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Decontamination of Sugar Syrup by Pulsed Light

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ABSTRACT

The pulsed light produced by xenon flash lamps was applied to 65 to 67 °Brix sugar syrups artificially contaminated with suspensions of *Saccharomyces cerevisiae* and with spores of *Bacillus subtilis, Geobacillus stearothermophilus, Alicyclobacillus acidoterrestris,* and *Aspergillus niger*. The emitted pulsed light contained 18.5% UV radiation. At least 3-log reductions of *S. cerevisiae, B. subtilis, G. stearothermophilus,* and *A. acidoterrestris* suspended in 3-mm-deep volumes of sugar syrup were obtained with a fluence of the incident pulsed light equal to or less than 1.8 J/cm^2, and the same results were obtained for *B. subtilis* and *A. acidoterrestris* suspended in 10-mm-deep volumes of sugar syrup. *A. niger* spores would require a more intense treatment; for instance, the maximal log reduction was close to 1 with a fluence of the incident pulsed light of 1.2 J/cm^2. A flowthrough reactor with a flow rate of 320 ml/min and a flow gap of 2.15 mm was designed for pulsed light treatment of sugar syrup. Using this device, a 3-log reduction of *A. acidoterrestris* spores was obtained with 3 to 4 pulses of incident pulsed light at 0.91 J/cm^2 per sugar syrup volume.

Sugar syrups have a wide range of possible use in the food industries *(4)*. Many microorganisms, mainly spore-forming bacteria, yeasts, and molds, contaminate sugar syrups. With the exception of osmophilic yeasts and xerophilic molds, most microorganisms are unable to multiply because of the low water activity of sugar syrup. After dilution of sugar syrup in the final products, for preparation of fruit drinks and other soft drinks for example, microorganisms may find favorable conditions for growth and ultimately cause spoilage, such as off-odor, off-flavor, particle formation, and package swelling. Moreover, the risk of food poisoning cannot be fully excluded; for instance, *Clostridium botulinum* has been detected in corn syrup *(15)*. Microbial contaminants in sugar syrups can be eliminated by heating *(4)*. However, pasteurization of the final product can cause changes in color, flavor, and other organoleptic qualities of the drinks. Pasteurization may also be restricted by the high resistance of some food contaminants *(2, 22)*. Pulsed light (PL) appears as an alternative to conventional thermal and chemical decontamination processes. Microorganisms are inactivated by short-time (10^{-6} to 10^{-3} s), UV-C–rich light pulses of high energy (with a peak power density that can exceed several thousand watts per square centimeter) *(9)*. PL is able to inactivate a range of molds, viruses, and bacteria in solid foods, such as food powders, vegetables, or salmon *(7, 8, 17)*, in water *(11)*, in liquid foods, such as milk and fruit juices *(21)*, and on the surface of material in contact with foods, such as packaging material *(6, 23)*. Up to several log reductions of microbiological populations have been obtained in many instances. This demonstrates the possibilities of application of the technology *(6, 23)*. The objective of this work was to evaluate the efficiency of PL for the decontamination of sugar syrup. The selected microorganisms (*Saccharomyces cerevisiae, Aspergillus niger, Bacillus subtilis, Alicyclobacillus acidoterrestris,* and *Geobacillus stearothermophilus*) are among the major microbiological contaminants of sugar syrups, fruit drinks, and soft drinks.

MATERIALS AND METHODS

Bacterial strains and preparation of spore suspensions. A loopful of a stock culture stored at −80°C in a 30% (vol/vol) glycerol solution of *A. acidoterrestris* strain ATCC 49025T (American Type Culture Collection, Manassas, VA), *B. subtilis* DSM 402 (=*B. subtilis* 168) (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany), or *G. stearothermophilus* CIP 6623T (Collection de l'Institut Pasteur, Paris, France), respectively, was spread on orange serum agar (pH = 4.5) (OSA; Oxoid, Cambridge, UK) plates at 45°C for 72 h *(16),* on Luria-Bertani agar (LBA) plates at 30°C for 24 h (pH = 7.0), or on fortified nutrient agar plates at 55°C for 24 h (pH = 7.0) *(5)*. A colony of each strain was picked and suspended in 1 ml of sterile distilled water, and then a volume of 200 μl was spread for spore production on OSA, on LBA (Sigma Aldrich, Steinheim, Germany), or on fortified nutrient agar (all ingredients from AES Laboratoire, Bruz, France) for 7 days at the temperatures indicated above. After 7 days, the cultures contained >90% free spores. Three 1-ml volumes of sterile demineralized water were poured

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onto the OSA, LBA, or fortified nutrient agar plates. The agar surface was scraped with caution with a spreader to detach and suspend spores. The suspensions were pipetted and centrifuged for 15 min at 7,000 × *g*, and the pellet was then suspended again in 25 ml of sterile water. This operation was performed twice. The pellet was suspended again and centrifuged twice at 5,000 × *g* and twice at 4,000 × *g* (Biofuge15R, Heraeus, Osterode, Germany). After the last wash, the pellet was suspended in 2 ml of sterile distilled water *(18)*. Spores were kept at 4°C for up to 6 months. Immediately before use, the spore suspensions of *B. subtilis* were heated for 10 min at 70°C and those of *A. acidoterrestris* and *G. stearothermophilus* for 10 min at 80°C to inactivate vegetative cells *(18)*. Spores were counted by spreading volumes of 0.1 ml of decimal serial dilutions on OSA or LBA plates. The spore suspensions contained 10^7 to 10^8 CFU/ml. The absence of spore clumps and of phase dark spores revealing germination was checked after each spore preparation and before use by phase-contrast microscope observation under ×1,000 magnification (Olympus BX50, Olympus, Rungis, France).

***A. niger* spore suspensions.** A loopful of a stock culture stored at 20°C in a 30% (vol/vol) glycerol solution of *A. niger* ATCC 9642 was spread on malt extract agar (MEA; Sigma Aldrich, Steinheim, Germany) plates at 30°C for 5 days. The mycelium of these plates was then spread on malt extract agar and incubated for 7 days at 30°C. After 7 days, two 5-ml volumes of a sterile Tween 80 solution (0.1%, wt/vol) were successively poured onto the mycelium. Spores were detached by smooth agitation of the plates. The solutions containing the spores were pipetted, pooled, and dispensed into tubes for storage at 4°C until use. Spores were detached from the mycelium with two 5-ml volumes of a sterile Tween 80 solution (0.1%, wt/vol). The spore suspension was stored at 4°C until use.

***S. cerevisiae* cell suspensions.** A loopful of a stock culture of *S. cerevisiae* stored at −20°C in a 30% (vol/vol) glycerol solution was spread on malt extract agar plates and incubated at 30°C for 48 h. A colony was picked, suspended in malt extract broth, and incubated for 48 h at 30°C with shaking at 100 rpm/min in a bench-top incubated shaker (model MaxQ 4000, Thermo, Dubuque, IA).

Preparation of sugar syrup. The sugar syrups tested were (i) a pure sucrose syrup at 65 °Brix (prepared with sucrose from VWR International, Leuven, Belgium, diluted in demineralized water) and (ii) industrial sugar syrups at 65 and 67 °Brix (Beghin-Say, Origny Sainte Benoite, France). The absorbance spectra were determined with a Cary 1E UV-visible light spectrophotometer (Varian, Palo Alto, CA). Sugar syrup was filter sterilized to avoid (i) potential interference in microbial counts between the spiked microorganism and the natural microflora and (ii) color changes caused by heat sterilization. The filtration treatment lasted approximately 1 h for a 250-ml sugar syrup volume. Sterile sugar syrups were obtained by filtration with 0.22-μm-pore-size filters (Millipore, Bedford, MA). Sugar syrups were then artificially contaminated with 10^5 to 10^6 CFU/ml bacterial spores or *S. cerevisiae* cells or with 10^4 CFU/ml *A. niger* spores.

Treatment of sugar syrup by PL. PL was delivered by a laboratory-scale PL system (Claranor SA, Avignon, France). All components were identical to those installed in industrial systems. Briefly, pulses (approximate duration, 250 μs) of broad-spectrum (200- to 1,100-nm) white light rich in UV (200 to 400 nm) were produced by a xenon flash lamp. The lamp input voltage was 2.5 kV. The optical energy of the lamp was estimated to be 103 J

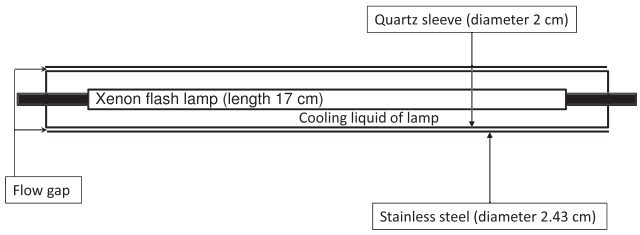

FIGURE 1. *Schematic of the thin flowthrough reactor used for the PL treatment of sugar syrup. The sugar syrup flows in the 0.215-cm gap between the 2-cm-diameter internal quartz sleeve and the 2.43-cm-diameter external stainless sleeve.*

(3). The percentage of UV in the 220- to 385-nm range was 18.5% of the PL emitted by the PL equipment used. Treatments were performed on sugar syrup volumes of 5.5 and 20 ml poured into 5-cm-diameter petri dishes, corresponding to sugar syrup thicknesses of approximately 3 and 10 mm. The PL fluences at the top of the sugar syrup (incident fluence) were from 0.3 to 1.86 J/cm^2. Fluences up to 0.8 J/cm^2 were delivered in one pulse and higher fluences in two pulses at a 1-s interval. Previous experiments have shown that the fluence was homogeneously distributed at the surface of the plates (data not shown). Variations in fluences were obtained by varying the distance between the samples and the lamps (from 7 to 23 cm) using an adjustable laboratory elevator (top plate dimension, 20 by 20 cm; open height, 30 cm; Fisher Scientific, Illkirch, France). The PL fluences were measured using a Gentec QE 12 LP Joulemeter with a 1.1-cm-edge square probe connected to a SOLO 2 power and energy meter (Gentec Electro-Optics, Inc., Quebec, Canada). An attenuator (model QEAS12, Gentec Electro-Optics, Inc.) was used for measurements at the highest fluences. Untreated samples were used as controls.

A pilot-scale flowthrough reactor was designed for PL treatment of sugar syrup (Claranor, Avignon, France). Sugar syrup inoculated with *A. acidoterrestris* spores ran in a 0.215-cm-wide flow gap between an outer stainless steel cylinder and an inner quartz sleeve containing a xenon flash lamp at its center (Fig. 1). The optical energy delivered by the lamp at 2,500 V was 103 J. The whole optical energy was distributed to the reactor. The theoretical incident and transmitted fluence (in the absence of sugar syrup) were obtained by dividing the optical energy by the inner and outer surface area of the cylindrical reactor, 106.8 and 129.8 cm^2, respectively. Consequently, the fluence of the incident PL was 0.96 J/cm^2 per pulse and the fluence of the transmitted PL was 0.79 J/cm^2 per pulse. The inactivation of *A. acidoterrestris* spores as a function of the number of pulses per volume of sugar syrup flowing through the reactor (from 1 to 4) was measured. The flow of 5.3 ml/s was determined by the rotations per minute of the volumetric pump supplying the sugar syrup. The volume of the reactor was 25.7 ml. According to the Napier-Stokes equation and assuming a linear flow, the velocity at the center of the flow gap is twice the mean velocity. The lowest residence time is therefore half of the mean residence time. A 1 flash per volume treatment was based on this lowest residence time and was equal to 0.5 times the volume of the reactor per flow rate, i.e., one flash each 2.4 s. Before and after each experiment, the system was decontaminated with 500 ml of a chlorine solution (10%) and 500 ml of hydroalcoholic solution (25% ethanol, vol/vol) and washed with 500 ml of sterile distilled water. The absence of surviving microorganisms was checked before and after each experiment by sampling approximately 20 ml of the last washing water in a sterile tube and spreading 100-μl volumes on 5 to 10 OSA plates incubated at 30°C for 48 h.

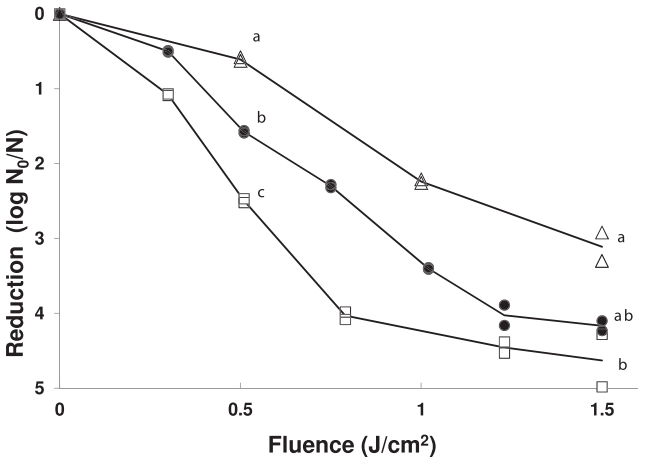

FIGURE 2. *Effect of a PL treatment on* B. subtilis *spores in water (□), 65 °Brix pure sucrose syrup (●), and 65 °Brix industrial sucrose syrup (△). The input voltage was set at 2.5 kV. The thickness of the liquid layer was 10 mm. Each symbol represents data from one experiment. Solid lines represent the average value for each tested medium. Reduction is expressed as log* N_0/N, *where* N_0 *is the concentration of the inoculated suspension and* N *the number of survivors after treatment. At selected fluences, different letters indicate a significant difference at* $P < 0.05$ *(Tukey's HSD test).*

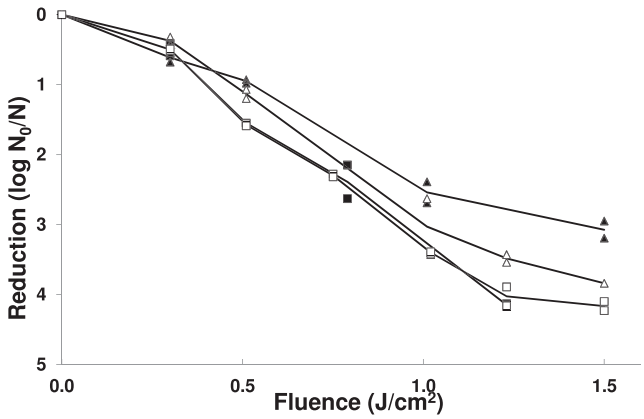

FIGURE 3. *Effect of a PL treatment on* B. subtilis *(□, ■) and on* A. acidoterrestris *(△, ▲) spores suspended in a 65 °Brix pure sucrose syrup. The sugar syrup layers were 3 mm (open symbols) and 10 mm (closed symbols) thick. The input voltage was set at 2.5 kV. Each symbol represents data from one experiment. Solid lines represent the average value of experimental data obtained on each species and with each thickness of sugar syrup. Reduction is expressed as log* N_0/N, *where* N_0 *is the concentration of the inoculated suspension and* N *the number of survivors after treatment.*

Microbial counts. After treatment by PL, decimal dilutions of the sugar syrup samples in sterile distilled water were spread on OSA and incubated at 45°C for 48 h when spiked with *A. acidoterrestris,* spread on malt extract agar and incubated at 30°C for 48 h when spiked with *A. niger* spores or *S. cerevisiae,* or spread on LBA and incubated for 24 h at 30 or 55°C when spiked with *B. subtilis* or *G. stearothermophilus* spores, respectively.

Experimental design and expression of microbial inactivation. Each inactivation curve (reduction as a function of fluence or number of flashes per volume) was replicated at least twice and up to four times. The replications were carried out on different dates with different suspensions of cells or spores. The reduction was expressed as log N_0/N, where N_0 is the initial concentration of the inoculated suspensions and N the number of survivors after a PL treatment, as a function of the applied fluence.

Statistical analysis. Significant differences in means were determined by one-way analysis of variance and Tukey's honestly significant difference (HSD) test at the 5% level using SYSTAT, version 9 (SPSS, Chicago, IL).

RESULTS AND DISCUSSION

Inactivation of *B. subtilis* spores by PL in water and sugar syrup. A 3-log reduction of *B. subtilis* spores suspended in a 10-mm-thick layer of 65 °Brix industrial sucrose syrup was obtained with a fluence of 1.5 J/cm^2. A similar reduction was obtained with the same sugar syrup at 67 °Brix (data not shown). At this fluence of 1.5 J/cm^2, significantly higher log reductions of 4.2 and 4.6 ($P < 0.1$, Tukey's HSD test) were obtained on spores suspended in 65 °Brix pure sucrose syrup and in sterile distilled water, respectively (Fig. 2). Factors that affect the inactivation of microorganisms in liquid foods include color, viscosity, and opacity *(10, 13, 19)*. The reduction in the sugar syrup was lower than that obtained in water. A lower light transmission in sugar solutions, in particular of the UV-C wavelengths, might explain this difference in inactivation. The coefficient of absorption of clear syrup at 254 nm has been estimated to be at least 200-fold greater than that of distilled water *(10, 12)*. The absorbance spectra of both tested sugar syrups were highly similar, and it is difficult to conclude how minor differences in absorbance could have resulted in a pronounced difference in the inactivation of *B. subtilis* spores. This could be due to lower purity of the industrial sugar syrup, without further evidence from our data.

The coloration of retail and industrial syrups may differ. The efficiency of PL was therefore tested on a range of sugar syrups of different colors. The microbial inactivation was observed only in uncolored sugar syrups (data not shown). For instance, a suspension of *B. subtilis* spores in a 3-mm-deep layer of light-brown sugar syrup at 65 °Brix was unaffected by a PL treatment at 1.8 J/cm^2. The absorbance values of this light-brown sugar syrup in the UV wavelengths 235 to 300 nm was higher than 2.0, while the absorbance of the uncolored industrial and pure sugar syrups remained below 0.5 in the same wavelengths.

Effect of the depth of the sugar syrup layer on *B. subtilis* and *A. acidoterrestris* inactivation by PL. A log reduction higher than 3 was obtained with both *B. subtilis* and *A. acidoterrestris* spores suspended in 3-mm- and 10-mm-deep sugar syrup layers exposed to one pulse at 1.5 J/cm^2. Increasing the depth of the sugar syrup layer from 3 to 10 mm did not significantly affect *B. subtilis* inactivation ($P > 0.1$) (Fig. 3). In water, the reduction was lower in a 10-mm-deep layer than in a 3-mm-deep layer only with fluences higher than 1 J/cm^2 (data not shown). In sugar syrup, the reduction in *A. acidoterrestris* spore numbers was also lower in a 10-mm-deep layer with

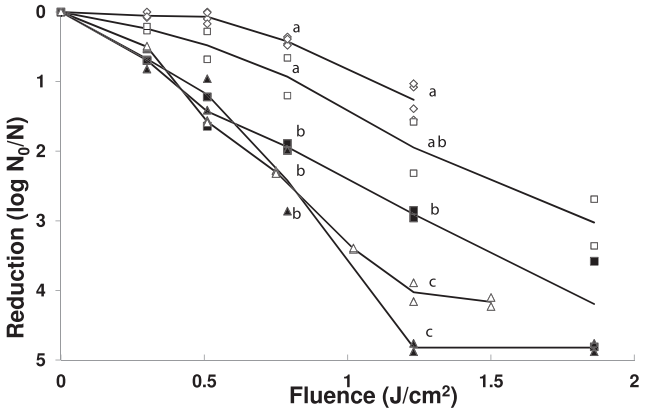

FIGURE 4. *Effect of PL treatment on* A. niger *spores (◇),* A. acidoterrestris *spores (□),* G. stearothermophilus *spores (■),* B. subtilis *spores (△), and* S. cerevisiae *vegetative cells (▲) suspended in 65 °Brix industrial sucrose syrup. The depth of the sugar syrup layer was 3 mm. The input voltage was set at 2.5 kV. Each symbol represents data from one experiment. Solid lines represent the average value obtained with each strain. Reduction is expressed as log* N_0/N, *where* N_0 *is the concentration of the inoculated suspension and* N *the number of survivors after treatment. At selected fluences, different letters indicate a significant difference at* $P < 0.05$ *(Tukey's HSD test).*

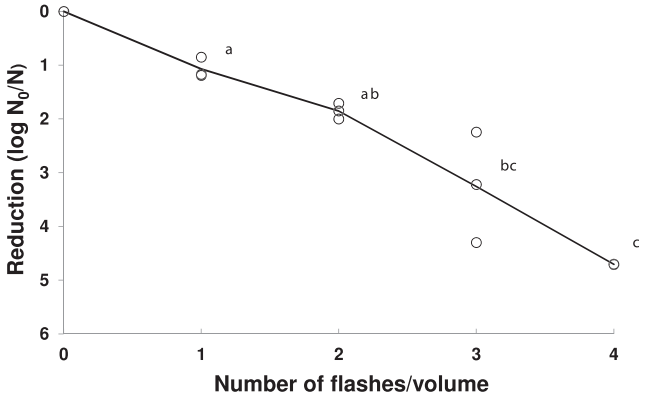

FIGURE 5. *Inactivation by PL and using a flowthrough reactor of* A. acidoterrestris *spores (○) suspended in 65 °Brix industrial sucrose syrup. The fluid gap in the reactor was 2.15 mm thick. The input voltage was set at 2.5 kV. Each symbol represents data from one experiment. The solid line represents the average value.* n = *4, except at 4 flashes per volume, where* n = *2. Reduction is expressed as log* N_0/N, *where* N_0 *is the concentration of the inoculated suspension and* N *the number of survivors after treatment. Different letters indicate a significant difference at* $P < 0.05$ *(Tukey's HSD test).*

fluences higher than 1 J/cm^2. The inactivation rate will depend on the distribution of fluence in the depth of the liquid and on the location of microorganisms. According to the Beer-Lambert law, the light intensity exponentially decreases as the optical path length increases. However, the inactivation of both microorganisms was still significant (i.e., a log reduction greater than 3 with one pulse) in a sugar syrup layer that was 10 mm deep.

Diversity in microorganism susceptibility to PL inactivation in sugar syrup. The decontamination curves of different microorganisms treated by PL are shown in Figure 4. *S. cerevisiae* showed the highest inactivation in sugar syrup, with a 5.4-log reduction at a fluence of 1.23 J/cm^2. The log reductions of *B. subtilis* and *G. stearothermophilus* spores with a fluence of 1.86 J/cm^2 were greater than 4. PL treatment of *A. acidoterrestris* spores achieved a 3-log reduction with a fluence of 1.86 J/cm^2. The most resistant microorganism of the panel was *A. niger,* with a reduction of only 1.3 log at 1.2 J/cm^2. *A. niger* is among the most UV-C irradiation-resistant microorganisms *(1, 10).* The relative sensitivities to PL of the microorganisms suspended in sugar syrup were in agreement with the sensitivities to PL of cells and spores of the same strains and species spread on agar *(14).*

Inactivation of *A. acidoterrestris* in a flowthrough PL reactor. Previous inactivation tests were performed on microorganisms suspended in static solutions. Industrial treatment by PL has to be applied to a continuous flow of large volumes of sugar syrup. The inactivation of *A. acidoterrestris* in a flowthrough PL reactor was almost linearly linked to the number of pulses delivered per sugar syrup volume (Fig. 5). A 1-log reduction was obtained on average for each flash per volume. A 3-log reduction was obtained in three flashes per volume on spores suspended in the 2.15-mm-thick sugar syrup layer. A similar reduction was obtained in static tests with a fluence of 1.86 J/cm^2 in a 3-mm-deep sugar syrup layer. This 3-log reduction is the common recommendation for commercial canning in which sugar syrup may be used *(20).* From different sources reporting *D*-values for *A. acidoterrestris* in fruit juices *(20),* a heat treatment of 5 to 20 min (corresponding to 2 to 3 *D*-values) at 95°C would be necessary to achieve the same reduction. This heat treatment could be even longer because of the low water activity of sugar syrup that increases the heat resistance of microorganisms.

In conclusion, significant inactivation (i.e., several log reductions) of spore-forming bacteria and *S. cerevisiae* cells was obtained in uncolored samples of sugar syrup treated with PL. This was achieved with a low number of pulses (from one to four) and in up to 10-mm-thick sugar syrup. The same efficiency was obtained with *A. acidoterrestris* in a continuous flow system. PL delivered by xenon flash lamps is rich in UV. Applications of PL to liquid foods are potentially similar to applications of UV light *(12).* A major advantage of PL is the short duration of the treatment. PL requires shorter residence time for microbial inactivation and, consequently, higher flows of liquids could be treated using several parallel modules of PL treatment.

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