Unexpected Thermal Destruction of Dried, Glass Bead-Immobilized Microorganisms as a Function of Water Activity

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To help us understand the factors and mechanisms implicated in the death of microorganisms or their resistance to temperature in a low water activity environment, microorganisms were dried on the surface of glass beads before being subjected to high temperatures for a short period followed by rapid cooling. Two microorganisms were studied: the yeast Saccharomyces cerevisiae and the bacterium Lactobacillus plantarum. Experiments were carried out at 150, 200, and 250°C, with four durations of heat treatment and seven levels of initial water activity between 0.10 and 0.70. We observed an unexpected range of water activity, between 0.30 and 0.50, at which microorganisms were more resistant to the various treatments, with maximal viability at 0.35 for L. plantarum and 0.40 for S. cerevisiae.

In dried food products, microorganisms cannot grow and multiply because of the small amount of available water. Nevertheless, these microorganisms are still viable and retain the potential to multiply if rehydrated. Thus, it is important to decontaminate food powders efficiently, especially if these powders must be incorporated into a more complex preparation with higher water content. Processes used to decontaminate dried food products often induce organoleptic degradation or allow only a low destruction rate. We propose that exposing dried food products to very high temperatures for a short time followed by rapid cooling might lead to good decontamination with less damage. Before a new process for decontaminating pulverulent substances is tested, it is important to understand better the factors and mechanisms involved in microorganism death or resistance to temperature in a low water activity (\(a_w\)) environment. For this study, two microorganisms were chosen: the yeast Saccharomyces cerevisiae and the bacterium Lactobacillus plantarum. The microorganisms were placed on the surface of glass beads to allow us to study the effects of heat treatment directly, without any interaction with supporting food powders.

Several authors have studied the destruction of microorganisms by heat, sometimes with conditions of low \(a_w\) (2, 15, 24). However, most of these studies were performed with liquid media by using solutes to depress the water activity (5, 6, 8, 13, 18, 23). Particular attention has been paid to spore-forming organisms compared with non-spore-forming species (1, 3, 19, 19a), because of their greater resistance to heat, and also to food-borne pathogens such as Listeria monocytogenes (16) and Escherichia coli O157:H7 (10). We studied here the effects of high hot and cold temperature shocks on the viability of dried vegetative cells.

S. cerevisiae strain CBS 1171 cells were maintained on petri dishes with modified Malt Wickerham medium supplemented with 20 g of agar (VWR International, Fontenay sous Bois, France)/liter. The yeast was grown aerobically, as previously described (12), in 250-ml conical flasks containing 100 ml of modified Malt Wickerham medium with 10 g of glucose (VWR International)/liter, 3 g of pancreatic peptone (VWR International)/liter, 3 g of yeast extract (Institut Pasteur, Paris, France)/liter, and 1.5 g of NaH₂PO₄ (VWR International)/liter. The pH was adjusted to 5.35 by the addition of orthophosphoric acid (Sigma, Saint-Quentin Fallavier, France) before sterilization by autoclaving at 121°C for 20 min. The flasks were shaken at 250 rpm on a rotary shaker (New Brunswick Scientific, Edison, N.J.) at 25°C for 48 h. An aliquot (1 ml) of culture was transferred into a conical flask containing the same medium and allowed to grow to early stationary phase (for 48 h, to a final concentration of \(10^9\) cells/ml as determined by counting CFU).

L. plantarum strain 103151 T cells (Institut Pasteur) were maintained on petri dishes with MRS medium (Biokar Diagnostics, Beauvais, France) supplemented with 20 g of agar (VWR International)/liter. The bacteria were grown in 250-ml conical flasks containing 100 ml of MRS medium at 30°C for 18 h without shaking. An aliquot (1 ml) of culture was transferred into a conical flask containing the same medium and allowed to grow to the early stationary phase (for 20 h, to a final concentration of \(10^9\) cells/ml as determined by counting CFU).

Cultures of microorganisms in the early stationary phase were harvested by centrifugation (2,000 × g, 5 min), and cells were washed twice in a binary water-glycerol solution at an \(a_w\) of 0.992. The pellet was mixed with 30 to 40 g of glass beads (average diameter, 0.75 mm; Fisher-Bioblock, Illkirch, France) that had been sterilized by autoclaving at 121°C for 20 min. The mix was dried in a climatic chamber (type 320H60/1.5; ClimaTech-Sapratin, St. Médard d’Eyran, France) coupled with a dryer (type MLC450; Munters, Colombes, France) for various periods of time depending on the \(a_w\) to be reached, as previously described (20). A temperature of 5°C and a relative humidity of 1% were maintained in the chamber during drying.
After drying, the samples were stored in aluminum bags sealed with a vacuum to prevent rehydration.

Experiments were performed at various initial \( a_w \) values (0.10, 0.20, 0.30, 0.40, 0.50, 0.60, and 0.70). During drying, changes in the \( a_w \) values of the samples were monitored to determine the duration of drying necessary in each case. The \( a_w \) values of solutions and samples were verified by using a dew point osmometer (Decagon Devices Inc., Pullman, Wash.).

Dried samples obtained were heated by being blown with hot air (150, 200, or 250°C) from a hot-air generator (Vulcanic, Neuilly sur Marne, France) for a short time (5, 10, 20, or 30 s) and immediately cooled with a cold gas (CO\(_2\), –70°C). For heating and cooling, samples (10 g) were placed in a metallic spherical grid, which could retain particles of more than 0.5 mm in diameter. The sphere, held with pliers, was placed under the hot airflow and shaken slightly to distribute the particles homogeneously. The sphere was then placed under the –70°C CO\(_2\) flow to cool. The temperature of the sample was monitored during each experiment by using a thermocouple in the center of the sphere, near the glass beads, and temperature acquisition occurred at increments of 3.34 ms.

After heating, 1 g of the treated glass beads was mixed with 9 g of water-glycerol solution (\( a_w = 0.992 \)) and shaken for 30 min to wash beads and reconstitute cellular suspensions. Decimal dilutions were made and spread on Malt Wickerham agar medium (for \( S.\) cerevisiae) or MRS agar (for \( L.\) plantarum). Viability was determined by counting CFU and related with the viability of controls, which were samples that had been dried to the same \( a_w \) but not heated.

The aim of the first set of experiments was to obtain dried and viable microorganisms in sufficient quantities to achieve heat destruction. For the two types of microorganisms, cells suspensions were mixed with glass beads at a concentration of about 6 \( \times \) 10\(^8\) cells per g. During drying, the viability of \( S.\) cerevisiae decreased slightly more than that of \( L.\) plantarum. Four hours of drying allowed the mix to reach an \( a_w \) of 0.07, with a cellular concentration of about 10\(^2\) to 10\(^3\) cells per g of mix. These experiments have permitted us to observe mortality as a function of drying duration and to adjust the amount of glass beads, enough to prepare a mix containing 10\(^8\) cells/g (±10%), at each \( a_w \).

The viabilities of \( L.\) plantarum cells as a function of \( a_w \) for the four durations of treatment and the three temperatures are presented in Fig. 1. Experiments were performed at least three times, and mean values as well as 95% confidence intervals of the means were calculated. These confidence intervals were found to be less than 15% of the mean viabilities. The first observation was that a short, hot treatment followed by rapid cooling permits significant reductions of up to 10\(^3\) cells per g, depending on the conditions. For each of the three temperatures tested, and whatever the initial \( a_w \) of the sample, viability was lower with longer treatment. However, viability was better maintained around an \( a_w \) of 0.35, regardless of the temperature used. For \( a_w \) values near 0.20 or 0.60, viabilities were similar, but they were lower by about 10- to 10\(^2\)-fold than the viability found at 0.35. For the lowest \( a_w \) (0.10), viability was lower by about 10-fold more, but the lowest viabilities were found for treatments at an \( a_w \) of 0.70. Thus, the viability of \( L.\) plantarum is closely dependent on the \( a_w \), and the microorganism shows an optimum resistance at around 0.35.

As for \( S.\) cerevisiae, for each of the three temperatures tested, and whatever the \( a_w \) of the sample, viability was lower with increased duration of treatment. There was an optimum resistance between initial \( a_w \) values of 0.20 and 0.50, with the maximum at around 0.40, but the \( a_w \) was less important than it was for \( L.\) plantarum. Moreover, unlike \( L.\) plantarum, the viability of \( S.\) cerevisiae at around 0.70 (more than 7 logs destruction) was higher than or equal to that found at the lowest \( a_w \) (0.10, a maximum of 6 logs destruction).

To better understand the influence of the temperatures used, the results are presented differently in Fig. 2, where we have shown viability as a function of \( a_w \) for two durations. For 5 s of treatment (Fig. 2a and b), we observed that the viability of \( S.\) cerevisiae was lower when the temperature was higher. However, for \( L.\) plantarum, at an \( a_w \) of 0.30 (near optimum resistance), viability was independent of the temperature treatment. This phenomenon was also observed for heat treatments of 10, 20, and 30 s, and for longer durations of treatment, we observed destruction levels that did not vary significantly with the temperatures applied (Fig. 2c and d).

At 150°C, the viability of \( S.\) cerevisiae at an \( a_w \) of 0.10 was lower than that of \( L.\) plantarum whatever the duration, except with a 5-s treatment after which the viability of \( L.\) plantarum was lower by about 1 log. At the opposite end of the \( a_w \) range, at an \( a_w \) of 0.70, \( S.\) cerevisiae seemed to be more resistant to heat. Moreover, the viabilities of \( S.\) cerevisiae and \( L.\) plantarum at \( a_w \) values of 0.30 and 0.50 were approximately the same.

At 200°C, we observed that the viability of \( S.\) cerevisiae at an \( a_w \) of 0.10 was lower than the viability of \( L.\) plantarum, whatever the duration of treatment. As observed at 150°C, \( S.\) cerevisiae seems to be more resistant than \( L.\) plantarum at an \( a_w \) of 0.70. The viabilities of \( S.\) cerevisiae and \( L.\) plantarum are approximately the same at \( a_w \) values of 0.30 and 0.50.

At 250°C, we observed again that the viability of \( S.\) cerevisiae at an \( a_w \) of 0.10 was lower than that of \( L.\) plantarum, whatever the duration tested. At an \( a_w \) of 0.50 or 0.70, \( S.\) cerevisiae was more resistant than \( L.\) plantarum. At an \( a_w \) of 0.30, for 20 or 30 s of heating, the viabilities of \( S.\) cerevisiae and \( L.\) plantarum were approximately the same, whereas with treatments of 5 or 10 s, \( L.\) plantarum seemed to be more resistant.

Several authors have shown that the resistance of microorganisms to thermal stress is influenced by growth temperature and growth phase. However, the thermostability of microorganisms also varies with the \( a_w \) of the medium. Early studies by Calhoun and Frazier (5), Goepfert et al. (14), and Corry (8) have shown that the addition of salts or sugars can raise viability at lethal high temperatures. Precht et al. (21) observed that superheated steam acting as dry air at 140 to 150°C was less lethal than wet steam at 100°C, showing the importance of water in the heat inactivation process. Most studies on the influence of \( a_w \) level on the heat resistance of microorganisms were carried out with sporulated microorganisms. These studies showed that the heat resistance of microorganisms increases as their water content decreases (4, 7, 9) and that an optimum resistance is found between \( a_w \) values of 0.2 and 0.5, depending on the microorganism studied (7). For example, the thermostability of \( Salmonella\) enterica serovar Typhimurium was found to be maximal at an \( a_w \) of 0.2 (8). In the present study, the microorganisms used do not induce sporulated forms and always remain in a vegetative form.
In our experiments, for each strain and for the three temperatures tested, we identified an unexpected range of $a_w$ values at which microorganisms were more resistant. This range was generally between initial $a_w$ values of 0.30 and 0.50, with a maximal viability at 0.35 for L. plantarum, whatever the temperature studied, and at 0.40 for S. cerevisiae.

Cells are able to acquire resistance to a severe stress condition when they have been previously exposed to a mild form of the same or a different stress (17, 22). Dried and viable microorganisms in food powders have suffered many stresses (thermal, hydric, and ionic) and have developed adaptation mechanisms. Nevertheless, these mechanisms, such as trehalose accumulation or stress protein synthesis, require some nutrients. In our study, cells were washed and resuspended in a binary water-glycerol solution ($a_w, 0.992$) before being mixed with the glass beads and dried. Thus, the cells did not have any nutrients available to repair damage caused by the drying process or heat stress, and only the quantity of available water could have influenced the viability results.

It has been postulated that the water that is in close contact with the proteins could be a factor in determining cell inactivation (21). As the cell is heated, water molecules begin to vibrate, and this vibration causes the disulfide and hydrogen bonds in the surrounding proteins to weaken and break, altering the final three-dimensional configuration and possibly preventing the protein from functioning (11). When only a small amount of water is present, these vibrations are reduced, decreasing protein denaturation. In our case, the greater viability obtained for a sample at an initial $a_w$ of around 0.40 than for a more hydrated sample can be explained by this mechanism.

**FIG. 1.** Destruction of L. plantarum (a, c, and e) and S. cerevisiae (b, d, and f) cells on glass beads at 150°C (a and b), 200°C (c and d), and 250°C (e and f). Duration of heat treatment: ●, 5 s; ■, 10 s; ▲, 20 s; ○, 30 s.
However, at lower \( a_w \)s, viability decreased, and other experiments would be necessary to understand why.

This study has shown that the initial \( a_w \) of the sample is very important to the destruction or survival of dried microorganisms subjected to this kind of heat stress and to the efficiency of powder decontamination by this technique. However, it seems likely that a short, hot treatment followed by rapid cooling will permit significant reductions of up to \( 10^7 \) cells per g on the surface of particles. Nevertheless, microorganisms may sometimes be located in the center of the particles, in which case they may be less sensitive to treatment. It seems therefore necessary to study the destruction of microorganisms incorporated into food powders with the new drying method that has been developed in our laboratory, which permits us to obtain particles containing \( 10^8 \) viable cells per g at various \( a_w \) values. Finally, using successive heating and cooling sequences to decontaminate food powders must not modify the functional properties of the product. As color is a good indicator of the degradation due to Maillard reactions, it would be interesting to measure the color of food samples, such as flour and dried milk, after treatment and to determine the time and temperature combinations that permit a large decrease of microbial contamination without altering the properties of the product.

REFERENCES
Gompertz equation to predict the effects of temperature, pH, and NaCl on the inactivation of *Listeria monocytogenes* Scott A heated in infant formula. *J. Food Prot.* **59**:16–23.


