Effects of Flour Sources on Acrylamide Formation and Oil Uptake in Fried Batters

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ABSTRACT: Food batters were formulated using flours of long-grain rice, waxy rice, wheat, or corn. Acrylamide and oil analyses were conducted for the flour and the corresponding fried batter. During frying, the formation of acrylamide ranged from 82 ng/g for the long-grain rice batter to 263 ng/g for the corn batter. Oil uptake ranged from 21.4% for the long-grain rice batter to 47.3% for the wheat batter. The incorporation of 5% pregelatinized rice flour and 1.5–3.0% milk as functional additives into the long-grain rice batter only slightly increased the acrylamide and oil contents.

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KEY WORDS: Acrylamide analysis, additives, batter viscosity, oil absorption, rice flour.

Batters are useful and popular in the preparation of fried foods. However, whereas fried batters may enhance the sensory quality of the coated food, they also may introduce undesirable effects to the consumers. Normally, fried batters contain high amounts of oil and contribute to oil-related health problems such as obesity and heart disease. Recently, acrylamide found in food has become a health concern because acrylamide can be carcinogenic (1,2). Factors that favor the formation of acrylamide include high-temperature processing and the interactions of protein components (especially asparagine) and carbohydrates (3,4). These are also conditions under which fried batters are processed. Therefore, fried batters may contain elevated amounts of acrylamide in addition to that present in the oil. Even though there are as yet no guidelines or regulations on the safe range of acrylamide in food, excessive formation of acrylamide is undesirable and to be avoided.

Oil absorption in fried foods or fried batters and the frying mechanisms that are involved have been studied extensively, but little information is available in the literature on acrylamide in the frying of food. Oil contents in batters vary, depending on frying conditions such as temperature, oil source, and ingredients in the batter formulation (5,6). Similar factors most likely will affect the formation of acrylamide. Flour components have been reported to play a role in oil absorption of fried batters, and rice flour ingredients, in particular, have superior oil-resisting properties during frying (7,8). Effects of flour source on acrylamide formation need to be investigated. We also need to understand the relationship or the lack of it between oil uptake and acrylamide formation, and ultimately to develop fried batters that are low both in oil and in acrylamide.

In this report, we investigated the acrylamide formation and oil uptake of fried batters from various flour sources. Batters were formulated with selected flours and additives to minimize the acrylamide and oil content of the final products.

MATERIALS AND METHODS

Materials. Waxy rice flour, Short Remyflo S100, and pregelatinized rice flour, Remyflo R500P, were from A&B Ingredients (Fairfield, NJ). Long-grain rice flour was from Riceland Foods (Stuttgart, AR). Corn flour, CCF600 Corn Flour, was from Lauhoff Grain Co. (Danville, IL). Wheat flour (Pillsbury's Best All-Purpose Flour), frying oil (Wesson vegetable oil), and commercial potato chips (Golden Flake from Snack Foods, Birmingham, AL; Regular Potato Chips from Frito-Lay, Plano, TX) were purchased from local supermarkets. The potato chips were frozen in liquid nitrogen, ground in a blender, and the resulting powder was bagged and stored at 4°C before use. Deuterium-labeled acrylamide (d_3-acrylamide) was from Cambridge Isotope Labs (Andover, MA). All other chemicals used were of reagent grade.

Preparation of fried batters. A batter base was formulated containing 47.64 g flour, 1.50 g sodium chloride, 0.50 g sodium bicarbonate, and 0.36 g disodium pyrophosphate. Water (47–127 g) was added to achieve a slurry mix with an RVU (rapid viscosity unit) viscosity reading of 115–130 (1380–1560 cP) by using an RVA-3D analyzer (Foss Food Technology Co., Eden Prairie, MN). A 6-qt Dazey deep-fryer (Dazey Co., New Century, KS) with a strainer was used for the frying experiment. The heating was controlled by a temperature probe, Therm-O-Watch L6-1000SS (Instruments for Research and Industry, Cheltenham, PA). The oil bath, filled to a depth of 4.5 cm with 1.4 L of the Wesson vegetable oil, was heated to 177°C for 2 h before use. Spoonfuls of the batter slurry were introduced into the oil bath and fried with occasional stirring for 6 min using a metal spatula. The fried batters were removed from the oil bath and drained on a strainer for 30 min before being frozen in liquid nitrogen and ground to a fine powder in a Waring blender. The samples were stored at 4°C before use.

General batter analysis. Batter analysis was conducted in triplicate. Batter viscosity was determined using the RVA-3D analyzer by introducing 28 g of the batter into a sample cup;

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the viscosity in RVU units was read after spinning, first at 960 rpm for 10 s and then at 160 rpm for the remaining time, at the end of a total time of 4 min. Moisture content of the fried batter was measured using a Mettler LP 16 IR dryer moisture analyzer (Mettler Co., Highstown, NJ) by spreading the ground batter (about 1–2 g) on a sample pan at 130°C and drying until the sample reached constant weight. Oil content was analyzed using a supercritical fluid extraction system (SFX 220; ISCO, Lincoln, NE). The sample cartridge was loosely filled with 1 g of sand at the exit end of the cartridge, followed by 1 g of diatomaceous earth and 1.5 to 3.0 g of ground batter until the cartridge was full. Carbon dioxide (65 mL) was used to extract the sample at 7500 psi and 100°C, and the restrictors were set at 140°C. The flow rate was 2.5 to 2.7 mL/min. The oil content was calculated from the weight gain of the oil collected in tubes packed with 1.5 g of glass wool during the extraction.

Acrylamide analysis. Acrylamide content of the sample was analyzed essentially based on the method of Rosen and Hellenas (9). Samples were prepared by mixing the ground-up fried batters (4 g) in 40 mL aqueous solution containing, as an internal standard, 80 µL d_3-acrylamide (20 µg/mL). The mixture was homogenized using a Tekmar homogenizer at 9500 rpm for 2 min and then centrifuged at 9000 × *g* for 15 min. The supernatant was collected by decantation for further purification. An SPE Isolute Multimode column (300 mg; Argonaut Technologies, Foster City, CA) was pretreated with 1 mL acetonitrile and then 2 × 2 mL deionized water, before introducing 3 mL of the supernatant to the column. After discarding 1 mL of the initial eluate, 2 mL of the remaining eluate was collected and filtered through a syringe filter (0.22 µm). The filtrate was further filtered through a Centricon 3 centrifuge spin filter (Millipore, www.millipore.com) at 7500 × *g* for 1 h to a volume desirable for analysis with LC-MS/MS.

HPLC-tandem MS (LC-MS/MS). A Waters 2695 liquid chromatograph was coupled to a Finnigan LCQ Classic (Finnigan Co., San Jose, CA) *via* an electrospray (ESI) interface. The HPLC column was a Hypercarb, 5 µm 50 × 2.1 mm (Thermo Hypersil-Keystone, www.thermohypersil.com). With water as the mobile phase at a flow rate of 0.2 mL/min, a 50-µL sample was injected into the column, and the HPLC effluent was directed into the interface. The LC-MS/MS was operated in a positive ESI mode. Other conditions included source temperature at 200°C, capillary temperature at 210°C, capillary voltage at 23.00 V, sheath gas flow at 21.82 arbitrary units (arb), and spray voltage at 6 kV. Between sample injections, the column was washed with 80% aqueous acetonitrile (4 min at 0.4 mL/min) followed by reconditioning with water (10 min at 0.2 mL/min).

Acrylamide was identified by the LCQ MS detector operating in the selected reaction monitoring scan mode. The precursor ion to product ion transitions were *m/z* 72 > 55 for the acrylamide and 75 > 58 for d_3-acrylamide. The collision energy and activation *q* were 31.0% and 0.450, respectively, for *m/z* 72 (acrylamide), and 32.0% and 0.460, respectively, for the *m/z* 75 (internal standard). The ratio of peak areas for *m/z* 55 (acrylamide) and *m/z* 58 (internal standard) obtained for the samples was compared with that obtained for the standards. Quantification was achieved by plotting the acrylamide concentration vs. the peak area of response from the mass spectrometer. A six-point calibration curve was used, with the concentration of native acrylamide at 25, 50, 100, 250, and 1000 ng/mL and the concentration of d_3-acrylamide at 40 ng/mL. The peak area varied linearly with the acrylamide concentration ($r^2 = 0.95$). A zero standard was not included in the standard curve.

Statistical analysis. The samples were analyzed in triplicate. Data were assessed by the one-way ANOVA and mean comparisons using a Bonferroni correction factor with $P < 0.05$ in MS Excel, version 9.

RESULTS AND DISCUSSION

Batter viscosity. Batter viscosity is important for applications in food coating and frying (10). Thin batters may not adhere well to the food being coated, resulting in low and loose batter pickup during frying. On the other hand, thick batters may end up with too much and uneven coating. Either way, poorly coated food adversely affects the textural and sensory quality of the product. Because the batter was formulated using a set amount of flour and leavening agents, different amounts of water were required to achieve a comparable viscosity for the resulting slurry, depending on the flour used. Figure 1 shows the change in viscosity as a function of the water added for various batters. Viscosity dropped as water was added. For effective quality control, a consistent batter viscosity needs to be established for all products. With the viscosity of traditional wheat batter providing a convenient reference, a batter viscosity in the range of 115–130 RVU (1380–1560 cP) was chosen for the following experiments. Generally, the water needed to prepare the batters ranged from 46–47 g for the long-grain rice batter to 83–84 g for the waxy rice batter.

Acrylamide analysis. Acrylamide in foods has been ana-

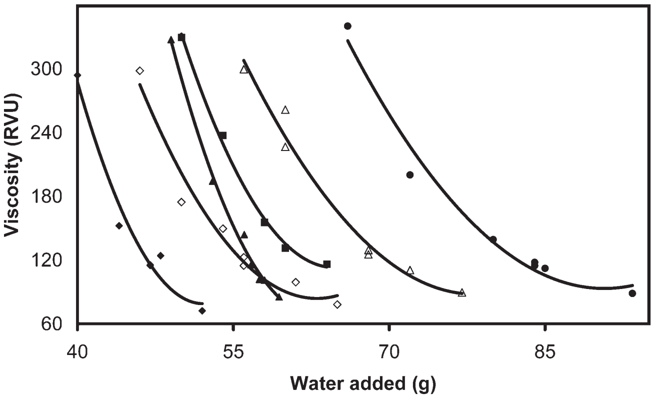

FIG. 1. Effects of water content on the viscosity of the batter slurry prepared with various flours. Analyses were conducted in triplicate. Symbol indications are for batters of long-grain rice (◆), waxy rice (●), wheat (■), corn (▲), long-grain rice with 5% pregelatinized rice flour (◇), and long-grain rice with 10% pregelatinized rice flour (△). RVU, rapid viscosity units.

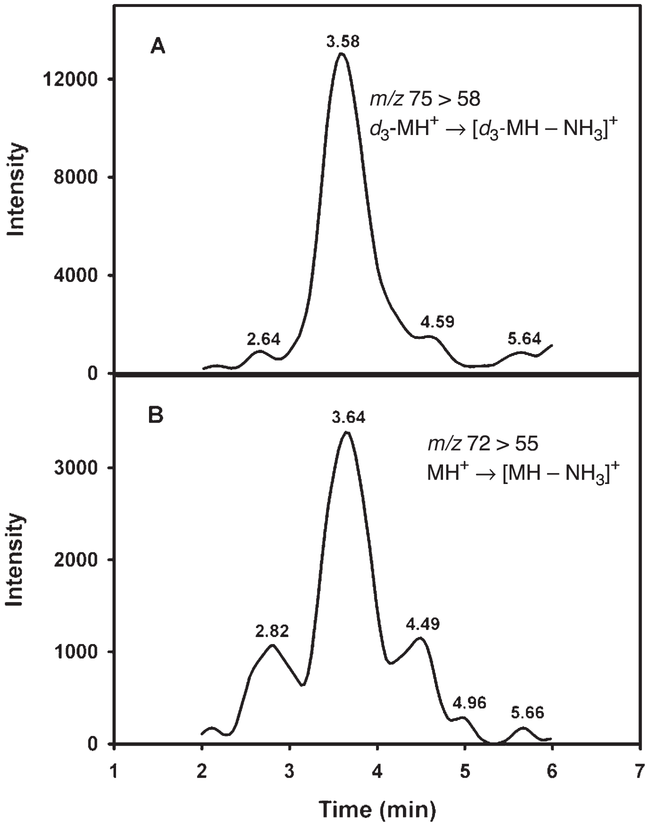

FIG. 2. Chromatograms in selected reaction monitoring (SRM) (MS/MS) scan mode for the analysis of acrylamide in the fried batter of 95% long-grain rice flour and 5% pregelatinized rice flour. (A) SRM scan of internal standard, d_3-acrylamide in sample (m/z 75 > 58, d_3-MH$^+$ to [d_3-MH – NH$_3$]$^+$). (B) SRM scan of acrylamide in sample (m/z 72 > 55, MH$^+$ to [MH – NH$_3$]$^+$).

lyzed by HPLC isolation and separation followed by MS/MS identification and quantification (3,9). Sample preparation can be difficult, depending on the source of the product. Filtration of the waxy rice sample, for example, can be time-consuming if the sample is overhomogenized, resulting in extra-fine particles. We conducted our analyses essentially according to the Rosen and Hellenas method and found it quite effective overall. The LC-MS/MS procedures were straightforward. Typically, desirable transition peaks were well-resolved (Fig. 2). An ion transition of m/z 75 > 58 was monitored for the internal standard d_3-acrylamide (Fig. 2A), whereas an ion transition m/z 72 > 55 was used for the sample acrylamide (Fig. 2B). Quantification of the sample can readily be calculated using a standard curve (linear coefficient = 0.9623).

Acrylamide formation and oil uptake. Contents of acrylamide and oil were analyzed for the flour components as well as the corresponding fried batters. The results are listed in Table 1. Acrylamide contents of the flour were relatively low, ranging from 86 ng/g of the waxy rice flour to 115 ng/g of the corn flour. They greatly increased during frying, ranging from 180 to 378 ng/g for the fried batters. The increase in acrylamide or its formation during frying was calculated by subtracting the acrylamide content of the flour from that of the fried batter. These values, as given in Table 1, further confirm that the frying of batters from rice flours, either long-grain rice or waxy rice, produced the least amount of acrylamide. Slightly greater formations were from batters with corn flour and wheat flour. For comparison, two commercial products of potato chips were also analyzed and found to have acrylamide contents of 554 and 815 ng/g, respectively.

Oil contents of the flour were minimal, ranging from 0.30 to 1.61% (Table 1). Oil uptakes for the fried batter varied, depending on, among other factors, flour components in the batter. Variations in oil uptake ranged from 21.4% of the fried long-grain rice batter to 47.3% of the fried wheat batter. Long-grain rice batters absorbed less oil than the waxy rice batter, most likely because long-grain rice is rich in amylose, whereas waxy rice is almost free of amylose, which resists oil absorption during frying (7). The relatively high oil uptake of wheat batters may be due to the presence of gluten in wheat, which is relatively lipophilic and capable of leavening, factors that enhance oil absorption. According to Mohamed *et al.* (6) in a related research report, fried foods with protein additives other than gluten had reduced oil absorption.

The results indicate that the flour source plays a role in determining the acrylamide and oil contents of the fried batter. When high acrylamide and oil contents occur in fried batters,

TABLE 1
Oil Uptake and Acrylamide Formation of Batters During Frying[a]

	Fried batter				Flour	
	Acrylamide		Oil			
	Content (ng/g)	Formation (ng/g)	Content (%)	Uptake (%)	Acrylamide content (ng/g)	Oil content (ng/g)
Long-grain rice	180 ± 21	82.1	22.4 ± 2.0	21.43	98 ± 11	0.93 ± 0.02
Waxy rice	194 ± 36.5	107.7	32.2 ± 0.3	31.93	86 ± 3	0.30 ± 0.02
Wheat	298 ± 11	210.7	47.9 ± 0.8	47.25	87 ± 2	0.62 ± 0.04
Corn	378 ± 56	262.5	29.5 ± 0.5	27.90	115 ± 6	1.61 ± 0.01
Potato chips[b]	815 ± 97	—	35.71[d]	—	—	—
Potato chip[c]	554 ± 53		35.71[d]			

[a] Oil and acrylamide analyses were done in triplicate and reported as value ± SD.
[b] A commercial product from Golden Flake Snack Foods (Birmingham, AL).
[c] A commercial product from Frito-Lay (Plano, TX).
[d] Oil content reported by the manufacturer.

it is often not because the flour that makes up the batter is originally rich in these components but because of the greater capacity of the batter to acquire them during frying. However, the formation of acrylamide does not seem to correlate with the oil uptake of the fried batters.

Effects of additives. Batter characteristics in terms of texture and sensory quality may be compromised when flours other than wheat flour are used to replace the traditional wheat batter. Additives have been used, such as pregelatinized rice flour or phosphorylated rice starch, to provide viscosity to the rice batter and improve its frying properties. Rice batters with up to 5% pregelatinized rice flour have been reported to show textural characteristics comparable to those of the traditional wheat batter (7). In the present study, experiments were conducted in which two levels of pregelatinized rice flour, 5 and 10%, were incorporated into the long-grain rice batter formulation. The fried batters were analyzed for their acrylamide and oil contents, and the results are shown in Figure 3. Whereas acrylamide content increased from 180 to 245 ng/g for the low-level incorporation and to 319 ng/g for the high-level incorporation, oil uptake increased only slightly from 21.4 to 25.2% for the 10% and to 26.5% for the 5% level of additive incorporation. The acrylamide contents in the final products remained reasonably low as compared with those of other fried foods such as potato chips. On the other hand, the oil uptake for both incorporation levels remained about 45% lower than the traditional wheat batter. Therefore, the addition of pregelatinized rice flour in the rice batter, particularly at the low level of about 5%, should be considered totally acceptable concerning oil and acrylamide contents of the fried batter.

Milk is another additive commonly added to batters for improved functional and frying properties. In a follow-up experiment, milk was added at two levels, 1.5 and 3.0%, to the rice batter, which was prepared using 95% long-grain rice flour and 5% pregelatinized rice flour. The fried batters were again analyzed for acrylamide and oil contents and the results are shown in Figure 4. The addition of milk, at 1.5–3.0% as normally used in the food industry, appeared to raise the acrylamide and oil contents of the fried rice batter slightly, to about 330 ng/g and 28–30%, respectively. The values remained relatively low, and they compared favorably with those of other fried products. Therefore, the batters as formulated with 95% long-grain rice flour, 5% pregelatinized rice flour, and 1.5–3.0% milk can be recognized as having low-acrylamide and low-oil frying properties.

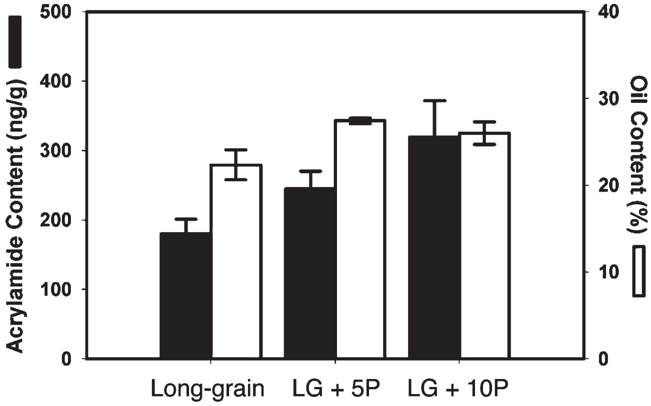

FIG. 3. Effects of pregelatinized rice flour as an additive on the acrylamide formation and oil uptake of the long-grain rice batter during frying. Analyses were conducted in triplicate. The labels (LG + 5P) and (LG + 10P) indicate batters of long-grain rice flour with 5 and 10% pregelatinized rice flour, respectively.

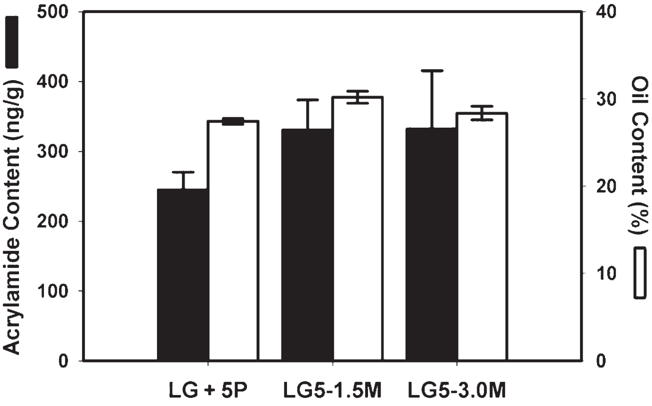

FIG. 4. Effects of milk as an additive on the acrylamide formation and oil uptake of fried batters with 95% long-grain rice flour and 5% pregelatinized rice flour. Analyses were conducted in triplicate. Labels indicate that milk was added to the batter at 0% (LG + 5P, the control), 1.5% (LG5-1.5M) and 3.0% (LG5-3.0M).

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