

Formation of new periderm cell layers and subsequent suberization have been recognized to be important for disease resistance in the tubers (Spark and Iritani, 1964; Spark, 1970; Kolattukudy, 1977). Radiation-induced soft rot has been attributed to the impaired periderm formation (Spark and Iritani, 1964; Spark, 1970). A dose as low as 2 krd has been found to be sufficient to impair periderm formation (Thomas, 1982). The reduced contents of free lipids, suberin, phenolics, and phytoalexins in suberized wound periderm, as observed in the present studies, could also contribute to the lowered resistance in irradiated tubers.

Analysis of aliphatic fraction of suberin component of potato wound periderm has been reported (Kolattukudy and Dean, 1974; Kolattukudy and Agrawal, 1974). The pattern of separation of this fraction by TLC observed in the present studies was similar to that reported by these workers. It has been shown that octadec-9-enedioic acid (a dicarboxylic acid) and ω -hydroxyoleic acid were the key components responsible for the ability of wound periderm to prevent moisture losses. In the present studies, though dicarboxylic acids were detected, their further characterization was not carried out. Similarly, ω -hydroxyoleic acid (compound E) in Figure 4 was tentatively identified based on its IR spectrum (absorption at 2.85 μ m characteristic of OH group), its stronger affinity for I_2 vapors (due to unsaturation), and its relative position (R_f) on TLC plate between more polar α -hydroxycaproic acid and relatively less polar 12-hydroxystearic acid.

Registry No. Suberin, 8072-95-5; rishitin, 18178-54-6; phytuberin, 37209-50-0; chlorogenic acid, 327-97-9; caffeic acid, 331-39-5; ferulic acid, 1135-24-6.

LITERATURE CITED

Clifford, M. N.; Wight, J. J. *J. Sci. Food Agric.* 1976, 27, 73.

- De Boer, S. H. Ph.D. Dissertation, University Microfilm Internationale, Ann Arbor, MI, 1976.
- Folch, J.; Lees, M.; Hostonstanley, G. *J. Biol. Chem.* 1957, 226, 497.
- Fricke, H.; Morse, S. *Am. J. Roentgenol. Radium Ther.* 1927, 18, 430.
- Ghanekar, A. S.; Nair, P. M. *Indian J. Biochem. Biophys.* 1974, 11, 233.
- Ghanekar, A. S.; Padwal-Desai, S. R.; Nadkarni, G. B. *Potato Res.* 1983, in press.
- Hanson, K. R.; Zucker, M. *J. Biol. Chem.* 1963, 238, 1105.
- Henfling, J. W. D. M.; Kuć, J. *Phytopathology* 1979, 69, 609.
- Huang, M. C.; Agrios, G. N. *Phytopathology* 1979, 69, 35.
- Kolattukudy, P. E. *Recent Adv. Phytochem.* 1977, 11, 185.
- Kolattukudy, P. E.; Agrawal, V. P. *Lipids* 1974, 9, 682.
- Kolattukudy, P. E.; Dean, B. B. *Plant Physiol.* 1974, 54, 116.
- Lewis, N. F.; Alur, M. D.; Nadkarni, A. R.; Gaonkar, S. G.; Kumta, U. S., presented at the International Symposium on Radiation Preservation of Foods, Bombay, IAEA/SM-166/13, 1972.
- Lyon, G. D.; Bayliss, C. E. *Physiol. Plant Pathol.* 1975a, 6, 177.
- Lyon, G. D.; Bayliss, C. E. *Physiol. Plant Pathol.* 1975b, 6, 43.
- Nair, P. M.; Behere, A. G.; Ramaswamy, N. K. *J. Sci. Ind. Res.* 1981, 40, 529.
- Nair, P. M.; Thomas, P.; Ussuf, K. K.; Surendranathan, K. K.; Limaye, S. P.; Srirangarajan, A. N.; Padwal-Desai, S. R. In "Radiation Preservation of Food"; IAEA: Vienna, Austria, 1973; IAEA-SM-166/11 Publ., p 347.
- Spark, W. C. *Res. Bull.—Idaho, Agric. Exp. Stn.* 1970, 520.
- Spark, W. C.; Iritani, W. M. *Res. Bull.—Idaho, Agric. Exp. Stn.* 1964, 69.
- Thomas, P. *Potato Res.* 1982, 25, 155.
- Thomas, P.; Srirangarajan, A. N.; Joshi, M. R.; Janave, M. T. *Potato Res.* 1979, 22, 261.
- Walter, W. M.; Purcell, A. E.; McCollum, G. K. *J. Agric. Food Chem.* 1979, 27, 938.

Received for review February 1, 1983. Accepted June 2, 1983.

Dietary Fiber Content of Eleven Tropical Fruits and Vegetables

Eric D. Lund,* John M. Smoot, and Nancy T. Hall

Dietary fiber was determined in the following samples: yam, malanga, cassava, taro, avocado, date, coconut, calabaza, grapefruit albedo, and kiwi. Values were obtained for cellulose, hemicellulose, lignin, cutin, ash, neutral detergent residue (NDR), and enzymatic (soluble and insoluble) fractions. Ranges were (percent of fresh weight) as follows: for NDR, 0.53–8.6%; for cellulose, 0.20–3.8%; for hemicellulose, 0.07–4.2%; for lignin, 0.051–2.01%; for enzymatic insoluble, 0.91–6.9%; for enzymatic soluble, 0.64–4.7%. Cellulose and hemicellulose were most concentrated in coconut, and lignin was highest in dates. The enzymatic-soluble fraction was exceptionally high in the yam, *Dioscorea rotundata*, and in grapefruit albedo.

Because of the lack of detailed information on fiber in many foods, we undertook a study of dietary fiber components in the more common tropical fruits and vegetables. We were also interested in certain *in vitro* physiological properties of isolated fiber components which required water-soluble and -insoluble fractions prepared by an enzymatic procedure for further studies *in vitro*.

In an earlier study, values for fiber components in 15 samples from 8 fruits and vegetables were reported (Lund

and Smoot, 1982). Since this study was completed, a more complete study of banana fiber (Kayisu et al., 1981) and the fiber composition of the yams *Dioscorea dumetorum* and *rotundata* have been published (Brillouet et al., 1981).

The modified forms of the Van Soest detergent and Hellendoorn enzymatic methods which we reported previously (Lund and Smoot, 1982) were employed in the current study. Several reports have recently appeared describing improvements in these methods for use with samples containing relatively large amounts of starch or pectin (Dovell and Harris, 1982; Selvendran and DuPont, 1980; Marlett and Lee, 1980; Collinge et al., 1980; Morrison, 1980; Jeltema and Zabik, 1980). Some of these procedures may eventually be incorporated into a standard method

U.S. Citrus and Subtropical Products Laboratory, Southern Region, U.S. Department of Agriculture, Agricultural Research Service, Winter Haven, Florida 33880.

Table I. Fiber Content As Determined by Nonsequential (N) and Sequential (S) Detergent Methods

	fiber content, % of fresh weight \pm SD			
	NDR (N)	NDR (S)	ADR (N)	ADR (S)
yam (<i>Dioscorea</i>)				
alata				
Florida	0.74 \pm 0.02	0.76 \pm 0.01	0.76 \pm 0.01	0.58 \pm 0.04
Veeven	0.92 \pm 0.03	0.93 \pm 0.05	0.89 \pm 0.03	0.75 \pm 0.02
esculenta				
Doli	0.93 \pm 0.01	0.94 \pm 0.02	0.95 \pm 0.02	0.74 \pm 0.04
rotundata	0.56 \pm 0.01	0.53 \pm 0.09	0.71 \pm 0.04	0.46 \pm 0.01
malanga				
small	0.70 \pm 0.01	0.74 \pm 0.02	0.76 \pm 0.04	0.61 \pm 0.03
large	0.98 \pm 0.02	1.15 \pm 0.04	0.96 \pm 0.02	1.03 \pm 0.04
cassava	0.83 \pm 0.02	0.86 \pm 0.01	0.78 \pm 0.03	0.72 \pm 0.03
taro	0.76 \pm 0.01	0.81 \pm 0.03	0.74 \pm 0.02	0.70 \pm 0.03
avocado	2.60 \pm 0.02	2.66 \pm 0.09	1.37 \pm 0.04	1.34 \pm 0.01
date	4.8 \pm 0.4	5.1 \pm 0.1	3.8 \pm 0.1	3.8 \pm 0.2
coconut	7.8 \pm 0.1	8.6 \pm 0.5	4.2 \pm 0.1	4.4 \pm 0.3
calabaza	1.28 \pm 0.03	1.32 \pm 0.10	0.85 \pm 0.01	0.55 \pm 0.03
grapefruit albedo	3.6 \pm 0.1	3.3 \pm 0.1	3.0 \pm 0.1	2.59 \pm 0.16
kiwi	1.92 \pm 0.24	1.85 \pm 0.15	1.74 \pm 0.02	1.42 \pm 0.08

for high-starch and pectin-containing samples.

In the current study, the dietary fiber composition was determined on 14 fruits and vegetables. This group includes yam (four varieties), malanga (two types), cassava, taro, avocado, date, coconut, calabaza, and kiwi. Grapefruit albedo was also analyzed. Debittered grapefruit albedo fortified with flavor and nutrients has been proposed as a new product (Roe and Bruemmer, 1977). Samples of water-soluble and -insoluble fiber fractions were also prepared from each fruit or vegetable.

EXPERIMENTAL SECTION

Materials. Malangas, cassava, avocado, date, coconut, calabaza, grapefruit, and kiwi were purchased at local markets. The yams and taro were obtained from the Mayaguez Institute of Tropical Agriculture, Mayaguez, PR.

Methods. The edible, fleshy portion of the fruit or vegetable was analyzed. Peel was included for dates only. For all other samples, the fresh fruit (800–900 g) was cut into small pieces, then frozen in liquid nitrogen, and homogenized in a precooled blender prior to analysis. The frozen, blended sample was extracted 3 times with 4-L portions of boiling 80% ethanol and filtered through a coarse sintered glass filter, and the residue was washed with 1–2 L of acetone. Residual acetone was removed in an evacuated desiccator at room temperature and 1 torr of pressure. A liquid nitrogen trap was included between the desiccator and pump. Samples containing large amounts of starch or pectin generally required extended periods for complete removal of acetone (1–2 days). The AIR (alcohol-insoluble residue) was dried in the desiccator over CaSO₄ at 0.1 torr and room temperature. Lipids were extracted from the coconut and avocado AIRs with three 1-L portions of Folch solvent (2:1 CHCl₃-methanol) before analysis.

Dry weight was determined by holding a 5-g sample of the fresh blended material in a vacuum oven at 70 °C and 1 torr for 6 h. The dried sample was allowed to cool to room temperature in a desiccator and weighed. Fiber composition (including ash values) were determined on triplicate samples of the AIR. Modified versions of the Van Soest detergent and Hellendoorn's enzymatic methods were used, as described previously (Lund and Smoot, 1982).

The analysis of *D. rotundata* was more difficult than that of other *Dioscorea* species. Filtration was difficult and the enzymatic insoluble results were extremely variable. The rate of filtration was increased by preheating the filter with boiling water (detergent method) or by

transferring the filtrate to a second crucible (enzymatic method). The procedure for the enzymatic analysis was modified by adding 2 or 3 times the specified amount of pancreatin.

RESULTS AND DISCUSSION

For most of the more common tropical fruits and vegetables studied, the dietary fiber composition could be determined by standard detergent and enzymatic procedures. However, interference from relatively large amounts of starch and pectin required modification of the analytical procedures for many samples [see Lund and Smoot (1982)].

Several difficulties (filtration, variable results) were encountered in the enzymatic analysis of *D. rotundata*. As mentioned above, filtration of the soluble fraction was hindered by the sticky consistency. The unique sticky quality is one reason for the use of this yam for "fufu", a traditional African dish which cannot be made from most other yams (Martin, 1979). The variable results for the enzymatic insoluble value could be caused by an amylase inhibitor similar to the inhibitor recently isolated from *Dioscorea alata* (Sharma and Pattabiraman, 1982). However, when we added additional pancreatin (see Experimental Section) to the mixture as suggested by these authors, reproducibility was not improved.

A comparison of NDR (neutral detergent residue) and ADR (acid detergent residue) from both nonsequential (N) and sequential (S) methods is shown in Table I. In the sequential method, acid-insoluble pectins and tannins are extracted by neutral detergent solution prior to the ADR determinations. Any error caused by these substances would appear as a lower ADR value from the sequential procedure. Sodium sulfite was not added in the sequential NDR determination because the sulfite is unstable in the subsequent acid extraction step. The NDR (S) and NDR (N) were generally comparable. In contrast, the ADR values for the yams, calabaza, grapefruit albedo, and kiwi all showed significantly lower values from the sequential method. This difference probably indicates the presence of acid-insoluble pectins or tannins [see Lund and Smoot (1982)].

As the data in Table II show, NDR values ranged around 1%. Notable exceptions were avocado, date, coconut and grapefruit albedo. The high fiber content of date, coconut, and grapefruit albedo is not surprising, but the high level in avocado was unexpected. The NDR in avocado includes cellulose, hemicellulose, and lignin in relative amounts typical of many fruits and vegetables.

Table II. Dietary Fiber Content of Tropical Fruits and Vegetables

	% of fresh weight \pm SD for										% dry weight \pm SD	
	detergent ^{a, b}					enzymatic fiber						
	cellulose	hemicellulose	lignin	ash	NDR	insoluble	soluble					
<i>Yam (Dioscorea)</i>												
<i>alata</i>												
Florida	0.52 \pm 0.01	0.178 \pm 0.031	0.065 \pm 0.032	0.028 \pm 0.004	0.76 \pm 0.01	2.96 \pm 0.02	0.66 \pm 0.02					23.9 \pm 0.2
Veeven	0.61 \pm 0.02	0.177 \pm 0.046	0.145 \pm 0.003	0.036 \pm 0.008	0.93 \pm 0.05	2.42 \pm 0.06	0.80 \pm 0.01					22.0 \pm 0.1
<i>esculenta</i>												
Doli	0.63 \pm 0.02	0.205 \pm 0.032	0.102 \pm 0.023	0.029 \pm 0.009	0.94 \pm 0.02	2.44 \pm 0.18	0.64 \pm 0.16					27.6 \pm 0.1
rotundata	0.20 \pm 0.03	0.07 \pm 0.03	0.134 \pm 0.004		0.53 \pm 0.09	2.34 \pm 0.70	4.10 \pm 0.96					27.2 \pm 0.2
<i>malanga</i>												
small	0.48 \pm 0.02	0.128 \pm 0.013	0.137 \pm 0.013	0.05 \pm 0.02	0.74 \pm 0.02	2.99 \pm 0.06	1.19 \pm 0.04					24.7 \pm 0.2
large	0.76 \pm 0.01	0.121 \pm 0.006	0.259 \pm 0.017	0.10 \pm 0.04	1.15 \pm 0.04	2.72 \pm 0.03	1.72 \pm 0.09					22.5 \pm 0.4
<i>cassava</i>												
taro	0.66 \pm 0.01	0.134 \pm 0.018	0.065 \pm 0.011	0.041 \pm 0.015	0.86 \pm 0.01	2.18 \pm 0.15	0.69 \pm 0.02					32.9 \pm 0.3
avocado	0.61 \pm 0.02	0.113 \pm 0.016	0.090 \pm 0.020	0.033 \pm 0.010	0.81 \pm 0.03	2.08 \pm 0.04	1.71 \pm 0.04					20.0 \pm 0.1
date	1.16 \pm 0.007	1.32 \pm 0.10	0.180 \pm 0.016	0.06 \pm 0.02	2.66 \pm 0.09	2.04 \pm 0.03	1.29 \pm 0.07					23.4 \pm 0.1
coconut	1.55 \pm 0.08	1.28 \pm 0.12	2.01 \pm 0.11	0.13 \pm 0.04	5.1 \pm 0.1	6.9 \pm 0.2	2.31 \pm 0.04					77.9 \pm 0.2
calabaza	3.8 \pm 0.2	4.2 \pm 0.5	0.57 \pm 0.06	0.10 \pm 0.06	8.6 \pm 0.5	6.9 \pm 0.1	2.12 \pm 0.17					57.9 \pm 0.4
grapefruit albedo	0.50 \pm 0.03	0.77 \pm 0.07	0.051 \pm 0.001	0.05 \pm 0.02	1.32 \pm 0.10	0.91 \pm 0.01	0.64 \pm 0.02					8.3 \pm 0.1
kiwi	2.37 \pm 0.29	0.74 \pm 0.36	0.12 \pm 0.03	0.11 \pm 0.13	3.3 \pm 0.1	5.3 \pm 0.7	4.7 \pm 0.4					19.9 \pm 0.1
	0.84 \pm 0.05	0.43 \pm 0.10	0.17 \pm 0.03		1.85 \pm 0.15	2.35 \pm 0.18	1.49 \pm 0.18					19.6 \pm 0.2

^a Values derived from the sequential method. ^b Cutin values: date = 0.257 \pm 0.031; kiwi = 0.44 \pm 0.04.

Although NDR should be comparable to the enzymatic insoluble fraction, the NDR values for high-starch vegetables were much less than the enzymatic insoluble values. Insoluble pectin or other insoluble noncellulosic polysaccharides probably account for the differences (Lund and Smoot, 1982).

Pectin is abundant in avocado (Kawabata, 1977), grapefruit albedo (Braddock and Graumlich, 1981; Sinclair, 1972), and kiwi (Kawabata, 1977; Robertson of Swinburne, 1981). As indicated in these previous works, soluble pectin probably constitutes most of the enzymatic soluble fraction of avocado (1.3%) and insoluble pectin accounts for most of the difference between NDR and enzymatic insoluble fractions for grapefruit albedo (2%) and kiwi (0.5%).

D. rotundata differed from the other yams, as reported here and in the previous paper (Lund and Smoot, 1982), by virtue of the relatively large enzymatic soluble fraction (4.1% vs. 0.6–0.9%) and the small amount of cellulose (0.2% vs. 0.5–0.8%) and hemicellulose (0.07% vs. 0.18–0.38%). The lignin value (0.134%) was close to that reported for stored *D. rotundata* tubers (0.128% of fresh weight) by Brillouet et al. (1981). The same authors reported a value of 0.60% of fresh weight for the total polysaccharides. Our value for NDR (0.53%) was very similar. On the other hand, our value for the enzymatic insoluble fraction was much higher than the total polysaccharides they reported. The difference could be residual starch (see earlier discussion of experimental difficulties with *D. rotundata*).

Ash values varied from 0.03 to 0.13%. These concentrations are within normally expected ranges for fruits and vegetables.

Several of the samples contained unusually high concentrations of various fiber fractions. Date contained a very high lignin concentration, presumably derived from the peel. Increased amounts of lignin were also found in coconut and the large malanga. Cutin was concentrated in date and kiwi. The kiwi contained a large number of seeds, which were probably the cutin source. The hemicellulose concentration was very high in coconut. In calabaza, hemicellulose was relatively concentrated when expressed as a percentage of the dry weight.

Many of the samples analyzed in this study contain relatively high amounts of certain fiber components, but the nutritional quality remains to be evaluated. Structural differences between fiber fractions isolated from different sources can greatly alter physiological effects of the fiber. In vitro and in vivo studies of fiber isolated from various fruits and vegetables based on quantitative fiber composition data must be carried out before the nutritive value can be quantitatively established.

ACKNOWLEDGMENT

We thank the following individuals for contributions of fruit and vegetable samples: Jose Vicente-Chandler of the USDA Agricultural Experiment Station, Rio Piedras, PR; Antonio Sotomayor-Rios and J. A. Santiago of the Mayaguez Institute of Tropical Agriculture, Mayaguez, PR. We also appreciate the technical assistance offered by Joseph H. Bruemmer of the USDA Citrus and Subtropical Products Laboratory, Winter Haven, FL, and Franklin E. Barton, II, of the USDA Richard B. Russell Research Center, Athens, GA.

Registry No. Cellulose, 9004-34-6; hemicellulose, 9034-32-6; lignin, 9005-53-2.

LITERATURE CITED

Braddock, R. J.; Graumlich, T. R. *Lebensm.-Wiss. Technol.* 1981, 14, 229–231.

- Brillouet, J.-M.; Treche, S.; Sealy, L. *J. Food Sci.* 1981, 46, 1964-1967.
- Collinge, S. K.; Randall, S. G.; Mahoney, A. W. *J. Food Biochem.* 1980, 4, 111-117.
- Dovell, C. J.; Harris, N. D. *J. Sci. Food Agric.* 1982, 33, 185-193.
- Jeltema, M. A.; Zabik, M. E. *J. Sci. Food Agric.* 1980, 31, 820-829.
- Kawabata, A. *Mem. Tokyo Univ. Agric.* 1977, 19, 155-200.
- Kayisu, K.; Hood, L. F.; Van Soest, P. J. *J. Food Sci.* 1981, 46, 1885-1890.
- Lund, E. D.; Smoot, J. M. *J. Agric. Food Chem.* 1982, 30, 1123-1127.
- Marlett, J. A.; Lee, S. C. *J. Food Sci.* 1980, 45, 1688-1693.
- Martin, F. W. In "Tropical Foods: Chemistry and Nutrition"; Inglett, G. E.; Charalambous, G., Eds.; Academic Press: New York, 1979; Vol. 1, p 249.
- Morrison, I. M. *J. Sci. Food Agric.* 1980, 31, 639-645.
- Robertson, G. L.; Swinburne, D. *J. Food Sci.* 1981, 46, 1557-1562.
- Roe, B.; Bruemmer, J. H. *Proc. Fla. State Hort. Soc.* 1977, 90, 180-182.
- Selvendran, R. R.; DuPont, M. S. *J. Sci. Food Agric.* 1980, 31, 1178-1182.
- Sharma, K. K.; Pattabiraman, T. N. *J. Sci. Food Agric.* 1982, 33, 255-262.
- Sinclair, W. B. "The Grapefruit, Its Composition, Physiology and Products"; University of California, Division of Agricultural Science: Riverside, CA, 1972; p 223.

Received for review December 6, 1982. Revised manuscript received March 28, 1983. Accepted April 19, 1983. Mention of a trademark of proprietary product is for identification only and does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, nor does it imply approval to the exclusion of other products that may also be suitable.

A Study of the Mutagenicity of Irradiated Sugar Solutions: Implications for the Radiation Preservation of Subtropical Fruits

Johannes G. Niemand, Laetitia den Drijver, Clasina J. Pretorius, Cedric W. Holzapfel, and Hendrik J. van der Linde*

A comprehensive investigation of the γ -radiolysis of sugars present in subtropical fruit was carried out and mutagenicity against *Salmonella* tester strain TA 100 demonstrated. An attempt was made to identify the products responsible for the mutagenic activity of irradiated sugar solutions and glucosone was implicated as a mutagenic agent. Irradiated mango fruit was found *not* to be mutagenic. The effect of fruit components on the mutagen glucosone was investigated and a decrease in activity demonstrated. The implications of these findings for the safety of radurized foods are discussed.

During the past 3 decades, proof of the wholesomeness of irradiated foods has been a major obstacle to the acceptance of food irradiation as a viable process. Recent animal feeding studies (International Food Irradiation Project, 1979) have explicitly proved the safety of irradiated subtropical fruits such as mango and papaya, while all foods irradiated to a dose of 10 kGy were recently declared wholesome for human consumption by a Joint Expert Committee of the International Atomic Energy Agency, the Food and Agriculture Organization, and the World Health Organization. Nevertheless, results indicating mutagenicity or cytotoxicity of irradiated, one-component sugar solutions in short-term mutagenic tests need further clarification.

For this reason a comprehensive investigation of the γ -radiolysis of the major sugars present in subtropical fruits, viz., fructose, glucose, maltose, and sucrose, together with ribose, which were shown (Aiyar and Subbo Rao, 1977) to become mutagenic upon irradiation, was carried out. At the same time an attempt was made to identify the specific products responsible for the observed effects. The investigation was further extended to the mutagenicity testing of an irradiated aqueous model mango solution (Basson et al., 1979) as well as irradiated mango fruit.

Chemistry Department, Nuclear Development Corporation, Pretoria, South Africa (J.G.N., L.d.D., C.J.P., and H.J.v.d.L.), and Rand Afrikaans University, Johannesburg, South Africa (C.W.H.).

EXPERIMENTAL PROCEDURES

Mutagenicity Testing. The *Salmonella* mutagenicity assay of Ames et al. (1975) was employed in the testing of synthesized compounds by using five tester strains, viz., TA 1535, 1537, 1538, 98, and 100. A modification of the preincubation mutagenesis assay described by Aiyar and Subba Rao (1977) was carried out for the testing of irradiated sugar solutions. Two milliliters of an 18-h nutrient broth culture of the tester strains was centrifuged (2000g, 15 min) and resuspended in 2 mL of minimal medium broth, supplemented by a trace of histidine (0.5 mM) and Biotin (0.5 mM). This facilitates the growth of the bacteria for a few generations, which is necessary for mutagenesis to occur. To this broth, 2 mL of the irradiated sugar solution to be tested was added and incubated for 3.5 h at 37 °C with constant shaking. After incubation the cells were centrifuged, washed with saline, and then resuspended in 2 mL of saline. Of the latter suspension, 0.1 mL was incorporated into 2 mL of molten (45 °C) supplemented top agar and, after being mixed, poured onto a minimal medium plate and incubated for 48 h at 37 °C, after which it was scored. Plates showing maximum mutagenesis were replicated onto minimal plates without histidine in order to check the true revertant nature of colonies. Viable counts were always made from the saline suspension to determine the toxicity of the solution being tested.

All experiments were carried out in duplicate and repeated at least twice.