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RADIATION STERILIZATION OF ORANGE JUICE

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ABSTRACT: Juice from commercial oranges has been treated with 1.0 MeV electrons and ^{60}Co gamma radiation with the purpose to study the effect of irradiation on its preservation. The analysis of organoleptic qualities, physical characteristics and content of the main vitamins, shows several changes non-acceptable for consumption.

A combined treatment of irradiation following by a thermal shock (125 Krad, 60-0°C, during 2 minutes at each temperature) does not produce changes of the organoleptic qualities, color, refractive index and absorbance. The content of the main vitamins in the treated juice is comparable with that obtained with conventional methods of preservation. This treatment, irradiation-thermal shock, offers a wide possibility of aplication.

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INTRODUCTION

On January 1965 an international project[1] began in Seibersdorf, Austria, carried out by the European Nuclear Energy Organization (ENEA), to examine the application of irradiation to food preservation in a "model substance". Fruit juices were selected for these fundamental studies for the following reasons:

a) the components are representative for most foods,

b) the components can be easily separated,

c) the microorganisms spoiling the fruit are mainly yeast,

and

d) the item has economic value.

The main problem is to reduce the dose as much as possible, with the purpose of minimizing the degradation due to radiation of the constituents of food, assuring organoleptic quality, wholesomeness and rendering the product economic.

The possibility of preserving fruit juices by irradiation has been outlined by some authors[2-5].

We have selected the juice of orange fruit for our initial studies in food preservation for two reasons:

1) Our country is rich in orange fruit production and its exportation, so any investigation connected with its preservation is useful.

2) Some research has been done before by other authors, mainly by Goldblith[6], Shimba[7] and recently Dharkar[8], so it is possible to compare with our studies.

As we know that the dose necessary to inhibit the multiplication and metabolic activity of microorganisms responsible for the deterioration of the juice, normally changes the chemical constitution of the foodstuff in such a way that undesired organoleptic qualities occur, we have selected a combined treatment of irradiation and heat, following the preliminary investigations of Dharkar[8] and Gaisch[9].

Also, another of our purposes was to develop a general technique for the use of high-energy electrons in these kind of studies, in which ^{60}Co gamma radiation is normally used.

EXPERIMENTAL TECHNIQUES

1. Orange juice samples.

The juice, from commercial non-treated Valenciana variety oranges, was extracted and pipetted into the irradiation containers described latter. In this way the juice contains the natural microflora, but if was observed during the experiments, that the contamination due mainly to manipulation of juice was not always constant, so the concentration of microorganisms varies from sample to sample.

However, all juice samples were incubated during 24 hours at $37 \pm 1^{\circ}C$ in order to guarantee a more uniform presence of microorganisms. It is useful to point out that in a normal industrial process, 24 hours is enough time for the extraction and manipulation of commercial juice.

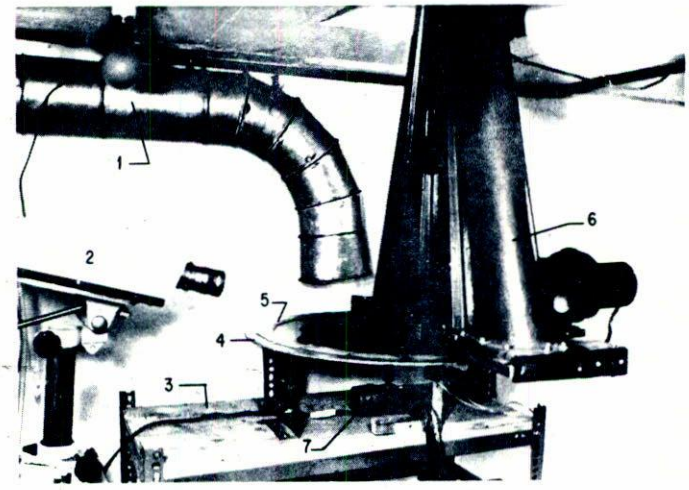

Fig. 1. Irradiation area at the Van de Graaff accelerator showing the array used for electron irradiation. Number 1 indicates the air-conditioned tube to prevent an excess of ozone in the area; 2 is the TV chamber to control the experiment; 3 is a support and 4 is the aluminium rotating table; 5 refers to the polyethylene sealed bags containing the juice; 6 the scanner extension of the accelerator and 7 a control remote motor to control the velocity of the rotating table.

2. Irradiation techniques

Electron irradiation was carried out with 1.0 MeV electrons from the Van de Graaff accelerator at the Instituto de Física, UNAM. 15cc of juice was pipetted into 10x4cm polyethylene bags, sealed and irradiated on a rotating table at a constant velocity in such way that the contact time was of one second. Figure 1 shows the details of the arrangement used in the irradiation at the accelerator laboratory.

Gamma irradiations were performed in two Gamma-cell units (Atomic Energy of Canada Ltd.), property one, model 200, of Laboratorio Nuclear, UNAM and another, model 220, of Programa de Aplicaciones de los Radioisótopos a la Agricultura, CNEN. In both cases, samples of 8cc of juice were pipetted in Pyrex test tubes, plugged with cotton or sealed with bakelite screws and supported in a rigid aluminium array[10] in order to introduce it into the units' cavities.

3. Dosimetry

Electrons.- Dose rate for the juice $(DR)_j$ was determined by the relation[11]:

$$(DR)_j = \frac{{}_mS_j}{{}_mS_d}\,(DR)_d \qquad (1)$$

where $(DR)_d$ is the dose rate for a secondary dosimeter, irradiated on the same conditions as the juice. ${}_mS_j$ and ${}_mS_d$ are the mass stopping powers for the juice and dosimeter respectively, which can be calculated from Bethe's relation[11].

$(DR)_d$ was determined using the common Fricke's solution[12]. The mass stopping power fraction for our system is very close to 1, so $(DR)_j = (DR)_d$. We use a dose rate of 24 Krad/sec.

Dose distribution in the sample was studied by means of a block of polyvinyl chloride plates (PVC) simulating the size of the juice sample. Irradiation induced coloration[13] and the optical density (OD) reading gives a measure of the dose. As the samples were rotated under the radiation beam, a 100% uniform dose distribution is obtained along the plane perpendicular to the electron beam direction. A more extensive study is necessary along the depth direction because of the low penetration of 1.0 MeV electrons. Curve 1 of the figure 2 shows the results obtained for a block

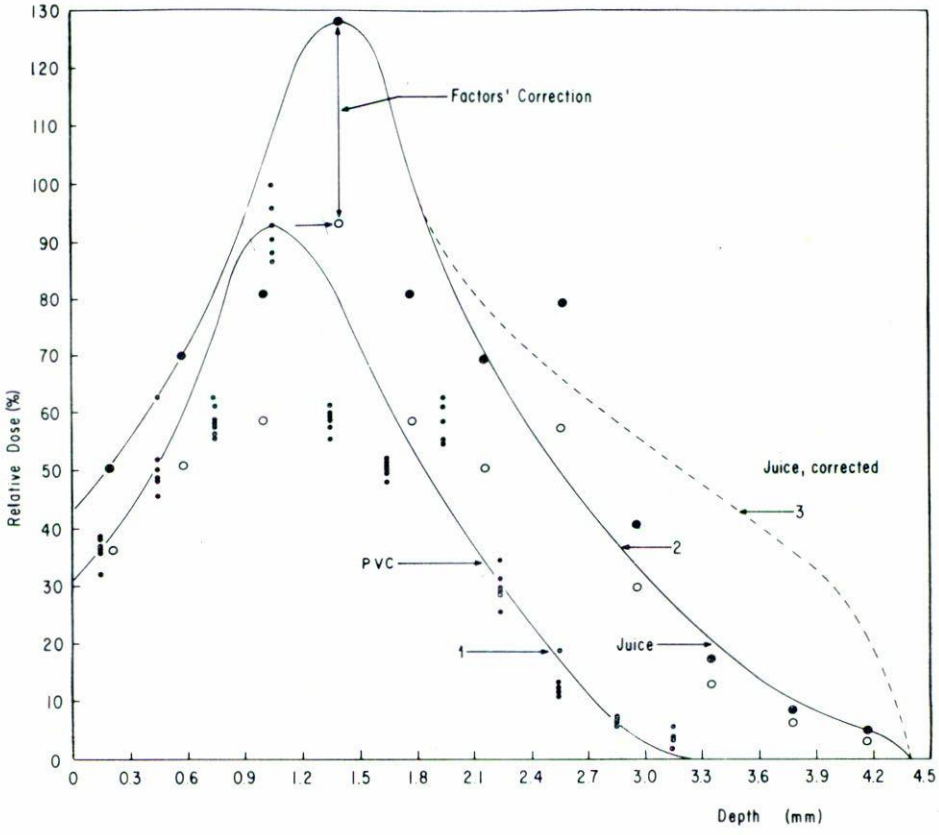

Fig. 2. Depth-dose distribution obtained for PVC and juice with 1.0 MeV electrons. Curve I (PVC) was obtained meassuring the optical density of each individual plate from a block simulating the juice sample and transforming to percentage of dose. Curve 2 is the relation to juice obtained from Curve 1, after correcting their values by the density fraction $\rho_{PVC}/\rho_j = 1.32$, in the horizontal axis, and by the mass stopping power fraction ${}_mS_j/{}_mS_{PVC}$ 1.38, along the vertical axis. Curve 3 is the final result, obtained after correcting Curve 2 by the dose contribution of the back-scattering electrons produced in the aluminium table.

of 0.51 cm thickness (same of the juice sample), contained 17 plates of PVC, irradiated with 1.0 MeV electrons. The optical density of each plate was reading and the fraction $(OD)_i/(OD)_{max}$ was plotted versus depth. $(OD)_i$ and $(OD)_{max}$ are the optical density for any plate and that who shows the maximum reading respectively.

Curve 2 of same figure shows the relative dose percentage versus depth for the juice, obtained from Curve 1 after taking in account the different density of PVC and of the juice. A factor of 1.32 is needed to correct the horizontal and 1.38 the vertical axes. Finally, Curve 3 shows the depth-dose distribution for juice samples with a small correction due to the contribution to the dose of the back-scattering electrons produced in the aluminium table. From latter results, a 55% dose uniformity is obtained in this direction, so dose must be corrected by this factor.

This depth-dose distribution for our juice sample implied many interpretation problems, due to the fact that some of the microorganisms in the sample do not receive enough dose to be inhibited, so we observed reproduction even at doses in which we assumed to have achieved a complete sterilization. For this reason we turned to gamma irradiations.

Some experiments performed by other groups[14] using 0.5 MeV electrons, show that when the juice is stirred by a magnetic rod inside the polyethylene bags, the dose of sterilization is lower, close to 1.0 Mrad. This result proves our assumption.

Gammas. - $(DR)_j$ was determined by means of

$$(DR)_j = \frac{\overline{(\mu/\rho)}_j}{(\mu/\rho)_d} (DR)_d \qquad (2)$$

where $\overline{(\mu/\rho)}_j$ and $\overline{(\mu/\rho)}_d$ are the mean mass total absorption coefficients for the juice and dosimeter, respectively. $(DR)_d$ was also measured using Fricke's solution; with ^{60}Co gamma radiation, with mean energy of 1.25 MeV, the ratio of the coefficients is equal to $(Z/A)_j / (Z/A)_d$, the mean electron density values, that is also close to 1; we obtain, finally, $(DR)_j = (DR)_d$. The value of $(DR)_j$ used was between 8 and 10 Krad/min.

4. Test for sterility of juice

From the growth of natural microflora in the treated, as well as un-

treated orange juice, a certain amount was plated on two growth mediums: a Bacto dehydrated Sabourand Dextrose Agar (Difco, 0109-01), excellent for propagation of molds and yeasts and a Bacto dehydrated Orange Serum Agar (Difco, 0521-01), recommended for the enumeration and cultivation of organisms causing spoilage in citrus products.

All glass material was carefully sterilized, the medium prepared and the juice samples saw as usual[10].

The number of colonies which appeared after 48-72 hours incubation were recorded.

Many growth samples of untreated orange juice were analized in order to identify the reproduced microorganisms and it was found[15] that yeasts were present, mainly Candida Parapsilosis, Candida Stellatoidea and Torulopsis Sp. The fermentation of orange juice after prolonged storage is attributed to contamination with yeasts. These organisms are active at low pH (3.5) and cause also acid production and off-flavour of the juice.

5. Vitamin assays

Analysis of vitamin C, vitamin A (carotene) and niacin were performed by the personnel of División de Nutrición, at the Instituto Nacional de la Nutrición, Secretaría de Salubridad y Asistencia Pública, by means of the techniques described in detail by Horwitz et al[16].

6. Physical measurements

The treated and untreated juice pH was determined with a pH-meter (Photovolt, model 111) with a precission of ± 0.1 pH units. A refractometer (R. Fuess) was also used to measure the refractive index at white light of the samples and finally we used a spectrophotometer (Hitachi Perkin-Elmer, model Coleman EPS-3T) to record the absorbance in the 300-1400 mμ wave-lenght interval for both samples.

7. Organoleptic test

A panel of 17 persons, which did not know that the juice samples

were treated, evaluated the changes in test and flavour.

RESULTS AND DISCUSSION

8. Electron sterilization of orange juice

The effect of electron irradiation was studied between 0 and 16 Mrad in order to study the sterility of juice in approximately 1200 samples at different doses; this gives a very good statistical approach to the relation between dose and the number of colonies of microorganisms. The amount of experimental work is necessary because of the different contaminations in commercial oranges. Up to 24 Krad, irradiation produces excitation of microorganisms and the number of colonies observed in the culture of the treated sample is greater than the number observed in the untreated one. This radioexcitation in the reproduction of microorganisms has also been observed by other authors. After 24 Krad, it was clearly observed that the number of colonies started to decrease until the dose of sterilization is reached at which no colonies were found. A picture published in reference (10) gives a clear picture of the aparence of the culture dishes where the reproductivility was observed. The value for the dose of sterilization was found at 2.5 Mrad; this value differs from those of other authors maybe due to the problem in the uniformity of the depth-dose distribution, or due perhaps to the value for the dose rate used in our experiments. The possibility of the variation of the dose of sterilization with dose rate has not yet been investigated.

9. Gamma sterilization of orange juice

The effect of gamma irradiation was also studied between 0.2 and 2.5 Mrad and after 1700 samples it was found that a dose of 1.0 Mrad is enough to sterilize the juice, incubated during 24 hours; it was also observed that when the juice is incubated 48 and 72 hours before irradiation, at 1.0 Mrad there is still reproduction of microorganisms. This latter observation is still not clearly explained. It could be, according to Szilvinyi[17], that the effect of irradiation depends, to a certain extent, on the phase of the cell cycle during which the irradiation is applied.

When the juice is irradiated at a temperature 0 and $-11\,^{\circ}C$ the dose

sterilization observed was the same; this result indicates that the dose of sterilization is independent of the temperature of irradiation in this range.

10. General analysis of electron sterilized orange juice.

The pH and refractive index were studied as a function of the dose, to observe whether irradiation produces a great change in these physical properties or not. No change was observed, however, with electron irradiation in the interval up to 3.0 Mrad.

Taste and flavour of the sterilized juice at doses as high as 2.5 Mrad were considered as "strange" by the panel. No further analysis was then carried out in electron-sterilized orange juice.

11. Vitamin content of gamma-sterilized orange juice

The vitamin content in gamma treated and untreated samples is shown in the third row of table 1 where, after several determinations, a mean loss of 22 % is observed for the content of vitamin C of the sterilized juice with respect to the untreated sample, 11 percent of the total content of carotene and 47 percent of niacin. It seems that irradiation affects strongly the structure of these vitamins, mainly the niacine, but no further work was done to study what products were formed during the degradation of this substance.

12. Effect of temperature of irradiation

As we observed this strong effect on the vitamins, and following the suggestions given in reference (1), we carried out some experiments irradiating the samples at two temperatures, -11 and 0°C with 1.0 Mrad of gamma radiation; again, the vitamin content was observed. The results are shown in the first and second rows of table 1, for -11 and 0°C, respectively, where it can be seen that the degradation of the vitamin is lower that when the samples were irradiated at room temperature. It is also observed that the lower temperature affects the vitamin content more than at 0°C.

TABLE I

Vitamin content analysis of several samples of untreated (T) and treated (I) orange juices.

Notation	Characteristics of treatment	Vitamin C		Total of carotene		Vitamin A (equiv)		Niacine	
		mg in 100 ml of juice	Loss (%)	µg (%)	Loss (%)	µg (%)	Loss (%)	mg in 100 ml of juice	Loss (%)
T-1		35.6	11	928.2	5	46.2	15	0.84	23
I-1	1.0 Mrad (+11°C)	31.8		884.52		39.34		0.65	
T-2		24.2	8	687.96	6	47.2	13	0.83	25
I-2	1.0 Mrad (0°C)	22.5		644.28		41.15		0.62	
T-3		36.2	22	600.60	11	45.9	29	0.78	47
I-3	1 Mrad (room temperature)	28.1		535.08		32.60		0.41	
T-4		38.7	13	764.40	10	33.93	11	0.79	20
I-4	125 Krad, 60, 0°C during 2 minutes	33.7		687.96		30.32		0.63	

13. Sterilization of orange juice by gamma radiation and heat treatment

From a preliminary investigation,[10] it seemed promising to carry out extensive studies on the combined effect of irradiation and heat on the most resistant yeasts. Doses ranging from 100 to 900 Krad alone, or heat treatment at the temperatures used, alone, was not effective in sterilizing the juice (colony count: million/ml). Heat treatment (60, 0°C, during 2 to 5 minutes at each temperature) reduces the number of colonies. However, juice exposed to gamma radiation followed by thermal shock, shows considerable reduction in the microflora.

The juice was irradiated in the same polyethylene bags, in order that the thermal shock was given uniformly to the sample, and then treated, first at 60°C during 2 to 5 minutes and second at 0°C during the same time. The samples were then incubated at 37°C during 48 hours and the number of colonies formed was counted.

Microorganisms in general are sensitized to heat by irradiation, so that sublethal temperatures become lethal to irradiated microorganisms. The radiation dose is considerably reduced, from 1.0 Mrad to 125 Krad. This dose was the minimum necessary to sterilize the juice, with heat treatment, so this reduces the degradation of the constituents of juice. Several physico-chemical as well as physiological factors have been pointed out by Dharkar[8] that, presumably, contribute to increase the mortality of the irradiated microorganisms by heat, e.g. change in the permeability of cell walls, heat inactivation of enzymes, etc.

14. General analysis of sterilized orange juice by combined treatment

The pH and refractive index show no change with the combined treatment (125 Krad, 60, 0°C, during 2 minutes); no color variation was observed and also no change in absorbance spectra in the 400-1400 mμ range was found. Browning in orange juice was observed pronounced only after 4 weeks after irradiation, when kept at room temperature. No gas development was observed in the sealed bags containing the treated juice, so it is possible to assume that the enzymatic activity was stopped. For the untreated juice, the gas evolution was pronounced. It has been suggested that during non-enzymatic browning of fruit juices[18], hydroxymethyl-

furfural, the degradation product of ascorbic acid accumulates, causing the change in color.

TABLE 2

Sample	Taste	Flavour
Untreated juice (room temperature)	normal	normal
Ice juice	normal	normal
1.0 Mrad irradiated juice at -11 °C	acid	medicine flavour
125 Krad, 60, 0 °C during 2 minutes	normal	normal
1.0 Mrad treated juice	acid	"strange"

(The taster panel, formed by 17 persons, did not know whether the samples were treated or not)

We show in Table 2 the general appreciations of the taster panel; we have included there some data about the test in taste and flavour of several samples. It is interesting to note that juice treated by the combined radiation-thermal shock presents no difference in taste and flavour with respect to the untreated juice. This indicates that the application of this technique to the preservation of orange juice, by a combined treatment, is promising.

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REFERENCES

1. K. Kaindl, Food Irradiation, IAEA, STI/PUB/127, pp. 701, et seq. Vienna (1966).
2. J. Farkas, et al., Fruchtsaftunion **6**, 350 (1961).
3. H. Gaisch, et al., Food Irradiat. **2**, A8, 3 (1962).
4. T. Tuchscheerer, Flüssiges Obst. **30**, X7, 10 (1963).
5. K. Kaindl, et al., Neue Technik. 6, B1, 36 (1964).
6. S.A. Goldblith, et al., US AEC NYO-9181 (1960).
7. R. Shimba, Nosankako Gijutsu Kenkyu Kaishi, **8**, 285 (1961).
8. S.D. Dharkar, Indian J. Technol. **2** (1), 24 (1964).
9. H. Gaisch, et al., Zeitschr. f. Naturf. **19b** (5), 430 (1964).
10. E. Adem Ch. Tesis Profesional, Facultad de Ciencias, UNAM, (1968).
11. H.A. Bethe and J. Askhin, in "Experimental Nuclear Physics", E. Segré, ed. Vol. **1**, Wiley, N.Y. (1953) pp. 166 et seq.
12. J.T.W. Spinks and R.J. Woods, "An introduction to Radiation Chemistry", Wiley, N.Y. (1964), pp. 106 et seq.
13. J. Reyes L., et al., Rev. Mex. Fís. **16**, 57 (1967).
14. Experiments performed by personnel of Departamento de Investigaciones Científicas at the Universidad de Guanajuato.
15. The analysis was done by the staff of Sección de Bacteriología, Departamento de Ecología Humana at the Facultad de Medicina, UNAM, and Laboratorio de Microbiología, Escuela Nacional de Ciencias Biológicas, IPN.

16. W. Horwitz (ed.), "Official methods of the Association of Official Agricultural Chemists", Association of Official Agricultural Chemists, Washington, D.C. (1965); see also, Interdepartamental Committee on Nutrition (ICN), "Manual for Nutrition Survey", ICN Washington, D.C. (1957).
17. A.V. Szilvinyi, et al., Mitteil. d. Versuchsst. f.d. Gärungsgewerbe, 17, 9/10 (1963).
18. J.B.S. Braverman, "Citrus Products", Interscience, N.Y. (1949).

RESUMEN

El jugo de naranjas comerciales ha sido tratado con electrones de 1.0 MeV de energía y radiación gamma del ^{60}Co, con el propósito de preservarlo. El análisis de las cualidades organolépticas, características físicas y contenido de las principales vitaminas nuestra que el jugo presenta cambios no aceptables para el consumo.

Un tratamiento combinado, a base de irradiación seguida por un choque térmico (125 Krad, 60-0°C, durante 2 minutos a cada temperatura) no produce cambios en las propiedades organolépticas, color, índice de refracción y absorbancia. La disminución del contenido de las principales vitaminas se encuentra dentro del intervalo obtenible por otros métodos de preservación convencionales, por lo que el tratamiento ofrece amplia posibilidad de aplicación.