prunes before sealing in the respirometer (Initial aerob) and after 35 days in sealed conditions in the respirometer at 35°C (Final aerob).

species were completely inhibited at low a_w, while at high a_w, because of residual O_2, conidia germinated but failed to grow. At any a_w value between 0.88 and 0.92, conidia failed to germinate when 100% CO_2 was applied. Similar results were obtained for *E. amstelodami* and *Fusarium oxysporum* in 80% CO_2/20% N_2 and in 60% CO_2/40% N_2. Under aerobic conditions (5% O_2), germination and growth occurred only at high a_w, while 10 or 20% O_2 combined with either 80 or 60% CO_2 only delayed conidial germination and mold growth when compared with the control in air. Our results on mold development are in agreement with the detailed study of El Halouat and Debevere (1997). Indeed, even after exposure for more than 5 months in the respirometer, mold development could not be detected at any tested moisture level.

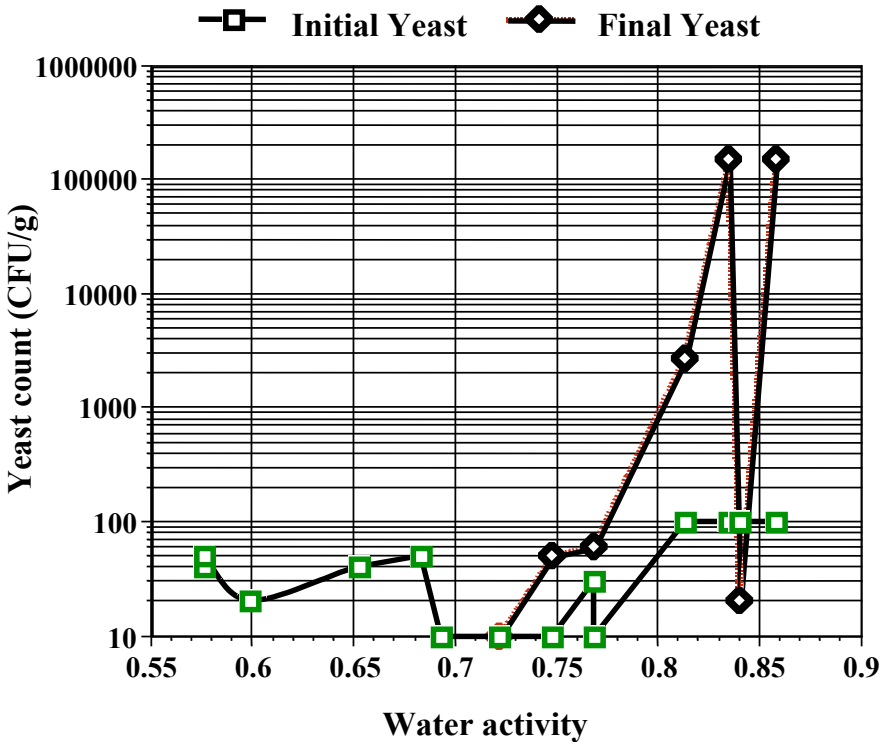

Fig. 2. Yeast count in CFU/g in relation to water activity of moisturized prunes before sealing in the respirometer (Initial Yeast) and after 35 days in sealed conditions in the respirometer at 35°C (Final Yeast).

Respiration of prunes

Physiologically, dried prunes, especially those dried in commercial dehydrators at elevated temperatures such as 80 to 86°C, are considered as non-living organisms. Therefore the observed respiration processes could be attributed only to the microorganism load and its composition on the prunes. These respiration rates are shown in Figs. 4 - 6. The higher the a_w, the more intense was the oxygen consumption. In most cases, a nearly linear relationship was observed between oxygen depletion and time. At the highest moisture content (0.858 a_w), a nearly anaerobic condition (<1% O_2) was achieved within two days at 25°C (Fig. 4). At 30°C, a similar condition (<1% O_2) was achieved within 40 h at 0.858 a_w, and reduction to about 1% O_2 was achieved within 70 h at 0.824 and 0.836 a_w (Fig. 5). At 35°C, nearly anaerobic conditions were achieved within 4 days at the 3 highest water activities (Fig. 6).

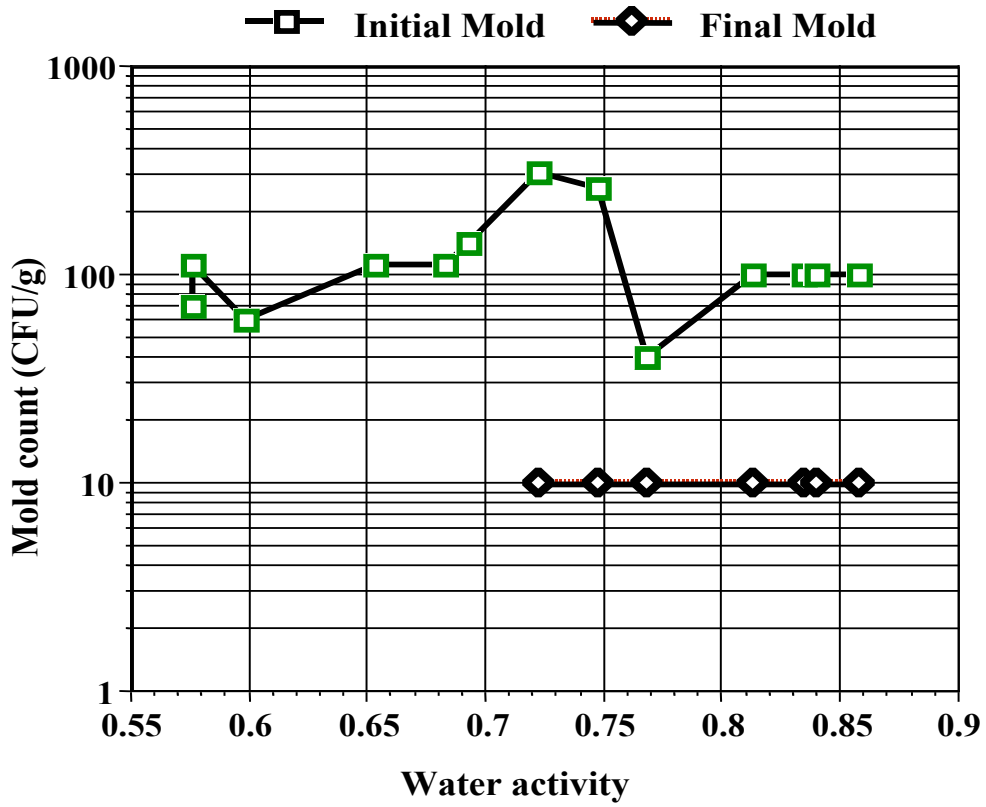

Fig. 3. Mold count in CFU/g in relation to water activity of moisturized prunes before sealing in the respirometer (Initial Mold) and after 35 days in sealed conditions in the respirometer at 35°C (Final Mold).

Respiration rates as a function of water activity and temperature are shown in Fig. 7. These data show that, regardless of the temperature, an intense deterioration of prunes may occur under sealed storage conditions at or above 0.81 a_w.

CONCLUSIONS

The results of our study indicate that, at aerobic conditions, microorganisms can flourish and cause deterioration of prunes if the a_w of the fruit is above 0.7 (m.c. of 24.6%). However, under sealed conditions, prunes can tolerate a higher water activity without microorganism growth and fruit deterioration. We conclude that California prunes can be safely stored at higher than normal moisture contents when under hermetic conditions compared with an unsealed environment. In addition, the microorganisms that naturally contaminate prunes stored at high moisture contents can produce a toxic MA capable of controlling microbial growth and subsequent deterioration of stored prunes.

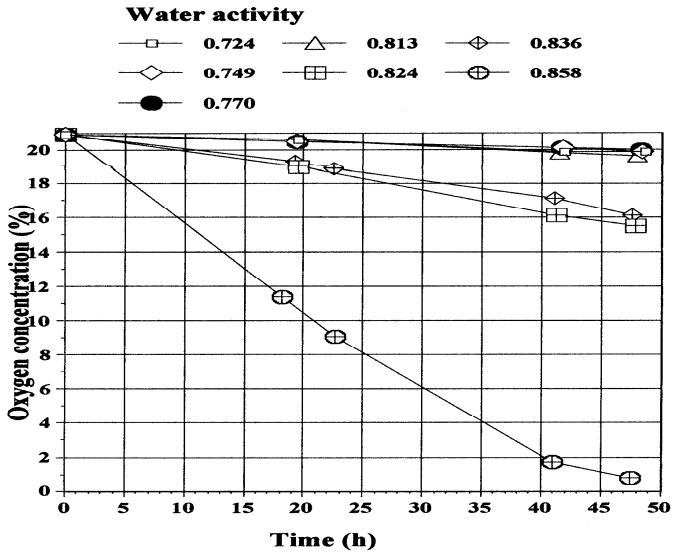

Fig. 4. Oxygen depletion in the respirometer as a result of respiration of moisturized prunes at 25°C and various water activities.

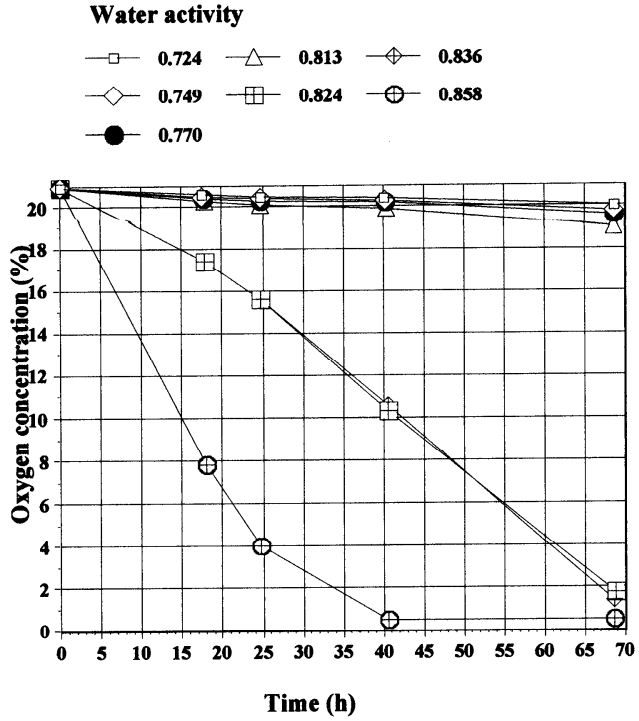

Fig. 5. Oxygen depletion in the respirometer as a result of respiration of moisturized prunes at 30°C and various water activities.

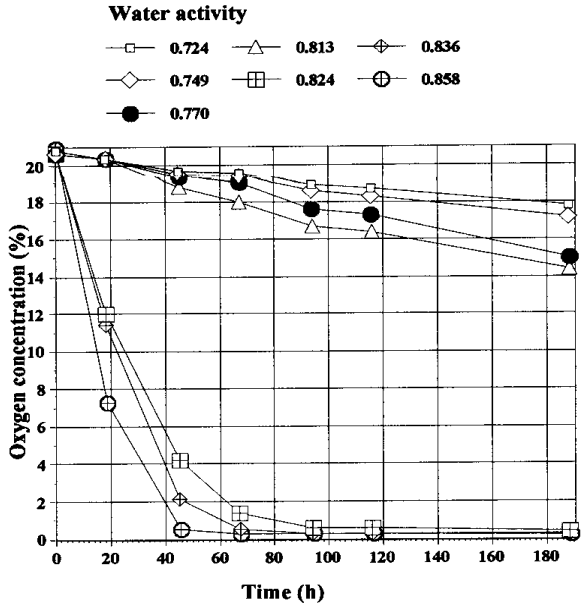

Fig. 6. Oxygen depletion in the respirometer as a result of respiration of moisturized prunes at 35°C and various water activities.

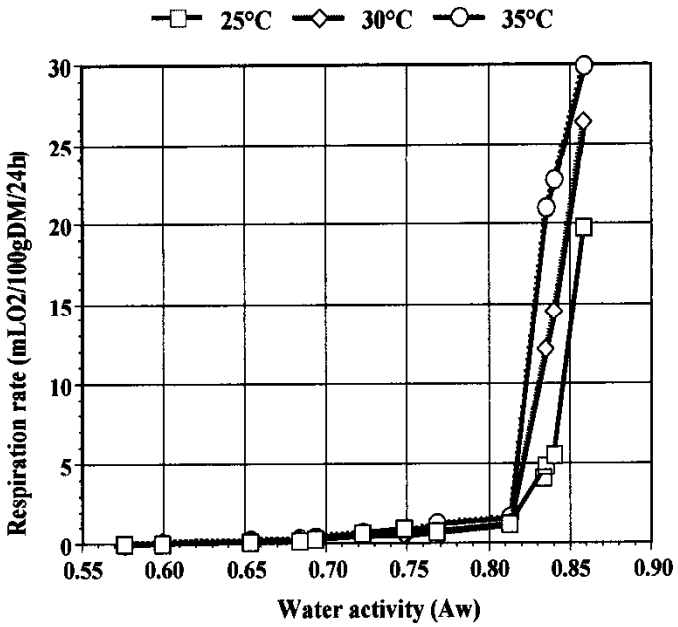

Fig. 7. Respiration rates of prunes stored at 25°, 30° and 35°C at different water activity (a_w) values. Rates were determined on "**moisturized samples**" after they had reached equilibrium moisture content.

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