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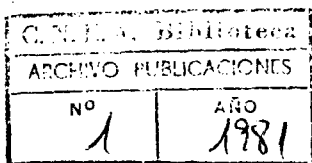
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CONSERVATION OF APPLE AND PEAR JUICE CONCENTRATES

Synergic effect of heat and radiation

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Abstract

CONSERVATION OF APPLE AND PEAR JUICE CONCENTRATES: SYNERGIC EFFECT OF HEAT AND RADIATION.

This paper aims at assessing the feasibility for conserving apple and pear-juice concentrates through the synergic action of heat and radiation. The material was packed in sterile 100- μ m polyethylene bags and, after the treatment was applied, the resulting fractions were stored under room temperature ($25^{\circ}\text{C} \pm 1^{\circ}\text{C}$). The temperature applied to the samples before irradiation was 50°C during 10 minutes and the doses were 100, 200, 300 and 400 krad. For such purposes, a ^{60}Co 165-krad/h source was used, located in a mobile irradiator. Periodical microbiological, chemical and organoleptic controls of the food were performed on both control and on irradiated samples, with or without heat. A single altering microorganism was isolated from all the samples, which was featured as *Saccharomices rouxii*. Adopting the temperature as an application variable and the absorbed dose as a constant, the above osmophilic yeast is considerably more sensitive to radiations when it is suspended in a 50% sucrose solution, after the latter was submitted to a 50°C temperature treatment. It has been proved that 72.5 krad are needed to attain the reduction of a logarithmic cycle in the *Saccharomices rouxii* population irradiated at room temperature, while 36 krad are needed if the sample has been previously heated to 50°C for 10 minutes. An attempt was made to apply the synergism of such process to the juice concentrate. Below 50°C associated with 400 krad gamma radiation, a total inactivation took place in the *Saccharomices rouxii* during the 150 days under analysis. Colour changes were detected in the concentrate; however, the acceptability features for consumption remained at a normal value (level 5) in a hedonic scale of 7 points.

INTRODUCTION

The fruit production in Argentina has led to the development of a juice industry with an important level of production. Consequently, a need has arisen for conservation of the juice under optimum hygiene conditions, avoiding the use of chemical conservation agents and the expenses involved in installing and maintaining cool storage and transport facilities for the finished products.

Within such a framework, tests were performed on the action of intermediate doses of ionizing radiations upon apple and pear juice concentrates. To reduce the doses to be used an association of heat action was attempted, thus adjusting the conditions to a greater synergic effect.

The doses tested resulted from the analysis of tentative effects of radiation upon a highly concentrated matter, almost exclusively constituted by carbohydrates, which are considerably affected by the direct dissipation of the radiation energy and, fundamentally, by the action of free electrons (e^-) and by hydroxyl radicals ($HO^\cdot$) [1-3].

Polysaccharides degrade into monosaccharides and these suffer oxidation processes, becoming acids and/or producing aldehydes (cellular toxics) through the rupture of their annular cycles. This latter phenomenon reaches its maximum, G, in very diluted pure solutions with a clearly alkaline pH (pH 11).

Therefore, considering the high solute concentration and the definite acid pH of the apple and pear juice concentrates, it was felt that no toxic substances would be formed. This consideration would be supported by the results obtained by Baraldi [1] in apple juice, indicating that a 1-Mrad dose does not lead to the formation of toxic aldehydes if the irradiation is performed within a medium with an acid pH (pH 3.5).

MATERIAL AND METHODS

The apple and pear juice concentrates used were obtained from one of the local suppliers, with no special preparation procedures and no addition of chemical conservation agents.

The juice concentration was performed by a heating process. A 10-litre sample of the finished product was obtained from the plant and packed in a hermetic plastic tank, which was then sent to our laboratories and submitted to the treatments scheduled for this project.

The sample was fractionated in aliquots of approximately 100 cm³ and packed in a 100- μ m-thick polyethylene bags that were immediately heat-sealed. All the operations were performed within a laminar air jacket in order to avoid any additional contamination to the finished product. The packed material was distributed in three lots with 60 bags each, which were submitted to the following treatments:

- Lot No.1 was stored at a temperature of $25^\circ\text{C} \pm 1^\circ\text{C}$, as control sample;
- Lot No.2 was submitted to various radiation doses; and
- Lot No.3 was treated at 50°C effective heat and immediately irradiated.

Later on, lots 2 and 3 were fractionated into sub-lots and submitted to doses of 100, 200, 300 and 400 krad in an irradiator with a ^{60}Co source and at a dose speed of 165 krad/h.

The treated and the control samples were also stored at a temperature of $25^\circ\text{C} \pm 1^\circ\text{C}$ and then checked after 0, 15, 30, 45, 60, 90 and 150 days.

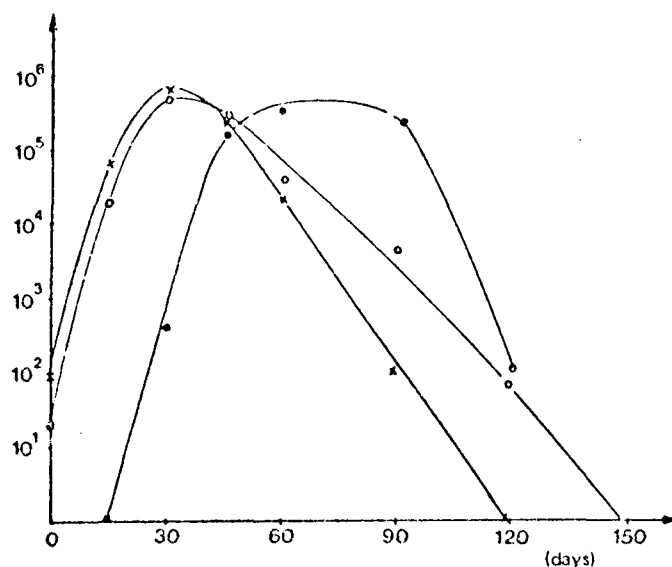


FIG.1. Survival curves of the *Saccharomices rouxii* shown as the number of microorganisms (logarithmic scale) as a function of the storage period shown in days.

X—X Control (no treatment).

o—o 200 krad (without heat treatment).

•—• 200 krad with heat treatment. The samples were stored at $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

MICROBIOLOGICAL STUDY

The following microorganisms were examined: *Lactobacillus* in selective media [4]; coliform bacteria (NMP) in McConkey's broth [5]; aerobic bacteria in TGY agar; and fungi and yeasts in potatoedextrose agar and malt agar.

PHYSICAL AND CHEMICAL STUDIES

The colour was assessed by means of ultraviolet spectrophotometry, using 1% vol/vol dilutions which showed maximum absorption at 282 mμ.

The viscosity, density and pH of the concentrate were assessed at a temperature of 25°C . The concentrations of total soluble solids and of soluble solids in ethanol at 50°C were assessed by means of refractometry.

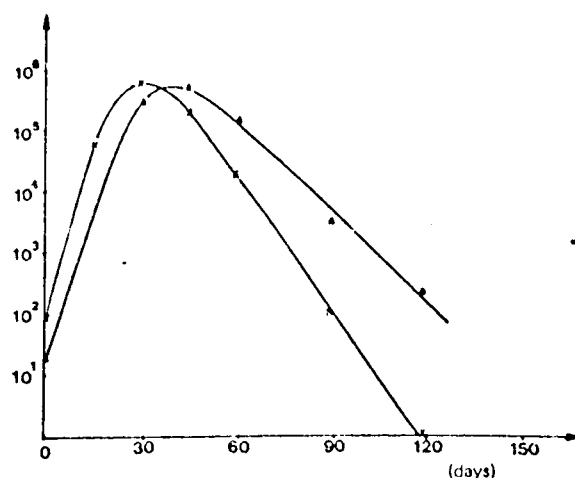


FIG. 2. Survival curves of the *Saccharomyces rouxii* shown as the number of microorganisms (logarithmic scale) as a function of the storage period shown in days.

X—X Control (no treatment).

▲—▲ 400 krad (without heat treatment).

The sample irradiated with 400 krad and heat-treated cannot be graphically shown, since it contained no microorganisms. The samples were stored at $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

The acidity degree was established through volumetry with NaOH (0.1N) in 10% vol./vol. dilutions.

The levels of pectin in the samples were detected by means of the colorimetric reaction produced by galacturonic acid following the method of McComb et al. [6].

PRODUCT ACCEPTABILITY

In addition to the product samples stored at 25°C , control samples stored at $4^{\circ}\text{C} \pm 1^{\circ}\text{C}$ were used. The data obtained was the result of applying the "rating" method, with a hedonic scale of seven points, the fifth of them being considered as normal acceptability.

The evaluation of the results obtained by the various participants was performed according to Dunnett's method [7].

RESULTS

The microbiological analysis showed the presence of a yeast as the only contamination agent of the concentrate. This yeast was featured as *Saccharomyces rouxii*, according to the classification of Lodder and Kreger Van Rij [8].

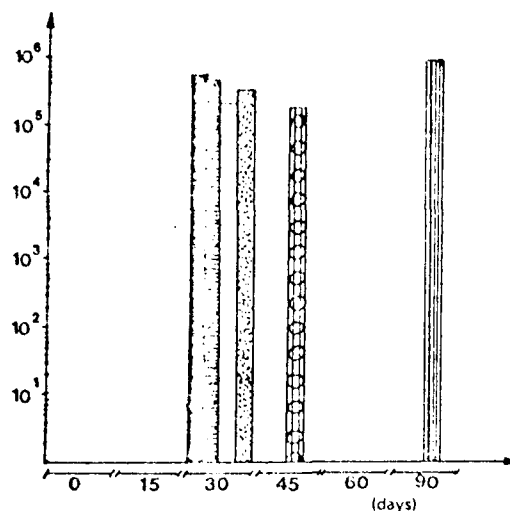


FIG.3. Maximum number of microorganisms of the various samples (in a logarithmic scale) as a function of the storage time expressed in days.

- Control
 - ▨ 200
 - ▩ 300
 - ▤ 400
- } krad without heat treatment
-
- ▨ 200
 - ▩ 300
- } krad with previous heat treatment.

The analysis of the results obtained from the control and from the irradiated samples with no additional heating shows no effects regarding the maximum levels of microbiological development nor the time needed to reach them (Figs 1–3).

After 30 days both the control samples and those irradiated without heat treatment reached their maximum population density in the range of 10^5 microorganisms/ml (Fig.3).

The samples treated with the combined process (heat and irradiation) showed that the time needed to reach their maximum population density had increased in relation to the radiation dose increase, resulting in 45 and 60 days for the samples treated with 200 and 300 krad, respectively (Fig.3).

At this stage of analysing the results, it must be noted that the heat-treated samples submitted to 400 krad irradiation showed no microbiological development at all during 150 days of storage.

Among the physical parameters under analysis, an evident discoloration of the samples was detected, occurring immediately after the irradiation process and in a direct ratio with the applied doses. This phenomenon was not so evident in the samples that were previously submitted to heat (Fig.4).

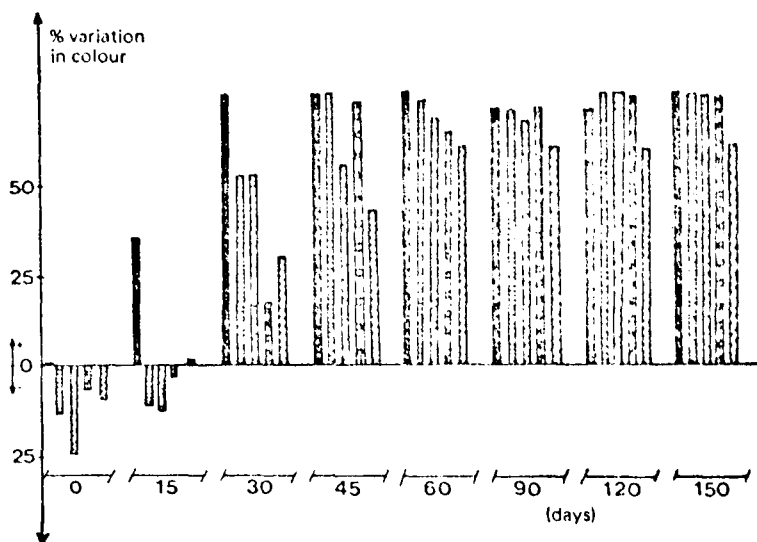


FIG.4. Representation of the percentages of colour variation as a function of time expressed in days. The zero value represents the original colour of the apple and pear juice concentrates. The negative values express discoloration. The positive values express darkening.

- Control
- ▨ 200 } krad without heat treatment
- ▩ 400 }
- 200 } krad with previous heat treatment.
- ▨ 400 }

During the storage period, all the samples suffered a darkening process, which was more accelerated among the control samples (Fig.4).

At day zero of this study, there were practically no differences in the viscosity values of the various samples (Fig.5). During the whole storage period, only the sample treated with 50°C and 400 krad maintained a constant viscosity value. In the rest of the samples, viscosity values decreased as time went by during the first 60 days and then stabilized at values below the initial figures.

In the strict correlation with the results obtained concerning viscosity, at day zero, only a 7% reduction was observed in the concentration of pectins of the treated samples, as compared with the control samples (Fig.6).

No significant differences were detected regarding the density values of the various samples within the period under analysis. The same behaviour was found concerning the analysis of total soluble solids and of those in ethanol at 50°C. It must be noted that the latter includes sugar, which remains soluble when the pectins precipitate.

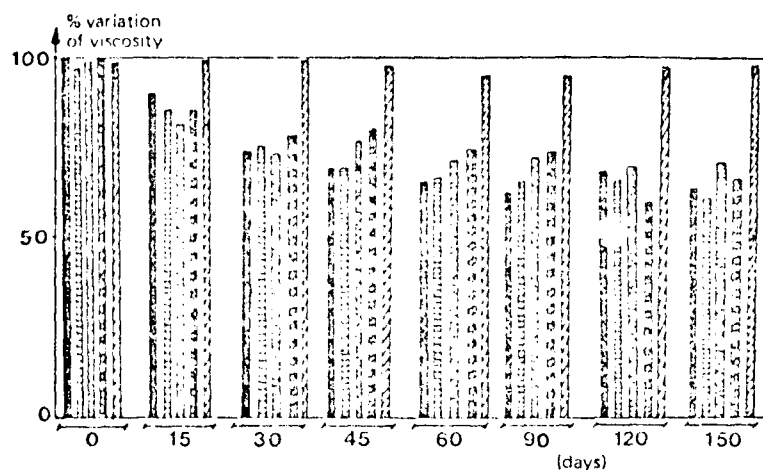


FIG. 5. Representation of the viscosity percentages found for the various samples shown in the graph, as per storage time function expressed in days. 100% corresponds to the original viscosity of the apple and pear juice concentrates.

- Control
- 200 } krad without heat treatment
- ▨ 400 }
- ▤ 200 } krad with previous heat treatment.
- ▥ 400 }

The initial variations observed for the pH, as a direct effect of the applied treatments, were always below 0.02 units. A slight tendency to alkalization was detected in the samples during the storage period. This phenomenon was more outstanding among the control samples than among the irradiated ones. Thus, at day zero, the control samples showed pH 3.56, while those treated at 50°C and with 400 krad showed pH 3.54. At 150 days, those values increased to 3.73 and 3.61, respectively.

The average flavour values obtained at day zero (Fig. 7) showed no detectable significant differences under the utilized evaluation method 6 and agree with those obtained by other researchers [9].

It was considered that the samples became unsuitable for consumption when the average flavour values — as compared with those of the control samples — showed a difference above value "A", with a reliability interval equal to 5%, or when the contamination level reached about 10^5 microorganisms/ml.

The value "A" is defined as:

$$A = ts \frac{P}{N}$$

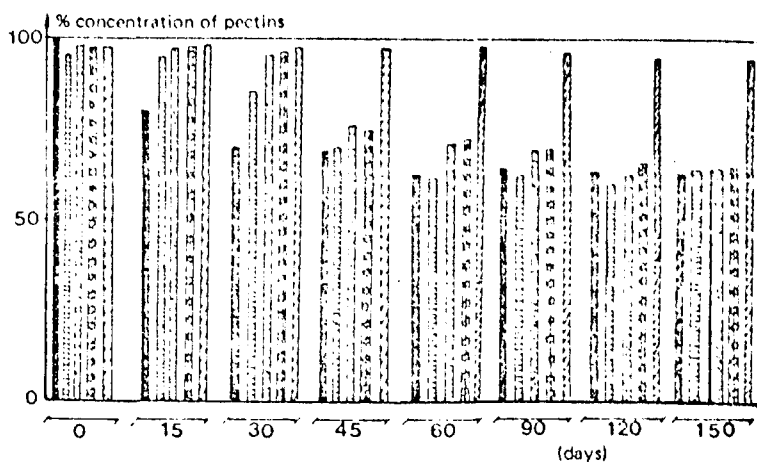


FIG.6. Representation of the variation in the percentual concentration of galacturonic acid (pectins) in the various samples shown in the graph, as a function of the storage time and shown in days; 100% corresponds to the original concentration of pectin galacturonic acid existing in the apple and pear juice concentrates.

- Control
- 200 } krad without heat treatment
- ▨ 400 }
- ▤ 200 } krad with heat treatment.
- ▥ 400 }

where:

t is a value extracted from Dunnett's table [7];
 P is the sample number, excluding the control sample;
 N is the number of observations for each test; and
 S is the "standard" deviation.

During the period of 150 days, the samples under combined treatment ($50^{\circ}\text{C} + 400$ krad), stored at 25°C and the control samples stored at 4°C showed the full average values in the flavour tests: 4.94 ± 0.36 and 4.86 ± 0.37 , respectively.

These differences are not significant if Students' test is applied, where a 5% reliability degree is admitted.

DISCUSSION

Considering the effect of the contaminating flora upon the decomposition of apple and pear juice concentrates, the objective of this project was centred on a

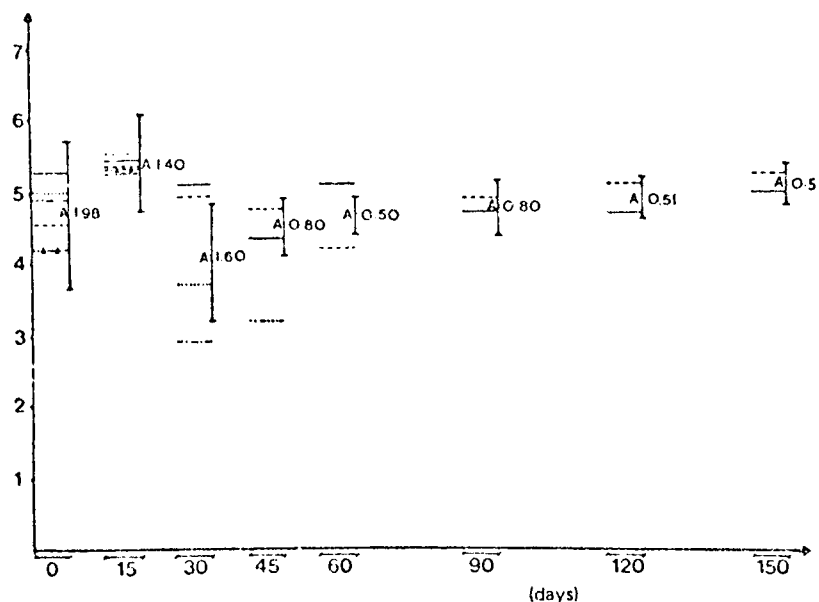


FIG. 7. Representation of the variation in the flavour quality of the apple and pear juice concentrates of the samples in the graph as a function of the storage time in days. Value 5 of the hedonic scale corresponds to a normal acceptability of the product.

- Control
- 400 krad with heat treatment
- 400 krad without heat treatment
- 200 with heat treatment
- ▲-▲ 200 without heat treatment.

search for the optimum irradiation conditions leading to an effective action upon the contaminating agents, avoiding significant changes among the natural components and/or the formation of toxic substances.

These factors already given indicate that the dose levels to be applied should be below 1 Mrad, in agreement with Baraldi's assessment [1]; consequently, the experiment was planned using intermediate doses of radiation.

The results obtained show that the treatment with only intermediate doses has practically no influence from a microbiological viewpoint. Thus, after 30 days, these samples showed a microbiological load equal to that of the control samples and became unsuitable for consumption.

All analysed samples showed a close correlation regarding yeast population levels, variation in the concentration of pectins and, consequently, viscosity.

This led to the inference that the only contaminating microorganism in the product - *Saccharomyces rouxii* - not only consumes sugar but exerts a digestive action upon the pectins, liberating, among other products, galacturonic acid.

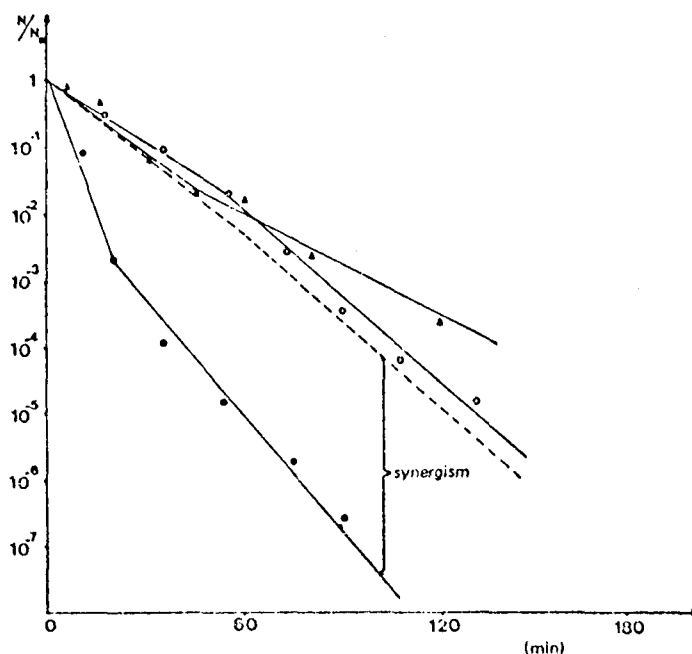


FIG. 8. Survival curve of the *Saccharomyces rouxii* (suspended in a 50% P/vol. sucrose solution) as a function of the time under treatment shown in minutes.

- ΔD_{10} : 33'42" (50°C)
- o D_{10} : 26' 6" (72.5 krad)
- D_{10} : 23' (50°C + 36 krad)
- Synergism

This hypothesis was confirmed by laboratory tests showing that *Saccharomyces rouxii* may develop in media that are exclusively constituted of apple pectins. Those conditions would explain the fact that no differences were detected in the refractometric measurements performed among solids soluble in ethanol during the storage period.

Thus, taking into account that those solids fundamentally constituted sugar, it was to be expected that their concentration would decrease as the population of contamination agents increased. This hypothesis was not confirmed by the refractometry measurements because the loss of solids soluble in ethanol at 50°C caused by sugar digestion is supposed to have been compensated for by the increase in galacturonic acid resulting from the digestion of pectins.

It must be pointed out that the samples treated with 50°C + 400 krad showed no development of contaminating flora and, consequently, no variations in the concentration of pectins, nor in viscosity, during the 150 days under analysis.

Aside from the contaminating flora, all the irradiated samples (heat-treated or not) showed only a 7% loss from the original concentration of pectins, owing to the treatment applied. As a matter of fact, the pure pectin solutions are highly sensitive to ionizing radiation effects, although this is not the case when they appear together with sugar. The sugar seems to exert a competitive action with the pectin when subjected to the effect mentioned earlier [10].

The incidence of radiation energy upon the material under analysis was also evident in the discoloration suffered by the juice at day zero. This coincides with what has been found in the bibliography [11], pointing out the radiosensitiveness of the existing pigments [12, 13].

After the above considerations, the treatment at 50°C and the immediate 400-krad irradiation would appear as the optimum synergism allowing apple and pear juice conservation at 25°C. Such a synergic effect was confirmed by tests performed in our laboratories with cultures of *Saccharomices rouxii* in a 50% wt./vol. sucrose solution [14].

CONCLUSIONS

- (a) The only contaminating organism found in the product under analysis was *Saccharomices rouxii*;
- (b) Heating at 50°C for 10 minutes, associated with 400 krad gamma radiation, proved to be effective in conserving the juice concentrate over a long period at 25°C;
- (c) Under the described treatment total inactivation of the population of micro-organisms occurs, at least during 150 days;
- (d) The quality of the product regarding sensory factors was excellent since after 150 days, no significant differences were found between the treated product and the reference samples.

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