

SHELF-LIFE EXTENSION OF GUMVINE (*SABA SENEGALENSIS*) PULP BY IRRADIATION PROCESSING

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(Reçu le 01-06-1999 - Révisé le 03-11-1999)

Résumé : Le made (*Saba senegalensis*, Apocynacée) est un fruit tropical largement consommé au Sénégal et qui contient des teneurs exceptionnellement élevées de vitamines B₂ (plus de 50 µg/g de fruit mûr). Après irradiation à une dose de 10 kGy, à l'état congelé et sous atmosphère inerte de la pulpe du fruit, celle-ci présentait une excellente qualité microbiologique après huit mois de conservation en réfrigéré à 6°C. Aucun changement majeur n'a été observé sur le plan organoleptique. Une disparition partielle des vitamines induite par le traitement et accentuée pendant le stockage a été notée. Cependant, le taux de rétention de la vitamine B₂ était supérieur à 50 %. Ainsi donc, le traitement ionisant rendait possible la conservation par simple réfrigération de la pulpe de made entre deux récoltes et permettrait ainsi la production industrielle de jus de fruit durant toute l'année.

Mots clés : *Saba senegalensis*, irradiation, conservation, réfrigération.

I - INTRODUCTION

The prolonged cooking of foods practised in African cuisine, while fully justified for health reasons (e.g. microbiological considerations), is associated with a high loss of vitamins. Fresh fruits, which are practically the only foods eaten raw by African populations, thus become essential as a major dietary source of vitamins.

Senegal is an important producer of tropical fruits and a fruit juice industry has recently been created in Dakar. The main difficulty limiting the development of this industry results from fruit overproduction

during a relatively short period. Due to the action of weevils and the development of bacteria and moulds, favoured by the climate, a large part of the production is lost. Only a small quantity of fruits can be stored in the form of frozen pulp, at a very high cost. Because of this, regular production of juice during the year is impossible.

Ionising treatments, at doses generally lower than 1 kGy (for organoleptic reasons), are effective for insect disinfestation and ripening alteration of fresh fruits⁽¹⁾, but cannot be considered for their microbial decontamination. On the other hand, irradiation of the fruit pulp in the frozen state, in an inert atmosphere and at a relatively high dose (with γ -rays or electron beam) would possibly result in food with a very low degree of contamination. This could then be conserved for several months by refrigeration, thus permitting fruit juice production throughout the year at a reasonable cost.

Gumvine (*Saba senegalensis*), one of the fruits whose valorisation has been undertaken in Senegal (production of juice and nectar), was chosen to test the efficiency of this latter treatment.

This work was carried out to study the favourable effects of an ionising treatment on the microbiological contamination of the gumvine pulp, and also the possible adverse consequences of such treatment on its organoleptic and nutritional properties. Irradiated pulp was observed immediately after treatment and during a long period of storage at 6°C (at least 8 months, e.g. the period between two harvests).

II - MATERIAL AND METHODS

Samples

Gumvine fresh pulp was prepared at the Institut de Technologie Alimentaire of Dakar (Senegal). Ripe and wholesome fruits were first washed and deseeded. The pulp obtained after straining to eliminate fibres and debris was put in 1 kg polyethylene bags, frozen at - 20°C and brought by air in an ice box to the laboratory in Illkirch (France) the same day. For irradiation treatment the pulp was thawed, put in

multilayer CSX plastic bags (AFP-Cenpa, Dax, France) (40 g for microbiological analysis and 10 g for physico-chemical analysis), degassed by a Multivac A300 apparatus (Wolfertschwenden, Germany), flushed with nitrogen and heat-sealed. A part of these samples was refrozen at -20°C and irradiated at this temperature. The irradiated and reference samples were then stored at $(6 \pm 2)^{\circ}\text{C}$.

Irradiation equipment and dosimetry

A Van de Graff electron accelerator, 2.2 MeV and 100 mA (Vivirad High Voltage Co, Handschubeim, France) were used for the ionising treatment. A 100- μm copper scattering foil was put over the sample in order to obtain a good homogeneous dose distribution [2]. Irradiation doses were controlled with radiachromic dosimeters FWT 60.00 (Far West Technology, Goleta, USA), previously calibrated with an alanine dosimeter (laboratoire de Métrologie des Rayonnements Ionisants, Gif-sur-Yvette, France).

Liquid chromatographic system

The chromatograph consisted of a Model 600 E pump, a 700 injector, a 991- arrays diode detector and a 470-fluorimetric detector (Waters Associated, Milford, USA).

Vitamin assay's

Analyses of vitamins were carried out according to the HPLC methods described by Arella et al [3] for vitamins B_1 and B_2 , Bergaentzle et al. [4] for vitamin B_6 and Arella et al. [5] for L-ascorbic acid and vitamin C. α - and β -carotene were extracted from the samples according to the method of Reeder and Park [6]. Separation of the two carotene's was performed isocratically with a Lichrospher 100 RP18 column (Merck, Nogent-sur-Marne, France), using acetonitrile / methanol /dichloromethane (55: 35: 10 v/v) at a flow rate of $1 \text{ ml} \cdot \text{min}^{-1}$.

The absorbance was measured at 470 nm.

For all vitamins, the data were quantified using external calibration.

Carbohydrate assays

Analysis of glucose, fructose and sucrose were carried out according to the enzymatic methods retained as french official methods for the analysis of fruit and vegetable juice [7].

pH measurement

Ten (10) g of pulp were diluted with 50 ml of distilled water. pH values were measured with a CG 837 Schott pH meter (Hofheim, Germany).

Sensory analysis

Sensory analysis were conducted in a specialised laboratory (AERIAL, Strasbourg, France) by a panel composed of judges (14 to 16) including Senegalese, and carried out according to the AFNOR (Association Française de Normalisation) standard concerning the triangular test methodology [8].

Microbiological analysis

The determination and the counting of bacteria were performed according to the AFNOR standards : Plate Count agar (72 h incubation at 30°C) for aerobic mesophiles [9], Violet Red Bile agar (24 h incubation at 30°C) for coliforms [10], OGA agar plus 0.1% chloramphenicol (72 h incubation at 25°C) for yeast's and moulds [11], TGY agar (48 h incubation at 37°C, after dilution of the inoculum with peptone water and heating at 75°C during 15 min) for aerobic spores, Baird Parker agar (24 h incubation at 37°C) for *Staphylococcus aureus* [12], TSC medium (48 h incubation at 46°C) for sulphite reducing anaerobes [13], Hecktoen and Kristensen media (24 h incubation at 37°C) after enrichment on Rapaport and Selenite media (24 h incubation at 37°C) for *Salmonella* [14].

III - RESULTS AND DISCUSSION

Initial state of non-irradiated fruit pulp

The vitamin levels found in gumvine pulp are shown in Table-I.

TABLE I : *Effect of the irradiation dose on the amount¹ of vitamins in frozen (-20°C samples of gumvine pulp*

Dose (kGy)	Amount (µg.g ⁻¹)						
	L-Ascorbic acid	Vitamin C	Vitamin B1	Vitamin B2	Vitamin B6	αcarotene	βcarotene
0	116 ± 18	210 ± 6	0.71 ± 0.01	54.7 ± 5.0	1.90 ± 0.15	19.7 ± 2.4	28.3 ± 1.8
5	117 ± 4	220 ± 21	0.65 ± 0.01	51.2 ± 1.3	1.65 ± 0.20	17.2 ± 1.6	28.5 ± 1.2
10	109 ± 3	203 ± 32	0.46 ± 0.02	50.5 ± 0.9	1.35 ± 0.15	14.5 ± 1.8	25.5 ± 1.0
15	106 ± 3	199 ± 25	0.37 ± 0.01	50.2 ± 1.7	1.10 ± 0.25	14.1 ± 1.8	21.2 ± 1.8

1. Mean of three measurements for each vitamin and dose RSD

The aerobic mesophile count of the gumvine pulp studied, which had an acid pH (2.70-2.80), was relatively high (7.0×10^4 CFU.g⁻¹). The majority of these microorganisms were yeast's and moulds (6.0×10^4 CFU.g⁻¹), their growth being favoured by the pH conditions. It should be noted that none of the pathogens under investigation could be identified. According to French official standards concerning frozen vegetable products having undergone a handling^[15] (aerobic mesophiles $< 1.5 \times 10^5$ CFU.g⁻¹ and yeast's / moulds $< 3.5 \times 10^3$ CFU.g⁻¹), the yeast's / moulds count appeared too high.

Immediate effects of an ionising treatment.

Neither pH modification nor significant organoleptic changes (taste, smell or colour) of gumvine were observed after irradiation of the frozen pulp (-20°C) at a dose of 15 kGy. According to the results of the triangular test, the irradiated samples were not considered to be different from the unirradiated samples, at a significance level of 1%, by the sixteen members of the jury (10 correct and 6 false answers).

For vitamins C and B₂, the degradation rates were statistically insignificant, even after irradiation at 15 kGy. It was difficult to compare the results obtained with that already published ^[16] because the treatments were carried out at -20°C and in the absence of oxygen. However they confirmed that vitamin B₂ is highly resistant to irradiation. The most surprising results concerned vitamin C. At 10 kGy, there was no noticeable degradation either of L-ascorbic acid or of total vitamin C.

The ionising treatment reduced microbiological contamination of this pulp appreciably since the aerobic mesophile count was always less than 10¹ CFU.g⁻¹ after irradiation at 10 kGy (Table II). After irradiation at 5 kGy, the aerobic mesophile count was still 1.5 x 10¹ CFU.g⁻¹. In order to ensure a satisfactory microbiological decontamination in all cases, a dose of 10 kGy therefore seemed necessary.

Alteration of the fruit pulp (reference and irradiated samples) during storage at (6 ± 2) °C

The change in the microbiological contamination observed in the reference samples of gumvine pulp resulted in a rapid growth of the aerobic mesophile germs during the first two months of storage (Table II). The growth of yeast's and moulds which constituted almost all the aerobic mesophile germs was encouraged by a favourable growth medium : a stable acidic pH (around 2.6 - 2.8), and a high carbohydrate concentration (114 mg.g⁻¹ comprising 32 mg.g⁻¹ of sucrose, 63 mg.g⁻¹ of glucose and 19 mg.g⁻¹ of fructose).

A regular, moderate but nonetheless unexpected increase in the number of aerobic spores was observed during storage (Table II). This phenomenon could be explained by the initial presence of aero-anaerobic microorganisms in the fruit pulp capable of reproducing spores during their development.

TABLE 2 : Change in the microbial contamination (CFU g⁻¹) of gumvine pulp samples in relation to the irradiation dose and the storage duration (at (6 ± 2)°C)

		Retention rate ¹ (%)						
	Dose (kGy)	1	30	60	90	130	175	260
	0	< 10 ¹	2.9x10 ⁵	1.1x10 ⁷	1.8x10 ⁷	2.0x10 ⁷	2.4x10 ⁷	1.5x10 ⁷
Aerobic	5	1.5x10 ¹	2.0x10 ³	2.5x10 ²	8.0x10 ²	5.0x10 ³	7.0x10 ³	4.5x10 ⁵
mesophile	10	< 10 ¹	< 10 ¹	< 10 ¹	< 10 ¹	< 10 ¹	1.0x10 ²	1.0x10 ²
germs	15	< 10 ¹	< 10 ¹	< 10 ¹	< 10 ¹	< 10 ¹	< 10 ¹	< 10 ¹
	0	6.0x10 ⁴	2.3x10 ⁵	1.3x10 ⁷	2.0x10 ⁷	1.2x10 ⁷	2.0x10 ⁷	1.0x10 ⁷
Yeast's	5	< 10 ¹	< 10 ¹	< 10 ¹	< 10 ¹	5.0x10 ¹	1.0x10 ²	5.1x10 ⁵
/Moulds	10	< 10 ¹	< 10 ¹	< 10 ¹	< 10 ¹	< 10 ¹	< 10 ¹	1.0x10 ²
	15	< 10 ¹	< 10 ¹	< 10 ¹	< 10 ¹	< 10 ¹	< 10 ¹	< 10 ¹
	0	1.0x10 ²	3.0x10 ²	8.0x10 ²	5.0x10 ²	1.2x10 ³	2.0x10 ³	5.0x10 ³
Aerobic	5	< 10 ¹	< 10 ¹	< 10 ¹	< 10 ¹	< 10 ¹	< 10 ¹	< 10 ¹
spores	10	< 10 ¹	< 10 ¹	< 10 ¹	< 10 ¹	< 10 ¹	< 10 ¹	1.0x10 ²
	15	< 10 ¹	< 10 ¹	< 10 ¹	< 10 ¹	< 10 ¹	< 10 ¹	< 10 ¹

1. One measurement for each storage duration and dose

In the irradiated samples of gumvine pulp, only the samples irradiated at 15 kGy had a microbiological contamination lower than 10¹ CFU.g⁻¹ after storage. A slight recurrence of bacterial growth was observed after 6 months storage in the samples irradiated at 10 kGy but these were still microbiologically satisfactory at the end of storage according to the French official standards. On the other hand, a dose of 5 kGy was not sufficient to produce microbiologically satisfactory samples. A definite growth increase in yeast's and moulds was effectively observed during the last weeks of storage.

A triangular test applied to the organoleptic analysis of the fruit pulp irradiated at 10 kGy and stored 8 months at 6°C (by comparison with a non-irradiated sample stored frozen), carried out by 14 judges, did not indicate any difference (at a significance level of 1%) between the two samples (9 correct and 5 false answers).

In the same way, the change in the vitamin amounts of the gumvine pulp has been followed throughout storage. Table III shows the reten-

tion rates of the more important vitamins (vitamins B₂ and B₆, - and -carotene's) at the end of storage.

TABLE 3 : *Retention of vitamins in reference and irradiated pulps of gumvine at the storage period (260 days at 6 ± 2 °C) in relation to the irradiation dose*

Dose (kGy)	Retention rate ¹ (%)			
	Vitamin B2	Vitamin B6	α -carotene	β -carotene
0	53.6	15.3	42.9	39.6
5	58.2	22.5	32.5	21.4
10	55.3	30.6	30.7	20.2
15	57.4	37.11	30.6	17.3

1 Mean of three measurements for each vitamin and RSD (%)

In the case of vitamin B₂, the losses were moderate (around 45%) and of the same order in the reference and irradiated samples. (independent of the irradiation dose). The α - and β -carotene's appeared less stable than vitamin B₂ and the fact that the samples were irradiated seemed to slightly accentuate their instability during storage. However, no effect relative to irradiation dose has been demonstrated. The analysis of vitamin B₆ has given rise to more unexpected results. In the reference samples, the average losses after storage were high (85%). They were less in the irradiated samples and the higher the irradiation dose, the higher the vitamin B₆ concentration appeared to be (average degradation rate of 63% after irradiation at 15 kGy). This phenomenon is difficult to explain, but could possibly result from the release during storage of unavailable bound forms of vitamin B₆ in food samples submitted to an irradiation treatment.

As a result of irradiation at 10 kGy and at a temperature of -20°C in an inert atmosphere, gumvine pulp of excellent microbiological quality could therefore be obtained after a storage period of 8 months at 6°C. The irradiation treatment thus enables an industrial production of juice and nectar throughout the year to be envisaged while retaining the organoleptic characteristics and, to a large extent, the remarkable nutritional properties of this fruit.

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