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Effects of X-ray irradiation on the microbial growth and quality of flue-cured tobacco during aging

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HIGHLIGHTS

- 2 kGy dose improved sufficiently the microbial safety of flue-cured tobacco.
- The doses up to 3 kGy did not affect the chemical components.
- A dose <3 kGy had no effect on sensory scores.
- The recommended dose to irradiated flue-cured tobacco is the range of 2–3 kGy.

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ABSTRACT

X-ray irradiation was evaluated for improving microbial safety and the quality of flue-cured tobacco during aging. Tobacco samples were irradiated at doses of 0, 1, 2, 3 and 5 kGy and stored for 12 months under normal storage conditions or in a high-humidity (RH $>70\%$) room. Microbiological data indicated that the population of total aerobic bacteria was significantly decreased with increasing irradiation doses. In particular, a dose of 2 kGy was effective for the decontamination of fungi from the tested samples, with a 0.93 log CFU/g reduction for bacteria. The control and 1 kGy X-ray treated tobacco samples were became rotted and moldy after the 12th month, whereas those treated with 2, 3 and 5 kGy had no detectable mold during 12 months of storage at high humidity. Chemical measurements showed that irradiation up to 3 kGy did not affect the total nitrogen, nicotine, reducing and total sugars, ratio of total nitrogen to nicotine and sugar-to-nicotine ratio. Furthermore, sensory evaluation results also showed that X-ray irradiation did not affect sensory scores with irradiation at a dose <3 kGy. Based on these results, X-ray irradiation dose in the range of 2–3 kGy is recommended for the decontamination of fungi from flue-cured tobacco.

1. Introduction

The flue-cured tobacco leaves is one of the most important type of tobacco in the world. Cured but unaged tobacco is unsuitable for cigarette products because it has a sharp, disagreeable odor and an undesirable aroma; it also produces harsh, irritating smoke. During industrial production, a further process called aging or fermentation for approximately 1–2 years is typically applied to improve the quality of flue-cured tobacco. Aging allows the leaf to go through more chemical changes, resulting in the smooth flavor of the tobacco. However, because of the high relative humidity ($>70\%$) on rainy days and the hygroscopic nature of dry tobacco leaves, cured leaves are more susceptible to the development of mold at temperatures between 10 and 32 °C, and the appearance of mold on tobacco greatly reduces the quality and marketability of the crop (Bailey, 2005). Furthermore, the presence of mold on tobacco products poses great health risks to consumers, as some of these molds produce potent mycotoxins (Pauly and Paszkiewicz, 2011).

By means of the direct, undiluted application of various alcohols, some growers have succeeded in preventing mold on tobacco (Bailey, 2005). However, due to the highly flammable nature of alcohols, a resulting fire can pose a serious danger to persons and property. Although chemicals registered for the control of mold on

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cured tobacco are rare, there are rumors of attempts made using common reagents, which could result in serious and illegal residue problems, hindering the usefulness of the tobacco to the industry. Based on a properly conducted experiment, the spraying of Ridomil on the floor and walls of the storage and the tobacco itself to prevent mold is ineffective; moreover, this product is not labeled for such use. This fungicide is not even effective to control the group of fungi involved (Nesmith et al., 2000). Any fungicide able to control mold would need to be applied in the field, with sufficient residues remaining during curing and marketing. If some fungicides have a similar residual activity, these residues would not be accepted by the tobacco companies (Bailey, 2005). Currently, judicious management of the storage barn's ventilation system is commonly used to prevent moldy tobacco (Nesmith et al., 2000), but this approach is troublesome and not cost effective.

Ionizing radiation is a well-established method for providing hygienic quality by reducing the microbial spoilage of foods (Farkas and Mohácsi-Farkas, 2011). As X-ray irradiation does not produce radioactive waste and can pass through thick materials (approximately 30–40 cm) (Oner and Wall, 2013), it can be applied as a more efficient preservation method for foods to achieve microbial decontamination. Previous studies have demonstrated that X-rays can result in very high microbial reduction efficacy for different pathogens on spices, strawberries, ready-to-eat shrimp, milk, spinach leaves, tomatoes, smoked catfish and sweet potatoes (Van Calenberg et al., 1998, 1999; Mahmoud, 2009a, 2009b, 2010; Mahmoud et al., 2010, 2012; Oner and Wall, 2013). However, there is no report on the decontamination of microorganisms on tobacco by X-ray irradiation, especially for flue-cured tobacco during aging.

Therefore, the objectives of this study were to examine the effects of X-ray irradiation on the microbial safety and quality of flue-cured tobacco during aging and to suggest appropriate processing conditions.

2. Materials and methods

2.1. Tobacco

The tobacco used for treatments was an all-lamina flue-cured tobacco blend. Unaged flue-cured tobacco (grade C2F) was collected from Tianchang International Tobacco Co., Ltd. (Xuchang, Henan Province, China) in 2012, and the samples (1 kg) were placed into polyethylene bags.

2.2. X-ray irradiation treatment

The bags of tobacco were taken to a commercial irradiation facility at Irradiation Technology Co., Ltd. of Tianjin Binhai, Tianjin, China, which uses an electron linear accelerator (7.5 MeV, model ISO750; NUCTECH Co., Ltd., Beijing, China) and converts the e-beam into X-rays for the treatment of produce. The tobacco samples were treated with 0 (control), 1, 2, 3 and 5 kGy X-ray irradiation at ambient temperature. The absorption dose was determined using a dosimeter (FWT-60-00-Radiochromic Film; Far West Technology, Goleta, CA, USA) assessed at 510 nm with a Gold Spectrumlab 54 reader (Lengguang Technology Co., Ltd., Shanghai, China). Twelve packages of tobacco were irradiated for each treatment. After irradiation, the samples were divided randomly into two equal lots of six packages each. One sample (I) was maintained under normal storage conditions unfavorable to mold development. To hasten the development of the mold, the other sample (II) was kept in a high-humidity (RH > 70%) room. Both treatments were sampled after 0, 6 and 12 months. Sample II was used for microbiological analysis, and Sample I was used for chemical measurements and sensory evaluation.

2.3. Microbiological analysis

Each sample (25 g) was mixed with 225 ml sterile physiological saline (0.85% NaCl) in a sterile stomacher bag, homogenized for 2 min using a Lab-blender stomacher SH-400 A (Hogon Scientific Instrument Co., Ltd., Shanghai, China), and serially diluted (1:10). Total aerobic bacterial and mold/yeast were plated and enumerated using plate count agar (PCA, Aoboxing Bio-Tech Co., Ltd., Beijing, China) and potato dextrose agar with chloramphenicol (PDA, Aoboxing Bio-Tech Co., Ltd.) by incubating plates at 37 °C for 48 h and at 28 °C for 96–120 h, respectively. Each microbial count was the mean of three determinations and is expressed as log CFU g^{-1}.

2.4. Chemical measurement

Total nitrogen, nicotine, and reducing and total sugars were determined by colorimetric methods with a continuous flow automatic chemical analyzer (Auto Analyzer 3, SEAL Analytical GmbH, Norderstedt, Germany). Nicotine and reducing and total sugars were extracted in 5% acetic acid solution. The reducing and total sugars were analyzed through the formation of a yellow complex after reaction with p-hydroxybenzoic acid hydrazide (YC/T 159, 2002). The content of nicotine was determined after a reaction with on-line-generated cyanogen chloride and sulfanilic acid to form a yellow compound (YC/T 160, 2002). Total nitrogen was determined as ammonia by the Berthelot reaction after Kjeldahl digestion of tobacco powder; ammonia reacts with sodium salicylate in the presence of hypochlorite to form a blue indophenol (YC/T 161, 2002).

2.5. Sensory smoking

The tobacco samples were made into cigarettes (length 84 mm, circumference 24.5 mm, average weight 0.9 g), and sensory smoking of the cigarettes was identified by the standard of YC/T 138, 1998 of China. Sensory quality indicators included quality (A) and quantity (B) of aroma, richness of smoke (C), offensive taste (D), strength of smoke (E), irritancy (F), after taste (G), burn rate (H) and gray (I). The sensory qualities of the samples were evaluated using a nine-point scoring method. Sensory scores of 6.1–9 were given for good, 3.1–6 for fair, and ≤3 for poor. The equation of the total score was as follows (Li, 2007):

$$\text{Total score} = (A \times 25\% + B \times 25\% + C \times 5\% + D \times 10\% + E \times 3\% + F \times 10\% + G \times 15\% + H \times 5\% + I \times 2\%) \times 11.11$$

2.6. Statistical analysis

The experimental design was a completely randomized block design with irradiation dose and storage times as the main factors. The data were analyzed by an analysis of variance (ANOVA) using SPSS 16.0 (SPSS Inc. Chicago, IL, USA). Tukey test multiple comparison at the 95% level was performed to separate the means among the irradiation doses and storage times. All results are expressed as the mean ± standard deviation.