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# Effect of sugar concentration and pasteurization on survival of *Rhodotorula rubra* and *Candida intermedia* in orange juice

# Efecto de la concentración de azúcar y de la pasteurización en la resistencia de *Rhodotorula rubra* y *Candida intermedia* en jugo de naranja

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## Resumen

*Rhodotorula rubra* y *Candida intermedia* aisladas de naranjadas pasteurizadas comerciales, fueron inoculadas en jugo de naranja estéril con una concentración total de azúcares de 12, 20, 30, 40, 56 y 62 °Brix e incubadas por 48 horas a 28 °C. Los azúcares fueron fructosa, glucosa y sacarosa en una proporción 1:1:2. Los cultivos de las levaduras a cada concentración de azúcares fueron pasteurizados e incubados durante 48 y 72 horas a 28 °C. Los resultados mostraron que ambas levaduras crecieron en todas las concentraciones de azúcar. Después de la pasteurización Rhodotorula rubra pudo sobrevivir cuando el contenido de azúcar fue mayor o igual a 40 °Brix y *C. intermedia* no pudo sobrevivir para ninguna concentración de azúcar.
**Palabras claves:** jugo de naranja, *Rhodotorula rubra*, *Candida intermedia*, concentración de azúcar, resistencia térmica, sobrevivencia.

## Abstract

*Rhodotorula rubra* and *Candida intermedia* isolated from commercial pasteurized orangeades, were inoculated in sterile orange juice with total sugar concentrations of 12, 20, 30, 40, 56, and 62 °Brix, and incubated for 48h at 28 °C. The sugars were fructose, glucose and sucrose at a 1:1:2 proportion. The cultures of the microorganisms at each sugar concentration were pasteurized and incubated for 48 and 72h at 28 °C. Results showed that both yeasts grew at all sugar concentrations. After pasteurization Rhodotorula rubra could survive only when sugar content was equal to or higher than 40 °Brix, and *C. intermedia* could not survive at any sugar content.
**Key words:** Orange juice, *Rhodotorula rubra*, *Candida intermedia*, sugar concentration, thermal resistance, survival.

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## Introduction

Pasteurized orangeades results from dilution of orange juice concentrate from 58 to 12 °Brix. (11). This product is widely consumed in Venezuela, principally by children. The shelf life of this product is very short, about 2 to 3 days, after which it presents changes in its organoleptic properties like large amount of sediments, redish color, and undesirable acidic flavor. Many investigations on microflora of citrus juices and by-products have reported that yeasts most frequently isolated from orange juice are *Candida* sp, *Candida intermedia*, *Candida maltosa*, *Candida mongoliae*, *Candida tropicalis*, *Rhodotorula* sp., *Rhodotorula rubra*, *Pichia membranaefaciens* and *Saccharomyces cerevisiae*, among others (3,8,16). In Venezuela, Guerra (8) and Delgado (3) isolated and identified the microflora of pasteurized orangeades and found that *R. rubra* and *C. intermedia* and *Candida* sp. were present in a very high concentration (>104 ufc/ml). Yeasts are the major target of pasteurization when frozen orange juice concentrate is reconstituted for chilled juice production (10). The injury and recovery of yeasts after thermal treatments have been investigated (3, 6, 7, 9, 17).

Thermal resistance of microorganisms, as well as kinetics of microbial spoilage were reported by several authors (3, 9, 14). Well-defined thermal inactivation studies are necessary for those microorganisms present in pasteurized orangeades in order to define process heating requirements and avoid quality defects associated with yeast growth. In addition, it has been shown that sugar concentration affects thermal resistance (3, 9).

The objective of this work is to study the growth and survival of the yeasts *R. rubra* and *C. intermedia* over a wide range of sugar soluble solids concentration, and the recovery of the yeast after a bach pasteurization process in those high soluble solid medium. This work will be useful to design thermal studies to find appropriate heating process conditions for these products.

## Materials and methods

**Yeasts.** Strains of *Candida intermedia* (LMA188) and *Rhodotorula rubra* (LMA288) isolated from commercial pasteurized orangeades by Delgado (3) and Guerra (8), were obtained from the Laboratorio de Microbiología de Alimentos, Universidad del Zulia, Venezuela, and stock cultures were maintained on malta agar (Difco, Detroit, MI) at 4 °C. Inocula for the experiments were prepared by adding a fresh culture of the yeasts, adjusted to have between 40 and 60 cells per field with the 40X objective of the microscope.

**Preparation of orange juice at different concentrations of soluble solids.** Orange juice was extracted from healthy, ripe and clean oranges (*Citrus sinensis*). Density, titratable acidity, pH, and soluble solids concentration were determined (1). According to the initial soluble solids concentration, fructose, glucose and sucrose (SIGMA,St. Louis, MO) in a 1:1:2 proportion were added to obtain the following concentrations of total sugars: 20, 30, 40, 56, and 62 °Brix. A temperature-controlled ABBE refractrometer was used (Cole-Parmer, Niles, IL). Orange juice with an original soluble solid content of 12 °Brix was used as a control. All juices were sterilized at 105 °C for 15 min, cooled and stored at 4 °C, to be used no more than 30 min after preparation.

Determination of high sugar concentration resistance. Both strains of yeast were cultured in triplicate by adding 1 mL of inoculum to 100 mL orange juice aliquots prepared at 12, 20, 30, 40, 56, and 62 °Brix. The cultures were then incubated at 28 °C for 48 h and stored at 4 °C. Cell concentration was evaluated by surface plating, in duplicate, on malta agar followed by incubation under aerobic conditions at 28 °C for 48 h.

Determination of pasteurization resistance. Three test tubes (6x50mm) interconnected by a glass rod, each containing 2 mL of culture for each concentration, were placed in a temperature controlled and shaking water-bath (Precision, Cole-Parmer, Niles, IL) and pasteurized for 30 min after reaching 63 °C. Vials were withdrawn after the selected time, cooled on ice, then incubated at 28 °C for 48 h and 72 h. After the incubation period, the vials were enumerated for both yeast as outlined above. The bath and sample temperatures were gathered during the thermal treatment. Temperature stability in the bath during the runs was 5 °C from the set point.

**Statistical Analysis.** The data were analyzed by analysis of variance according to Snedecor and Cochran (18).

## Results and discussion

*R. rubra* and *C. intermedia* were chosen as the target organisms for this study because of their prevalence in pasteurized orange juice sold in Venezuela (3, 8, 19).

The orange juice extracted had 12 °Brix, 1.05 g/ml of density, 2.55 g/100 g of titratable acidity (expressed as citric acid), and pH of 3.5.

Effect of sugar concentration. Figure 1 shows that *R. rubra* could grow to a high cell count over all the sugar concentration ranges. *R. rubra* cell counts showed no significant differences at the different sugar concentrations ($P < 0.05$). On the other hand, *C. intermedia* could grow in the range of 12 to 40 °Brix up to 10E7 CFU/mL without significant differences ($P < 0.05$), but for 56 and 62 °Brix, there were lower cell counts, and these were significantly different from the cell counts at other sugar concentrations ($P < 0.05$). Although *C. intermedia* did not grow at these sugar concentrations, it could be detected on malta agar, showing that many were capable of surviving.

Effect of pasteurization. Figure 2 summarizes the effects of sugar on

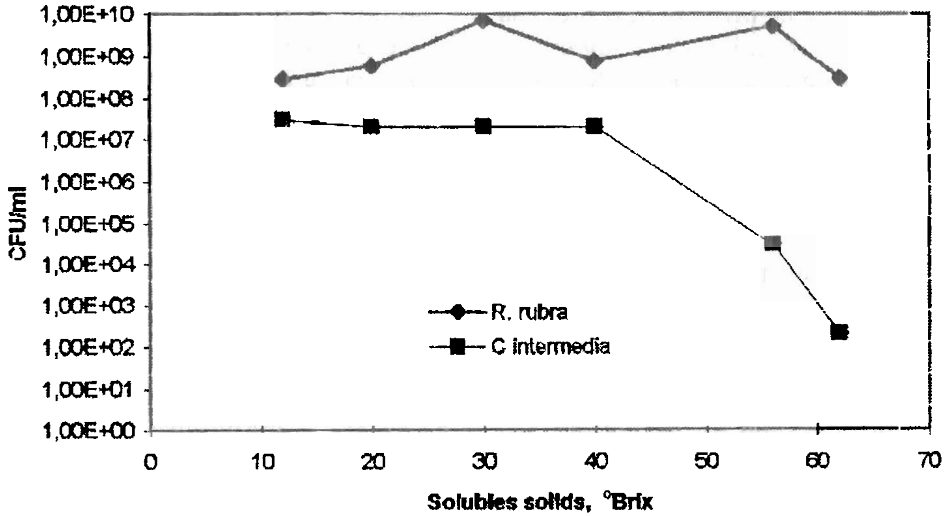


**Figure 1. Survivors of *Rhodotorula rubra* and *Candida intermedia* over a different soluble solids concentration.**

the cell concentration after heat treatment. There are only *R. rubra* cell counts, since *C. intermedia* did not grow at any sugar concentration after pasteurization. Some authors have reported that some species of *Candida* can lose their ability to ferment glucose due to a high temperature exposure, thermal inactivation of the pyruvate decarboxylase enzyme, or inactivation of ribosomes (5,15). Other authors reported that recovery of heat injured cells of *S. cerevisiae* decreased with increasing concentration of glu-

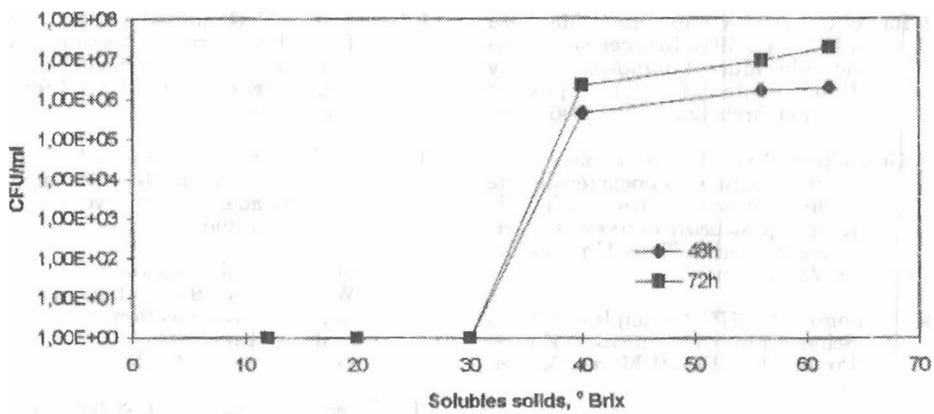


**Figure 2. Cell concentration of *Rhodotorula rubra* after pasteurization at two incubation periods.**

cose (7). The behavior of *R. rubra* was totally different. For a low sugar concentration as in orange juice, thermally injured cells of *R. rubra* could not be recovered (13). In this work, there were not survivors for 12, 20 and 30 °Brix. Barreiro and Vidaurreta (2), reported that *R. rubra* was not resistant to the pasteurization process in orange juice. For the highest sugar concentrations, 42, 56 and 62 °Brix, thermal resistance of *R. rubra* increases for both incubation periods, 48 and 72h, after pasteurization. Juven *et al.* (9) determined that high sugar concentration in orange concentrate increases the heat resistance of yeast, and stated that citric acid also played a role in heat resistance of spoiled yeast in orange concentrate. Since in this experiment juices had the same acid content and only differed in sugar content, the differences were due to differences in sugar concentration. Sadler *et al.* (17) have reported that yeast and mold endured thermal treatments (light and full pasteurization) procedures. In addition, Medina (12) studied by electron microscopy the cellular protoplasm of *R. rubra* cultured at high sugar concentration, showing that cellular protoplasm decreased without plasmolysis. Fennema (4), also indicated that an increase in thermal resistance may be caused by the passive dehydration of cellular protoplasm, providing a mechanism for cell protection at high sugar content. This could explain the increase in thermal resistance of *R. rubra* at a sugar content equal or higher than 40 °Brix, found in this work.

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