Evaluation of γ-Irradiation in Cocoa Husk

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γ-Irradiation was investigated as a technique to improve the hygienic quality of cocoa husk. Cocoa husk is a byproduct of cocoa bean processing industry. It contains approximately 57.5% (w/w) dietary fiber (nonstarch polysaccharides plus lignin), 15% (w/w) crude protein, 10.7% (w/w) mineral elements, 2.32% (w/w) cocoa butter, and 2.8% (w/w) carbohydrates (free sugars plus starch). The effect of irradiation on the growth rates of microorganisms are reported. Total counts, *enterobacteriaceae*, coliforms, *Staphylococcus aureus*, S*treptococcus* "D" of Lancefield, and yeast and mold counts before and after irradiation at 5, 8, and 10 kGy were determined. Cocoa husk was irradiated in open containers. An irradiation dose of 5 kGy was already sufficient to decrease the microbial counts to a very low level. No alteration in dietary fiber was measured in the irradiated product and no significant differences were detected between irradiated and nonirradiated cocoa husk.

Keywords: *Cocoa husk; microorganisms; γ-irradiation; dietary fiber*

INTRODUCTION

Cocoa beans (*Theobroma cacao* L.) are divided anatomically into three parts, cotyledon, shell, and germ in proportions ranging between 87 and 88/11−12/0.7−1. Main crop West African cocoas normally have a shell content of 11−12%, a norm against which other cocoas are judged. Higher shell percentages mean less edible material and, hence, a lower value. It is technically impossible to supply cocoa powder entirely shell free. Cocoa beans are exposed to a fermentation process in order to remove the mucilage pulpe, to kill the germ, and to induce extensive modifications within the cotyledons. This process also decreases the bitterness, reduces the astringency and causes the appearance of chocolate flavor precursors within the beans (Serra Bonvehí and Ventura Coll, 1997a,b, 1998). When the fermentation is completed, cocoas are dried, roasted, deshelled, and ground for the manufacture of both cocoa powder and chocolate. In terms of fat content, cocoa butter represents the major constituent of the cotyledon (55% approximately of the dry weight), whereas shell and germ contain only 3−5% of fat calculated on a dry weight basis. Cocoa husk is composed mainly of nonstarch polysaccharides (NSP) (cellulose, uronic acids, and noncellulosic components) and Klason lignin, along with small quantities of phenolic compounds, tannins, purine alkaloids, and cocoa butter (Serra Bonvehí and Aragay Benería, 1998; Serra Bonvehí and Escolà Jordà, 1998; Serra Bonvehí and Ventura Coll, 1999). According to its composition, cocoa husk has been regarded as a possible alternative to conventional sources of dietary fiber and mineral elements. This includes the knowledge of the microbiological quality of cocoa husk. The shell, as the cover of the cocoa bean, is always greatly polluted, and its bacteriological conditions are very poor. Cocoa husk with high plate counts usually contains many spores also. This is as the result of the fermentation process of the beans, which is done in growing countries. At the end of the fermentation, the temperature of the cocoa bean runs up to about 50 °C and permits the development of thermophilic organism. This includes many sporulated bacteria which show a considerable resistance to heating. Furthermore, an insufficient cocoa drying permits quick degradation by the action of microorganisms (fundamentally fungi) and enzymes (lipoxygenase, lipase, peroxidase, and polyphenol oxidase) (Villeneuve et al., 1985; Schwan et al., 1995). The bacteriological specification of cocoa husk must be interpreted just as appropriately as chemical and physical specifications (microbiological characteristics in cocoa powder: total plate count, ≤5000 cfu/g; molds and yeasts, ≤50 cfu/g; *Escherichia coli*, negative in 1 g; *Enterobacteriaceae*, negative in 1 g; *Salmonellae*, negative in 25 g; thermoresistant sporulated bacteria, ≤200 cfu/g; *Staphylococcus coagulase*, negative in 0.01 g) (Meursing, 1983). When the roasting process is not controlled or recontamination is not prevented, it is possible that plate counts become unpermissibly higher. In such cases, it is a tempting idea to sterilize cocoa husk subproducts artificially with radiation by γ rays. Food irradiation has been recognized as an efficient technology to extend the shelf life of fresh foods, and to decontaminate foods because irradiation does not include either heat or harmful substances which could cause an adverse effect in the wholesomeness of the foods. Cocoa bean can be irradiated at 1−5 kGy without appreciable loss in quality (Appiah et al., 1982). Related to irradiated foods, it is important to determine whether the effects of irradiation cause a deficiency in any food attributes and compare it with other technological processes since this could be extremely important from both health and marketing viewpoints (Thayer, 1990; Rayas-Duarte and Rugnow, 1993; Abdellaoui et al., 1995; Howard et al., 1995). In 1989, two luminescence techniques were included in the official collection of methods under article 35 of the German Foods Act as the first official methods for detection of irradiated foods: a chemiluminesce technique and a thermoluminescence (TL) analysis of mineral contaminants (Schreiber et al., 1993) were finally established in the

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Table 1. Cocoa Husk Sampling: Site of Origin

sample	country
1	Ivory Coast and Nigeria
2	Ivory Coast and Nigeria
3	Ivory Coast and Nigeria
4	Nigeria
5	Cameroon
6	Cameroon
7	Ivory Coast
8	Colombia
9	Colombia
10	Ecuador
11	Cameroon and Guinea
12	Brazil

European Union (CEN, 1996). The aim of the present examinations was to carry out more precise quantitative bacteriological analyses of cocoa husk. Simultaneously, the influence of the irradiation on the growth of microorganisms were determined and evaluated the effects of irradiation on the shelf life of cocoa husk.

MATERIALS AND METHODS

Samples. Twelve samples of unroasted cocoa husk of the main varieties of cocoa beans (*Theobroma cacao* L.) cultivated in Ivory Coast, Nigeria, Cameroon, Colombia, Ecuador, Guinea, and Brazil were studied (Table 1). Industrial extraction of cocoa butter from well-winnowed cocoa nib (cotyledon) was performed by expeller pressing followed by a second solvent extraction until cocoa butter ≤3 g/100 g. Commercial-grade hexane was used as solvent extraction. All samples were collected in 1995 and 1996. The samples were stored in the darkness at room temperature and analyzed no more than 2 weeks after arrival to the laboratory. All samples were ground to pass a 0.3-mm screen.

Irradiation. Cocoa husk was irradiated with γ-rays from a ^{60}Co source in the irradiated pool at the Aragogamma S. A. (Granollers, Barcelona, Spain), at doses varying from 5, 8, and 10 kGy at 25 °C (1 Gy = 100 rad = 1 J/kg = 0.239 cal/kg). The irradiation dose was determined according to EEC (1999) with Radiachromic dye film (Far West, FWT 60−00) (maximal dose detected/minimal dose detected ≤3). The recommended dose level in applying this technology to food preservation is 10 kGy or less, and food irradiation doses greater than 10 kGy are safe for consumption (FAO/IAEA/WHO, 1999; EEC, 1999). Irradiation D values were calculated from the function D = dose (kGy)/log cfu_a − log cfu_b, where a was the microbiological count before and b the microbiological count after irradiation.

Physicochemical Analyses. *Moisture.* Water content was determined by gravimetry using 5 g of cocoa husk finely ground in a conventional kiln at 103−105 °C for 3−4 h, until a constant weight was reached (Serra Bonvehí and Escolà Jordà, 1998).

Water Activity (A_w). This parameter was determined at 25 ± 0.02 °C with a Termoconstanter instrument (Humidat-TH2, Novasina, Switzerland).

Ash. Ash percentage was measured by calcination overnight at 500−550 °C in a furnace, to constant mass [AOAC (972.15), 1999].

Cocoa Butter. Five grams was extracted with petroleum ether (40−60 °C) for 6 h using a Soxhlet apparatus with previous hydrolysis [AOAC (963.15), 1999]. Fat was measured as the difference in weight of dried samples before and after extraction.

Total Protein Content. Nitrogen content of 2 g of pulverized cocoa husk was determined by conventional acid hydrolysis and Kjeldahl digestion using a copper catalyst [AOAC (970.22), 1999]. The ammonia was distilled and collected in a solution of boric acid, which was then titrated against standard acid. Digestion and distillation were carried out using the Kjeltec apparatus (model 1002, Tecator, Höganäs, Sweden). Protein content was calculated as total Nx 6.25.

pH. pH was measured in a 10% aqueous solution in an Expandable ion Analyzer (EA 920 Crison Research; Barcelona, Spain) pH-meter.

Nonstarch Polysaccharides (NSP). The Englyst procedures utilized determined total, soluble (SDF), and insoluble dietary fiber (IDF) as NSP, in 90−100 mg of pulverized cocoa husk, using enzymatic-chemical methods (Serra Bonvehí and Aragay Benería, 1998). Starch was dispersed with dimethyl sulfoxide and subsequently hydrolyzed by incubation with amylase and pullulanase. After enzymatic digestion of the protein and precipitation of NSP with ethyl alcohol, the starch-free residue was hydrolyzed with sulfuric acid. The neutral sugars released by hydrolysis were measured by colorimetry. An estimation of the insoluble NSP present in a sample was obtained replacing the precipitation of soluble NSP with ethyl alcohol by extraction with sodium phosphate buffer (pH 7). The values obtained represent insoluble NSP; soluble NSP is given by the difference between total NSP and insoluble NSP. When this dispersion was omitted from the procedure for total NSP, cellulose was dispersed with 12 M sulfuric acid prior to hydrolysis. When this dispersion was omitted from the procedure and replaced by direct hydrolysis with 2 M sulfuric acid, cellulose was not measured, and a value for noncellulose polysaccharides (NCP) was obtained. The value for cellulose was calculated as the difference between the glucose content of total NSP and that of the NCP obtained a specific colorimetrc method using glucose-oxidase.

Uronic Acids. Uronic acids were determined by colorimetric reaction according to Serra Bonvehí and Aragay Benería (1998). The hydrolyzate was mixed, diluted (1:5) with 2 M sulfuric acid, with 0.3 mL sodium chloride-boric solution. Concentrated sulfuric acid was added and the solution vortex mixed, placed in a heating block at 70 °C, and left for 40 min, then cooled to room temperature in ice−water. Then 0.2 mL of 3,5-dimethylphenol solution (0.1 g in 100 mL glacial acetic acid) was added and vortex mixed immediately. Between 10 and 15 min later, the absorbance was read at 400 and 450 nm against a water reference. The reading at 400 nm was subtracted from that at 450 nm, to correct for the interference of hexoses. The difference in absorbance obtained was plotted against glucuronic acid standards (Fluka, Buchs, Switzerland) over the range 25, 50, 75, 100, and 125 mg/kg. Sample concentrations were calculated or read from the graph.

Neutral-Detergent Fiber (NDF). NDF was determined according to Serra Bonvehí and Aragay Benería (1998). The 1 g sample was boiled in water for 1 min at 100 °C to gelatinize the starch. After cooling, the starch in the sample was hydrolyzed with α-amylase (Sigma Chemical Co., St. Louis, MO) and treated for 1 h at 100 °C with an NDF reagent of pH 7 (30 g of sodium lauryl sulfate, 18.162 g sodium hydrogen-EDTA, 6.81 g sodium tetraborate, 4.56 g disodium hydrogen phosphate, and 10 mL of 2-ethoxyethanol in 800 mL of distilled water) in the Tecator Fibertec System M (1020 Hot Extractor; Tecator AB, Höganäs, Sweden). After filtration, the residue was washed with hot water, acetone, and petroleum ether and then dried and weighed. A correction was made for the amount of ash in the residue, and the amount of NDF was calculated.

Acid-Detergent Fiber (ADF). ADF was determined according to Serra Bonvehí and Aragay Benería (1998). A 1 g sample was hydrolyzed with 0.5 M sulfuric acid. After filtration, the residue was washed with water, acetone, and petroleum ether and then dried and weight. A correction was made for the amount of ash in the residue, and the amount of ADF was calculated.

Klason Lignin (LAD). It was determined gravimetrically as ash-free, acid-insoluble residue (Serra Bonvehí and Aragay Benería, 1998). The acid detergent residue was treated with 72% sulfuric acid to separate cellulose from cutin. The crucible was placed in an enamel pan and half-filled with 72% sulfuric acid. Glass rods were used to stir and melt all particles. Asbestos was not used. The crucible was continuously replenished with sulfuric acid which was then removed by suction, and the residue throroughly washed with hot distilled water. The crucible was then dried overnight at 100 °C and weighed

Table 2. Effect of the Dose Irradiation on Different Levels of Microorganisms (x = cfu/g) in Cocoa Husk[a]

microorganism	blank	5 kGy	8 kGy	10 kGy	blank	5 kGy	8 kGy	10 kGy
aerobic colony	48×10^6	34×10^3	7×10^3	5×10^3	27×10^6	12×10^3	3×10^3	4×10^2
Enterobacteriaceae	25×10^3	<10	<10	<10	12×10^3	<10	<10	<10
coliform (MPN/g)	>2400	<3	<3	<3	>2400	<3	<3	<3
Streptococcus (MPN/g)	>2400	<3	<3	<3	>2400	<3	<3	<3
yeasts and molds	4×10^3	<10	<10	<10	12×10^3	<10	<10	<10

[a] Blank, nonirradiated; MPN, most probable number; NE of samples, 18; replicates, 3.

hot. Further loss of weight upon ashing (4 h at 525 °C) was interpreted as loss of Klason lignin.

Water-Holding Capacity (*WHC*). Approximately 20 mL of water was added to a 30 mL centrifuge tube containing 0.8−1 g of neutral-detergent fiber (NDF). This was mixed using a glass rod and shaken for 1 h in a water bath at 37 °C, leaving the glass rod in the tube for better mixing. After centrifuging at 14000*g* for 1 h at 10 °C, the supernatant was discarded, and the tube drained for 15 min. The wet NDF was weighed, dried overnight at 18−20 °C, and weighed again to determine the water content (Serra Bonvehí and Escolà Jordà, 1998). The water-holding capacity (WHC) was expressed in grams of water held by 1 g of cocoa husk.

Thermoluminescence (*TL*). The European Standard EN 1788 for thermoluminescence was used (CEN, 1996). Minerals were washed out of cocoa husk samples by wet-sieving and isolated by a sodium polytungstate [$Na_6(H_2W_{12}O_{40})\cdot H_2O$] density centrifugation. After dissolving carbonates with HCl, the minerals were transferred onto a stainless steel disk, which has to be completely clean and free of dust. The mineral sample were stored overnight at 50 °C and fixed with silicon spray. The background intensities of the TL were read regularly to ensure that there are no changes in the intensity ranges. The stainless steel disk was placed in the TL reader and heated with minerals fixed onto it from 70 to 350 °C, at a rate of 6 °C/s (model Harshaw TLDM). Light emission was recorded as a function of temperature (first glow curve) (Schreiber et al., 1995). For clear identification of irradiation, therefore, TL intensities have to be normalized, which is done by reirradiation of the sample with a dose of at least 1 kGy and determination of the TL intensity of the sample in a second glow under the same heating conditions. Light intensity of the ^{14}C source for at least 10 s was measured, the intensity determined in picoamperes (pA) or counts per second and the minimum TL intensity value (second glow) calculated. If this minimum value was not exceeded by the total integral of the second glow, the sample values would be excluded from data analysis. The ratio of first-glow intensity to second-glow intensity was about one when the cocoa husk was irradiated with doses larger than 1 kGy prior to examination.

Determination of Viable Counts. Twenty-five grams of cocoa husk finely ground was dissolved in 225 mL of 0.2% w/w buffered peptone water (BPW) (Biolife 401278; Biolife S.r.l., Milano, Italy) according to ICMSF (1983).

Aerobic Colony Count. One milliliter (0.1 mL when samples had more than 3000 cfu/g) of the solution was poured into each of two Petri plates, and 10−15 mL of plate count agar (BK-098; Biokar Diagnostics, Beauvais, France), prepared as directed by the manufacturer, was then added. When solidified, plates were incubated at 30 °C for 72 h, and then bacterial colonies were recorded [AOAC(966.23), 1999].

Enterobacteriaceae. One milliliter of the solution used in the aerobic colony count determination was poured into each of two Petri plates, and 10−12 mL of violet red bile glucose agar (VRBG) (Biolife-402188), prepared as directed by the manufacturer, was then added. When solidified, a second layer of culture media (5−7 mL) was added. When solidified, plates were incubated at 37 °C for 24−48 h, and then Enterobacteriaceae colonies were recorded (ICMSF, 1983).

Coliforms. Using separate sterile pipets, decimal dilutions of 10^{-2}, 10^{-3}, and 10^{-4}, and others as appropriate, of food homogenate were prepared by transferring 10 mL of previous dilution to 90 mL of 0.2% (w/w) of buffered peptone water (BPW). One milliliter of the solution used was poured into each of the three tubes with 10 mL of lauril pepto bios broth (Biolife-401580), prepared as directed by the manufacturer, was then added. Tubes were incubated at 37 °C for 24−48 h. Colonies were recorded [most probable number (MPN/g)] (Best, 1990), assuming that each of the three sets of three tubes were properly incubated and inoculated with 0.1, 0.01, and 0.001 g of the sample and that the numbers of positive and negative tubes (showing growth or no growth) were determined (ICMSF, 1983).

Streptococcus "D" of Lancefield. One milliliter of the initial solution (10^{-1}) is diluted to 10^{-2} and 10^{-3} with 0.2% (w/w) of buffered peptone water. One milliliter of the solution used was poured into each of three tubes with 10 mL of azide dextrose broth (Biokar BK-060), prepared as directed by the manufacturer, was then added. Tubes were incubated at 37 °C for 24−48 h. Colonies were recorded (MPN/g) and confirmed with Slanetz-Bartley agar (ADSA Micro 1−178; ADSA Micro, Barcelona, Spain) (ICMSF, 1983).

Staphylococcus aureus. One milliliter of the initial solution was poured into two tubes, and 9 mL of *Staphylococcus* enrichment broth base acc. to Baird (Merck 7899, Darmstadt, Germany), prepared as directed by the manufacturer, was then added. The tubes were incubated at 37 °C for 24 h. Colonies were confirmed with Baird-Parker base agar (Biolife-401116) (presence or absence per gram) [AOAC (975.55), 1999].

Yeasts and Molds. A 0.1 mL aliquot of the solution used in the aerobic colony count determination was extended on the surface of each of three Petri plates solidified including 15 mL of rose bengal agar (ADSA Micro 1−301), prepared as directed by the manufacturer. Plates were incubated at 22 °C for 5 days. Colonies were recorded and identified (ICMSF, 1983).

Statistical Analyses. The study of the repeatability of the analytical methods was carried out according to ISO-5725 (1986). Anova and Duncan's multiple range tests weres carried out using the Statgraphics Statistical package, version 6.0 (SAS, 1990). Group differences were considered statistically significant at a level of $p \leq 0.05$. All determinations were carried out in triplicate.

RESULTS AND DISCUSSION

Table 2 shows the results obtained looking after the adequate irradiation dose of the product, bearing in mind high and medium levels of microbiological contamination. A previous block of six samples of cocoa husk irradiated at a dose of 5, 8, and 10 kGy showed reductions in the mesophilic aerobic counts (in log cfu/g) from 7.68 and 7.4 before irradiation to 4.23−4.61 (5 kGy), 3.48−3.85 (8 kGy), and 2.60−3.70 (10 kGy) after irradiation, giving irradiation D values (kGy) of 1.58−1.63, 2.04−2.09, 2.08−2.51, respectively. The D values for Enterobacteriaceae, coliform, *Streptococcus*, and yeasts and molds couldn't have been calculated since these were no irradiation survivors. The reduction in the mesophilic aerobic count was considerably higher. According to the results obtained, the samples irradiated at 5 kGy were considered to be as good bacteriological quality (Table 2). In resemblance, Zehnder and Ettel (1986) found that dehydrated vegetables, dehydrated soup mixes, and dehydrated instant soup mixes can be sterilized with a dose of 5 kGy. The composition and microbiological quality was compared in 12 samples of cocoa husk before and after irradiation at a sufficient

Table 3. Microbiological Quality and Physicochemical Properties of Cocoa Husk Nonirradiated[a]

parameter	1	2	3	4	5	6	7	8	9	10	11	12	*x*	SD
pH	5.55	5.55	5.65	4.89	4.97	5.26	5.48	5.40	5.20	5.28	5.23	5.20	5.31	0.23
A_w	0.425	0.454	0.454	0.472	0.463	0.457	0.453	0.456	0.492	0.404	0.336	0.326	0.433	0.052
water content	6.3	7.4	6.9	7	7.1	7.6	6.6	7.3	7.8	7.7	5.3	3.6	6.72	1.2
ash	11.6	10.5	11.9	9.8	9.3	10.2	10.8	10.9	11.4	12	10.7	9.7	10.73	0.88
fat	2.5	2.2	2.3	2.3	2.4	2.2	2.3	2.3	2.2	1.8	2.3	3	2.32	0.27
crude protein	15.5	13.9	14.2	12.8	15.3	14.8	15.2	15.8	15.8	12.5	17.6	16.1	14.96	1.43
NSP	44.5	41.8	43.2	45	43.6	44.2	43.2	39.9	43.8	49.4	43.3	44.3	43.85	2.21
IDF	30.6	27.3	27.2	27.6	28.6	30	28.5	24.2	26.7	30.8	30.2	28.6	28.36	1.91
SDF	13.9	14.5	16	17.4	15	14.2	14.7	15.7	17.1	18.6	13.1	15.7	15.49	1.60
uronic acid	10.7	13.4	10.9	10.8	12.8	14.5	11.3	12.5	14.2	12.4	13.4	13.4	12.53	1.33
LAD	13.5	13.1	12.2	16.1	17	13.2	15.7	11.5	12	13.8	12.5	13.3	13.66	1.73
cellulose	21.4	20.3	19.6	17.5	20.2	20.1	20.3	17	18.3	20.8	21.3	19.5	19.69	1.41
WHC	3.87	3.72	4.12	2.8	2.98	3.26	3.68	4.04	3.86	4.18	3.29	3.66	3.62	0.45
NDF	47.2	45.4	50.3	34.2	36.3	39.7	44.9	49.3	47.1	51	40.1	44.6	44.18	5.46
ADF	31.7	32	28.4	27.1	25.4	24.8	34.9	22.3	28.7	25.9	29.8	30	28.3	3.69
aerobic colony[b]	1.92	11.64	19.61	5.74	12.67	8.42	15.64	20.57	18.51	10	9.58	1.12		
Enterobacteriaceae[c]	0.67	4.83	7.21	3.11	5.43	3.87	6.82	9.31	8.43	3.82	3.32	0.28		
coliforms[d]	>2400	>2400	>2400	93	>2400	>2400	>2400	>2400	>2400	>2400	>2400	<3		
Staphylococcus[e]	nd	nd	nd	nd	nd	nd	nd	nd	nb	nd	nd	nd		
Streptococcus[d]	>2400	>2400	>2400	1100	>2400	>2400	>2400	>2400	>2400	>2400	>2400	>2400		
yeasts and molds[f]	160	8300	2200	10	10	6800	90	700	500	2500	1500	30		

[a] A_w, water activity; water content (g/100 g); ash (g/100 g); fat (g/100 g); crude protein (g/100 g); NSP, nonstarch polysaccharides (g/100 g); IDF, insoluble dietary fiber (g/100 g); SDF, soluble dietary fiber (g/100 g); uronic acid (g/100 g); LAD, Klason lignin (g/100 g); cellulose (g/100 g); WHC, water-holding capacity (g of H_2O/g); NDF, Neutral detergent fiber (g/100 g); ADF, acid detergent fiber (g/100 g). [b] 10^6 cfu/g. [c] 10^3 cfu/g. [d] Most probable number (MPN/g). [e] Absence per gram. [f] cfu/g; nd, absence per gram.

Table 4. Microbiological Quality and Physicochemical Properties Irradiated Cocoa husk (5 kGy)[a]

parameter	1	2	3	4	5	6	7	8	9	10	11	12	*x*	SD
pH	5.47	5.51	5.59	4.84	4.87	5.29	5.54	5.36	5.28	5.37	5.21	5.17	5.29	0.24
A_w	0.417	0.444	0.449	0.478	0.469	0.447	0.449	0.451	0.486	0.411	0.345	0.327	0.431	0.05
water content	6.22	7.35	6.78	6.84	6.93	7.45	6.38	7.19	7.67	7.63	5.28	3.42	6.59	1.21
ash	11.2	10.6	11.5	9.9	9.2	10.3	10.7	10.7	11.3	12.2	10.9	9.8	10.69	0.82
fat	2.46	2.14	2.37	2.26	2.34	2.17	2.33	2.27	2.17	1.89	2.27	3.08	2.31	0.28
crude protein	15.2	14.1	14.4	13	15.1	14.9	15.3	15.6	15.4	12.7	17.3	16.2	14.93	1.27
NSP	44.8	42.1	43.5	45.2	43.4	44.1	43.5	40.2	44.1	49.2	43.4	44.6	44	2.11
IDF	30.7	27.5	27.3	27.8	28.7	29.8	28.7	24.4	26.9	30.7	30.1	28.9	28.46	1.82
SDF	14.1	14.6	16.2	17.4	14.7	14.3	14.8	15.8	17.2	18.5	13.3	15.7	15.55	1.55
uronic acid	10.9	13.3	10.7	11	12.7	14.3	11.6	12.7	14.3	12.6	13.2	13.7	12.58	1.28
LAD	13.7	13.3	12.4	16.4	16.8	13.4	15.6	11.6	12.1	13.6	12.8	13.1	13.73	1.67
cellulose	21.3	20.1	20.1	17.7	20.4	20.2	20.7	17.1	18.1	20.5	20.9	19.4	19.71	1.35
WHC	3.81	3.68	4.21	2.84	3.04	3.31	3.62	4.07	3.92	4.21	3.31	3.77	3.65	0.44
NDF	47.4	45.2	50.4	34	36.6	39.6	44.7	49.6	47.4	50.8	40.4	44.2	44.19	5.48
ADF	31.8	31.9	28.6	27.2	25.6	24.6	34.6	22.4	28.9	26.1	30	29.8	28.46	3.46
aerobic colony[b]	0.56	3.4	4.6	1.3	3.7	2.3	3.9	4.9	4.3	3.7	3.4	0.42		
Enterobacteriaceae	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd		
coliforms[c]	<3	<3	<3	<3	<3	<3	<3	<3	<3	<3	<3	<3		
Staphylococcus	nd	nd	nd	nd	nd	nd	nd	nd	nb	nd	nd	nd		
Streptococcus[c]	<3	<3	<3	<3	<3	<3	<3	<3	<3	<3	<3	<3		
yeasts and molds[d]	nd	<10	<10	nd	nd	<10	nd	<10	<10	<10	<10	nd		

[a] A_w, water activity; water content (g/100 g); ash (g/100 g); fat (g/100 g); crude protein (g/100 g); NSP, nonstarch polysaccharides (g/100 g); IDF, insoluble dietary fiber (g/100 g); SDF, soluble dietary fiber (g/100 g); uronic acid: (g/100 g); LAD, Klason lignin (g/100 g); cellulose (g/100 g); WHC, water-holding capacity (g of H_2O/g); NDF, neutral detergent fiber (g/100 g); ADF, acid detergent fiber (g/100 g). [b] 10^3 cfu/g. [c] Most probable number (MPN/g). [d] cfu/g; nd, absence per gram.

dose of 5 kGy. Table 3 illustrates the composition and distribution of the microorganisms in unirradiated cocoa husk. The elevated dietary fiber content (43.8 ± 2.21 g/100 g) and the ratio between insoluble and soluble fiber (IDF/SDF = 1.82) found in the cocoa husk justifies its inclusion as a nutritional product. Cellulose (19.7 ± 1.41 g/100 g) is its main constituent, followed by hemilcellulose and galacturonic acid. Lignin values varied significantly among the fiber sources. The average content in cocoa husk was 13.7 ± 1.73 g/100 g. The most indigestible fraction of the dietary fiber, ADF, is basically cellulose, lignin and cutin, with average content of 28.3 ± 3.69 g/100 g. The NDF takes into account the vegetal cell wall components, mainly cellulose, lignin, and hemicellulose (HMC), giving an average content of 44.1 ± 5.46 g/100 g. The unirradiated cocoa husk contains a rather large initial microflora which may vary considerably depending on harvest time, preprocessing treatment, and hygienic measures in the processing of the cocoa bean. During fermentation of cocoa, a successsion of microbial populations occurs: first anaerobes, then facultative aerobes, and finally the result is an acidified and stable product (Schwan et al., 1995). The content of mesophilic bacteria ranged between 2.8×10^2 and 20.5×10^6 cfu/g, and more than 80% of the samples had contents of 5.7×10^6 cfu/g or higher. The results showed that there was a great fluctuation in the flora population encountered. The counts of *Streptococcus* and coliforms were high (>2400 MPN/g), in contrast, *S. aureus* was not identified (absence per gram). Significant differences ($p \leq 0.05$) were noted in total aerobic mesophilic plate count, yeasts and molds, between samples and origin of samples, but not for coliforms, S*taphylococcae* and

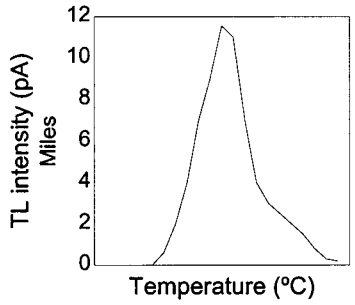

Figure 1. First glow curve of an irradiated cocoa husk (5 kGy). Heating from 70 to 350 °C was at a rate of about 6 °C/s.

Streptococcus counts. Table 4 shows the composition and microbiological quality in irradiated cocoa husk (5 kGy). The results illustrated that the composition and physicochemical properties were not affected. In general, the effects of low to medium doses (<10 kGy) of pasteurization or radurization have mild effects on carbohydrates which do not significantly alter either carbohydrates' functionality in foods or their nutritional values (Murano, 1995). A very distinct change of the composition of the microflora occurred after irradiation. Very low counts of mesophilic aerobic bacteria (not surpass microbiological limits established in cocoa powder) and absence of coliforms, *Streptococcus* "D" of Lancefield, and yeasts and molds were detected in agreement with the values obtained in Table 2. To facilitate the interpretation of these results, we also reported in these Tables the pH, water content, and A_w. To predict the shelf life of cocoa husk, it is essential to know these parameters that influence the survival and growth of microorganisms in the product. The water content does not surpass 7.67 g/100 g, and water activity ranged from 0.326 to 0.492 in both irradiated and no irradiated cocoa husk. The pH value of the samples a varied between 4.84 and 5.59. These A_w values do not allow the growth of microorganisms provided from recontamination or a postprocessing contamination. The moisture content was lower than the critical moisture content (8 g/100 g) for mold and yeast growth in cocoa (Schwan et al., 1995), depending on the relative equilibrium moisture contents in different environments and lengths of time of storage. The moderate water-holding capacity (WHC) values found in cocoa husk (3.65 ± 0.44 g water/g) appear to be related to their high concentration of cellulose (Serra Bonvehí and Aragay Benería, 1998). The chemical composition of the fiber plays a role in its ability to hold water and in the value of water activity. The absorbed dose of γ-irradiation was determinated according to thermoluminescence method. Moriarty et al. (1988) found that thermoluminiscence intensity displays a linear relationship with the adsorbed dose. However, even isolated minerals of irradiated products exhibit very different TL intensities (Schreiber et al., 1995). In recent years, as a consequence of that the relative intensity of TL signal, only allows a qualitative statement, defining if the product has been irradiated or not (CEN, 1996). The application of TL to the identification of foods which have been treated with ionizing radiation has been extensively studied (Raffi et al., 1994). Figure 1 shows the first glow curve of irradiated cocoa husk (5 kGy). In unirradiated samples, no thermoluminiscence signal was observed, the TL ratio (the quotient of the total glow curve integral of the first reading and the total glow curve integral of the second reading) was less than 0.60. Whereas in irradiated samples, a TL signal of 11 540 pA was seen, and the TL ratio was larger than 0.70. This signal intensity remained almost constant for 6 months of storage in agreement with electron spin resonance signal (Mangaonkar et al., 1997). Of the 12 samples irradiated at 5 kGy, none sample was incorrectly identified as nonirradiated. This study demostrated clearly that irradiation at doses of 5 kGy, as an alternative process to guarantine treatment, preserved the bacteriological quality of the cocoa husk.

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