Mutagen Formation in Deep-Fat Fried Foods as a Function of Frying Conditions

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ABSTRACT

Deep-fat fried foods possess low levels of mutagenic activity, and severely abusive frying conditions must be employed to obtain appreciable levels of mutagenic activity. With French fried potatoes, either excessively long frying times (35–40 min) or abnormally high frying temperatures (above 410 F or 210 C) were necessary to generate appreciable levels of mutagenic activity (> 2.5 times the spontaneous mutation rate). Repetitive frying of 45 batches of French fried potatoes or onion rings in the same batch of shortening elicited no increase in mutagen formation. Repetitive frying of fish fillets in the same batch of shortening resulted in the generation of appreciable levels of mutagen(s) in both the acidic and basic methylene chloride extracts. Increased mutagenic activity was noted by the 7th batch. Fish fillets obtained from a local restaurant at selected intervals during a one-week period of use of a particular batch of frying oil contained much lower levels of mutagenic activity.

INTRODUCTION

The formation of mutagens in cooked food has been associated with a variety of foods and cooking conditions (1–12). The earliest reports indicated that benzo(a)pyrene and other polycyclic aromatic hydrocarbons could be found in charbroiled or smoked meats (1–3). Later, Sugimura and colleagues (4) detected very potent mutagens in protein pyrolysates obtained by heating proteins at temperatures in excess of 300 C. The mutagens in these protein pyrolysates were pyrolysis products of amino acids (5). Concern over the presence and possible effects of mutagens in foods was heightened considerably by the observation that mutagens were generated during the surface frying of ground beef at typical frying temperatures (6–10) Mutagens were subsequently found to occur in other foods cooked at similar temperatures, although the level of mutagenic activity was generally lower than with surface-fried ground beef (11, 12). Uncertainties remain regarding the toxicological implications of the existence of these mutagens in cooked foods. However, the fact that mutagens can be generated in foods by normal cooking procedures has been and continues to be a source of concern.

Deep-fat fried foods are typically cooked at temperatures equal to or above those used in surface frying. Recently, we demonstrated that commercially available, deep-fat fried foods had little or no mutagenic activity (13). Deep-fat fried ground beef possessed mutagenic activity but at only one-fourth of the level found with surface-fried ground beef (13). A separate study confirmed that deep-fat fried chicken (14), tofu (15), and shrimp (15) had little mutagenic activity. Used deep-frying fats obtained from commercial establishments likewise lacked mutagenic activity (16,17). While these results seem to indicate that mutagens are not generated during deep-fat frying, the effect of variations in frying conditions was not determined. Most of the commercially available samples obtained in the earlier survey (13) were from large volume commercial establishments that would generally utilize optimal frying conditions. In smaller volume commercial establishments and homes, the potential for use of abusive frying conditions would be much greater. The purpose of the present study was to determine the effect of frying conditions on the formation of mutagens in deep-fat fried foods. An evaluation of the effect of abusive frying conditions such as abnormally long frying times, abnormally high frying temperatures, and repeated reuse of frying oil was included.

EXPERIMENTAL PROCEDURE

Foods

Frozen, breaded fish fillets and frozen, precut French fried potatoes were obtained from a local restaurant. In a later experiment, deep-fat fried fish fillets were obtained from the same restaurant at selected times during a one-week period of operation. The frying oil, a shortening composed of beef fat and cottonseed oil with monoglyceride citrate, butylated hydroxytoluene, butylated hydroxyanisole, propyl gallate and propylene glycol, was also obtained from this same restaurant. Frozen, breaded onion rings were purchased from a local retail outlet.

Frying Procedures

To determine the effect of frying time, frozen French fried potatoes were placed in the home-style, deep-fat fryer filled with shortening preheated to 335 F (168 C) and cooked for 5, 10, 15, 20, 25, 30, 35, and 40 min.

To determine the effect of frying temperature, the fryer was filled with shortening and heated to 310 F (154 C), 335 F (168 C), 375 F (191 C), 410 F (210 C), or 432 F (222 C). Frozen French fried potatoes were placed in the fryer and cooked for 10 min.

To assess the effect of repeated reuse of the same batch of frying oil, 15 batches of either French fried potatoes, fish fillets, or onion rings were fried each day during an 8-hr period for 3 successive days. The shortening was allowed to cool and solidify during the 16-hr period between cooking cycles. No addition of fresh oil or filtration of the used oil was done. The cooking times and temperatures were 8 min at 375 F (191 C) for French fried potatoes, 3.5 min (first 32 batches) or 5.5 min (batches 33–45) at 375 F (191 C) for onion rings, and 5 min (first 25 batches) or 5.5 min (batches 26–45) at 375 F (191 C) for fish fillets. Frying time was increased in the later batches of onion rings and fish fillets because heat transfer was poorer in reused oil. With onion rings and fish fillets, a sample of the crumb residue from the fryer was collected after frying the 44th batch of product. The 45th batch was fried in filtered oil.

In another experiment, fish fillets were obtained at

selected times from a local restaurant to determine the effect of typical practices on the generation of mutagens in this product. In this restaurant, the usual practice is to replace the oil in the fish fryer on a weekly basis and filter the oil on a daily basis. Fish fillets are fried during a 12-hr period; the fryer is covered and turned off during the other 12 hr of each day. Occasionally, fresh shortening may be added to the fryer. One sample was obtained immediately following the weekly replacement of the oil. Three additional samples were obtained immediately before filtration or replacement of the oil on the 3rd, 5th, and 7th days of use of that batch of oil.

Extraction of Foods

The procedure used to extract any mutagens formed during the deep-fat frying process has been described previously (13).

Extraction of Frying Oil

In the repetitive frying experiments, frying oil samples were collected following the frying of selected batches of product, and stored frozen until analysis. The polar and nonpolar fractions of the frying oil were obtained by a modification of the method of Billek (18). The polar fraction was taken to dryness in vacuo, dissolved in dimethyl sulfoxide, and used directly in the mutagenesis assays. The nonpolar fraction was concentrated in vacuo, saponified (19), and again concentrated in vacuo. The saponified concentrate was filtered and the residue was washed twice with boiling H_2O. The residue was the nonsaponifiable portion of nonpolar fraction. The filtrate was acidified with concentrated HCl and extracted twice with diethyl ether. The ether phase was dried, evaporated, dissolved in 1 mL of dimethyl sulfoxide, and used in the Ames test. An ether-insoluble portion of the saponifiable fraction was separated from this ether-soluble, saponified fraction.

Mutagenesis Testing

Assays were conducted using the bacterial mutagenesis test of Ames et al. (20), as modified by Pariza et al. (9). The test organism was *Salmonella typhimurium* TA98 plated at ca. 6.6×10^9 bacteria per plate. The rat liver S-9 fraction was obtained from Arochlor 1254 induced rats.

Reported values are the means of 4 plates. Two replicate plates were done with duplicate food samples obtained by dividing each cooked sample into equal portions. Results are reported as the ratio of induced revertants per plate to spontaneous revertants per plate. Since the cooked weight of the products varied with frying conditions, the amount of extract used in each assay has been provided as gram-equivalents of uncooked products. With the fish fillets obtained as fried product from the local restaurant, the amount of extract is given as gram-equivalents of cooked product.

RESULTS

Effect of Frying Time

The level of mutagenic activity in the basic methylene chloride extract of French fried potatoes increased as the frying time was extended (Table I). The number of revertants induced by the basic extract was not appreciably greater than the spontaneous reversion rate until the frying time reached 25 min. The level of mutagenic activity associated with the basic extract was highest for the samples fried for 35 and 40 min. An optimal frying time for French fried potatoes in our home-style fryer was ca. 10 min. By 25 min of frying, the potatoes had an unappetizing appearance with a dark golden brown to brown color and an extremely crisp texture. By 40 min of frying, the potatoes had developed a very dark brown color and an extremely crisp, thoroughly desiccated texture. Potatoes fried for over 15 min probably would be considered inedible. The acidic methylene chloride extract did not possess appreciable mutagenic activity. The criterion for appreciable mutagenic activity was a level sufficient to produce 2.5 times more revertants than were observed spontaneously.

Effect of Frying Temperature

As shown in Table II, changes in the frying temperature had little effect on the level of mutagenic activity associated with French fried potatoes. Appreciable, though low, levels of mutagenic activity were found with the basic methylene chloride extract of potato samples fried for 10 min at initial temperatures of 410 F (210 C) and 432 F (222 C). The acidic extracts and the basic extracts from potatoes fried at 310 F (154 C), 335 F (168 C), and 375 F (191 C) for 10 min did not induce appreciable levels of mutagenic

TABLE I

Effect of Frying Time on Mutagen Formation in Deep-Fat Fried, French Fried Potatoes

Frying time (min)	Gram-equivalents per plate[a]	Induced revertants per plate/ spontaneous revertants per plate[b]	
		Acidic extract	Basic extract
5	55	1.04	1.29
10	55	0.96	1.29
15	55	1.12	1.58
20	55	1.08	1.33
25	55	1.33	3.12
30	55	1.33	2.92
35	55	1.50	6.88
40	55	1.08	6.75

[a]Based on uncooked weight of product.

[b]The spontaneous reversion rate for the TA98 strain was 24.

TABLE II

Effect of Frying Temperature on Mutagen Formation in Deep-Fat Fried, French Fried Potatoes

Initial frying temperature (F)	(C)	Gram-equivalents per plate[a]	Induced revertants per plate/ spontaneous revertants per plate[b]	
			Acidic extract	Basic extract
310	(154)	55	0.82	1.00
335	(168)	55	0.96	1.29
375	(191)	55	1.03	1.30
410	(210)	55	1.17	2.73
432	(222)	55	1.40	2.73

[a]Based on uncooked weight of product.

[b]The spontaneous reversion rate for the TA98 strain ranged from 30 to 36.

activity. It should be noted that the actual frying temperatures were considerably lower than the initial temperatures. In Table I, the level of mutagenic activity was found to increase only after considerable overcooking. Because of the 10 min cooking period and the depressed frying temperatures, overcooking may not have occurred in this experiment. This possibility is supported by the outward appearance of these fried potatoes. The potatoes fried at the highest temperature, 432 F (222 C), had a dark golden brown color and a crisp texture but would not have been considered inedible. A frying temperature of 432 F (222 C) represented the highest temperature that could be safely achieved with our home-style fryer.

Effect of Repetitive Frying

Repetitive frying trials were performed with three foods—French fried potatoes, onion rings, and fish fillets—and the results are portrayed in Table III. Potatoes were chosen as an example of a starchy food, and because potatoes may be the most commonly consumed deep-fat fried product in the USA. Onion rings were chosen as an example of a food that contained appreciable levels of monosaccharides, which are thought to speed the decomposition of the frying oil. Fish fillets were chosen as an example of a proteinaceous food that also possesses a high level of polyunsaturated fat. Frying oil used for the frying of fish is known to decompose relatively quickly due to the presence of the polyunsaturated fats.

As indicated in Table III, repetitive frying of 45 batches of French fried potatoes in the same batch of oil with no filtration and no addition of fresh oil did not lead to an increase in the level of extractable mutagens in the potatoes. Low levels of mutagenic activity were found in both the acidic and basic methylene chloride extracts of all tested batches of the fried potatoes. After frying of the last batch of potatoes, the oil had a darkened appearance and numerous suspended particles. The polar and saponified nonpolar fractions of the frying oil possessed very low levels of mutagenic activity. In fact, the lipid fractions depressed the spontaneous reversion rate.

Likewise, the repetitive frying of 44 batches of onion rings in the same batch of oil did not result in the formation of appreciable levels of mutagenic activity (Table III). Neither the acidic nor the basic methylene chloride extracts of the fried onion rings produced revertants at a rate exceeding 2.5 times the spontaneous reversion rate. The 45th batch of onion rings was fried in filtered oil;

TABLE III

Effect of Repetitive Deep-Fat Frying on Mutagen Formation in French Fried Potatoes, Onion Rings, and Fish Fillets

	Induced revertants per plate/spontaneous revertants per plate[a]					
	French fried potatoes[b]		Onion rings[b]		Fish fillets[b]	
Batch no.	Acidic extract	Basic extract	Acidic extract	Basic extract	Acidic extract	Basic extract
1	1.31	1.58	1.34	0.83	1.38	1.52
7	–	–	1.17	1.53	3.62	10.0
15	1.42	1.36	1.32	0.98	4.29	12.2
30	1.03	1.33	1.00	1.90	5.33	21.2
37	1.22	1.86	1.46	1.34	6.42	26.7
45[c]	1.36	1.31	1.51	1.68	5.95	16.5
Crumb Residue[d]	–	–	Toxic[e]	4.63	2.48	79.1

[a]The spontaneous reversion rates for the potato, onion ring, and fish fillet experiment for strain TA98 were 36, 41, and 21, respectively.

[b]The amount of extract applied to the plate was based on the uncooked weight of the product except in the case of the crumb residue; 55 gram-equivalents per plate were used with French fried potatoes and onion rings and 47–49 gram-equivalents per plate were used with fish fillets.

[c]The frying oil was filtered for onion rings and fish fillets before frying batch no. 45.

[d]For the crumb residue samples, the gram-equivalents per plate (37 for onion rings and 22 for fish fillets) are based on cooked weight.

[e]This extract was toxic to strain TA98 so background lawn would not grow.

TABLE IV

Mutagenicity of Deep-Fat Fried Fish Fillets Obtained at Selected Intervals from a Local Restaurant

Sampling day[a]	Gram-equivalents per plate[b]	Induced revertants per plate/ spontaneous revertants per plate[c]	
		Acidic extract	Basic extract
1	39	1.19	1.96
3	39	1.35	2.35
5	40	1.69	2.81
7	40	1.50	3.42

[a]Details of the sampling schedule can be found in Experimental Procedures.
[b]Based on the cooked weight of the fish fillets.
[c]The spontaneous reversion rate for the TA98 strain was 26.

these onion rings did not have an appreciable level of mutagenic activity. The crumb residue obtained from the filtered oil after 44 repetitive frying operations did have an appreciable level of mutagenic activity. While the acidic methylene chloride extract was toxic to *S. typhimurium* TA98, the basic methylene chloride extract produced revertants at a level of 4.63 times greater than the spontaneous reversion rate. The crumb residue largely comprises breading material that detaches from the surface of the onion rings during frying. The crumb residue increases in mass with each repetitive frying operation. After frying of the 44th batch of onion rings, the oil had a darkened appearance and a substantial number of particles. The crumb residue was charred in appearance. Mutagens were not found in the polar and saponified nonpolar fractions of the frying oil, although in this case no depression of the spontaneous reversion rate was noted with these fractions.

In contrast to the situations observed with potatoes and onion rings, a substantial increase in the level of mutagenic activity was found in repetitive frying of fish fillets (Table III). Appreciable mutagenic activity was present in both the acidic and basic methylene chloride extracts of the fried fish, although several-fold higher levels of mutagenic activity were associated with the basic extract. Considerable mutagenic activity was observed after frying 7 batches of fish fillets. The level of mutagenic activity in the acidic extract increased as more batches of fish fillets were fried in the oil. The level of mutagenic activity in the basic extract was highest in the 37th batch of fish and decreased slightly thereafter. The 45th batch of fried fish which was fried in filtered oil possessed less mutagenic activity. The crumb residue obtained from the filtered oil after the 44th repetitive frying operation had substantial mutagenic activity, particularly in the basic methylene chloride extract. Again, the crumb residue mainly comprises breading material, and increases in amount with each repetitive frying operation. At the completion of the repetitive frying schedule, the oil had a darkened appearance and a substantial load of particles. The crumb residue was composed of these charred particles. Again, no mutagens were found in the polar and saponified nonpolar fractions of the frying oil, and no depression in the spontaneous reversion rate was noted with these fractions.

Since substantial levels of mutagenic activity were found in fish fillets after repetitive frying, fish fillets were obtained from a local restaurant at selected intervals over a one-week period and examined for mutagenic activity (Table IV). Much lower levels of mutagenic activity were found in the fish fillets obtained from the restaurant than in those fried in our home-style fryer (Table III). This difference is attributed to the daily filtration of the oil in the restaurant operation. Low levels of mutagenic activity were found in the acidic methylene chloride extracts from these samples. The basic methylene chloride extracts of samples obtained at the end of the week did induce revertants at a rate exceeding 2.5 times the spontaneous reversion rate. In this case, frying oil was not obtained for analysis.

DISCUSSION

Mutagen formation during the deep-fat frying of foods does not appear to occur to any appreciable extent unless very severe departures are made from common and optimal frying conditions. Appreciable mutagen formation with French fried potatoes was observed only after gross overcooking of the potatoes through use of prolonged frying times. Abnormally high frying temperatures and repetitive frying did not result in increased mutagen formation in French fried potatoes. The lack of an effect of frying temperature on mutagen formation was somewhat surprising, in light of the fact that mutagen formation in ground beef (7, 9) and other foods (11) is temperature-dependent. Repetitive frying did result in an increase in mutagen formation in fish fillets, but this was traced primarily to a highly mutagenic crumb residue. Analysis of fish fillets from a commercial operation where filtration is performed on a daily basis indicated that filtration would largely remove the potential for mutagen formation from this product. The crumb residue obtained on repetitive frying of onion rings was likewise mutagenic, but the level of mutagenic activity was far lower than that obtained with the crumb residue of fish fillets.

Abusive deep-fat frying conditions sufficient to induce significant mutagen formation would not likely occur in either home or commercial frying operations. The general lack of mutagens in deep-fat fried foods obtained at the retail level (13) supports this statement. The deep-fat frying conditions used in these experiments were designed to model commercial operations through the use of restaurant-ready frozen products and a commercial frying oil.

As indicated by the repetitive frying experiments (Table III), mutagen formation was greatest by far with fish fillets. This finding confirms earlier observations that indicated

greater mutagen formation with deep-fat fried, proteinaceous foods by comparison to starchy foods (13). Similar differences in the levels of mutagenic activity have been noted between proteinaceous and starchy foods cooked by other methods (9–12).

No mutagenic activity was found in the frying oil after repetitive frying of French fried potatoes, onion rings, or fish fillets. Previous experiments had shown that when a known amount of beef extract mutagen was added to ground beef before deep-fat frying, all of the mutagen was retained in the fried product (13). This finding had indicated that the mutagen in beef extract is not lipid-soluble and is not extracted into the frying fat. However, the possibility remained that other lipid-soluble mutagens would be generated, either by decomposition of the frying oil and its antioxidants (21), or in the food with partitioning into the oil. Analysis of the used frying oil in these experiments gave no indication of the existence of such mutagens. However, mutagenicity testing of lipid fractions, particularly the nonpolar fraction, is fraught with technical difficulties. The nonsaponifiable portion of the nonpolar fraction and the ether-insoluble portion of the saponifiable fraction were never examined for mutagenic activity because of their incompatibility with the Ames test system. Analysis of the saponifiable portion of the nonpolar fraction created problems on occasion due to the presence of interfering fats. The apparent depression of the spontaneous reversion rate seen with the oil samples taken from potato frying may have been the result of such difficulties. The suppression of mutagenic activity could also have occurred in the oil, since oleic acid has been found to inhibit the mutagenicity of the basic methylene chloride extract of cooked ground beef (22).

Generally, mutagens are absent or present at very low levels in deep-fat fried foods (13–15). In contrast, surface-fried and baked foods contain much higher levels of mutagenic activity (6–12). In a direct comparison of cooking methods, Bjeldanes et al. (14) showed that chicken deep-fat fried at 101 C for 12 min possessed virtually the same level of mutagenic activity as raw chicken, while other methods (particularly broiling for 34 min at 274 C) led to the generation of considerably more mutagenic activity. Several factors may be involved in preventing the formation of higher levels of mutagenic activity in deep-fat fried foods including the presence of fat, the presence of antioxidants in the oil, the uniform cooking temperatures, and the partial exclusion of oxygen. The ability of antioxidants to inhibit mutagen formation in model systems (23) and fried ground beef (24) has already been demonstrated. In addition, certain practices such as the use of reasonable frying times and temperatures, the limited reuse of frying oils, and the filtration of the oils are at least partially responsible for controlling mutagen formation. However, the critical difference between deep-fat frying and surface-frying that results in lower mutagen levels in deep-fat fried foods remains to be determined.

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