

Suitability of Real-Time Quantitative Polymerase Chain Reaction and Enzyme-Linked Immunosorbent Assay for *cry9C* Detection in Mexican Corn Tortillas: Fate of DNA and Protein After Alkaline Cooking

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Alkaline-cooked corn, called nixtamal, is the basis for many traditional corn products such as tortillas, chips, and taco shells that are used widely in Mexico and Central America and in the preparation of snack foods that are consumed globally. To assess the effects of alkaline and thermal treatments on the detectability of DNA and protein for the presence of genetically modified sequences, various nixtamalized products were prepared from blends of conventional white corn containing 0.1, 1.0, and 10% transgenic corn (event CBH 351, StarLinkTM). Real-time quantitative polymerase chain reactions (RTQ-PCR) and immunoassays were used to determine the *cry9C* gene and protein, respectively, in unprocessed corn kernels, freshly prepared alkaline-cooked and ground corn (masa), masa flour, tortillas prepared from masa by heat treatment, chips prepared from damp masa dough by deep frying, and from tortillas processed at high (200 C) and low temperatures (70 C). In spite of progressive degradation of genomic DNA during processing, RTQ-PCR genetic analysis allowed detection and quantification of the *cry9C* gene in all products prepared from 10, 1, and 0.1% StarLink corn, except deep-fried chips containing 0.1% StarLink. Enzyme-linked immunosorbent assays readily

detected <1 ppm *cry9C* protein in all blends of unprocessed corn (10, 1, and 0.1% StarLink) as well as in nonfried tortilla and masa products. This technique was not suitable for thermally treated nixtamalized products containing <1% transgenic corn.

Alkaline cooking of corn, also called nixtamalization, is an ancient process that is still used in Mexico and Central America for the preparation of a wide range of food products such as tortillas. It consists of cooking, steeping, and washing corn to produce treated grains called nixtamal, which is stone-ground with water, to obtain a soft, moist dough called masa. Daily per capita consumption of corn in Mexico is estimated to be from 285 to 480 g, and 40% of dietary protein is derived from this cereal, because it is the most economical source (1). In addition, products prepared with corn in the Mexican diet include not only nixtamalized foods, but also boiled corn cobs and grains, and many dishes that entail simpler, less stringent cooking procedures, compared with nixtamalization. This pattern of consumption puts in perspective the exposure to transgenic elements in the Mexican diet.

Recently, the use and economic importance of nixtamalized corn have expanded to other countries because of its role in the production of a wide variety of snack foods, including corn chips, tortilla chips, taco shells, and various extruded and fried corn products. Industrialized production of masa, in which the moist masa dough is dried and ground to powder, has now become common. This masa flour is highly

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