Oxidative stressed frying fats and oils
Potential role for health

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PART ONE

OXIDATIVE STRESSED FRYING FATS AND OILS

Deteriorated fried oil and fat products

Total Polar Materials (TPM)

- Mono- and di-glycerides
- Free Fatty Acids (FA)
- Dimeric and polymerized FA
- Dimeric and polymerized triglycerides (TG)
- Other?

3rd Int. Symp. DGF. Eur. J. Lipid Sci. Technol. 102 (2000)

Oxidative and degradated products of TPM

Volatiles (low MW)

- Aliphatic aldehydes (-enals, -dienals)
- Aliphatic cetones
- Epoxides
- Hydrocarbones
- Cyclic structures

3rd Int. Symp. DGF. Eur. J. Lipid Sci. Technol. 102 (2000)

- Other?
  (polycyclic aromatic hydrocarbons, …..)

Oxidative and degradated products of TPM

Non-volatiles

- Triglyceride hydroperoxides (not present in the frying bath)
- "Aldehydic" triglycerides containing attached oxo-, hydroxy-, epoxy-, cyclic acids
- Aldehydes (-enals, -dienals, …) of medium MW (2,4- decadienale,...)

3rd Int. Symp. DGF. Eur. J. Lipid Sci. Technol. 102 (2000)

- Other? (acrylamide, heterocyclic aromatic amines)

Which TPM degradation-fraction(s) are the most suspicious for health damage(s)?

- Cyclic fatty acids
- "Aldehydic" triglycerides
- Triglyceride hydroperoxides
- Aldehydes, cetones, …

} Cytotoxic, hepatotoxic, carcinogenic, mutagenic effect(s) on exp. animals

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Reviewed by G. Billek, Eur. J. Lipid Sci. Technol. 102 (2000) 587-593

- Acrylamide
- Heterocyclic aromatic amines
- Polycyclic aromatic hydrocarbons
- Other?

Here are some questions that might arise:

(a) How many times (or hours) a frying oil could be used successively? Under domestic conditions? (ex. in fast foods?)

(b) How dangerous for health could be TPM by-products under real consumption conditions? Either in domestic or commercial conditions?

(c) Which foods should be consumed together with fried foods in order to neutralize TPM by-products undesirable properties?

(d) Are the limits of TPM/ PTG protective for health?

Specifications for these questions will be given in follows while an effort to give some answers will be given in the second part of the lecture.

Oil uptake by fried foods

Product	% Oil
Potato chips	34,6
Corn chips	33,4
Tortilla chips	26,2
Doughnuts (plain)	22,9
Onion rings	18,7
Chicken breast-breaded	18,1
Fish fillet-battered or breaded	12,9

Reviewed by Saguy & Dana, *J. Food Engin. 56 (2003) 143-152*

Oil uptake by French-fried potatoes

Frying oil	% Oil in potatoes	Ref.
(Olive oil, vegetable shortening, sunflower oil)	5,7 – 12,8	Andrikopoulos et al., Int. J. Food Sci. Nutr. 53 (2002) 351-363
Soybean oil	6,0 – 14,0	Goburdhun & Jhurree Int. J. Food Sci. Nutr. 46 (1995) 363-371
Various	7,6 – 14,8	Reviewed by Saguy & Dana, J. Food Engin. 56 (2003) 143-152

- Mean fried oil absorption ~10% in the French-fries
- Mean fried oil intake ~15g by consuming one batch (150g) of French-fries

TPM uptake by French-fried potatoes

	% TPM		
Frying oil	**In the fried oil**	**In the oil absorbed**	**Ref.**
HOSO	5,5 16,0	4,9 15,4	Dobarganes et al. Eur. J. Lipid Sci. Technol. 102 (2000) 521-528
Sunflower oil	7,0 19,1	6,4 18,7	
Olive oil Sunflower oil	21,0 26,0	20,0 29,0	Andrikopoulos et al., Rev. Fr. Corps Grass 36 (1989) 127-129

- It seems that %TPM in the fried oil reflects the %TPM in the absorbed oil
- Preferential absorption of TPM-fraction has also been reported [Pokorny, Grasas y Aceites 49 (1998) 265-270]

How much does TPM loading count for?

- By eating a batch (150 g) of French-fries in oil within the limits of replenishment (20-25% TPM) a quantity of approx. 3 g of TPM are consumed

- How could the possible undesirable health effect(s) of 3 g of TPM be neutralized?

Based on TPM/PTG limits, how many times a frying oil could be used successively?

I. Frying in household conditions:

- None of the tested low linoleic vegetable oils exceeded the upper limits of TPM (25-27%) even after the 8th successive frying of potatoes (which is the usual upper limit when using a domestic fryer)
- In the following order of degradation:
  Virgin olive oil < Sunflower oil < Cottonseed oil < Corn oil
  Virgin olive oil < Vegetable shortening oil

Andrikopoulos et al., Rev. Fr. Corps Grass 36 (1989) 127-129

Andrikopoulos et al., Int. J. Food Sci. Technol. 37 (2002) 177-190

Based on TPM/PTG limits, how many times a frying oil could be used successively?

II. Frying in fast-food and restaurants:

Country	No of samples	Samples (%) with TPM>25%	Ref.
Finland	20	60	Reviewed by Saguy & Dana, J. Food Engin. 56 (2003) 143-152
France	31	48	
Brazil	60	30	
Sweden	100	38	
Germany	125	35	
Spain	174	34	
Greece	63	17	Andrikopoulos et al., Food Serv. Technol. 3 (2003) 49-59
Saudi Arabia	55	15	Al Kahfani JAOCS 68 (1991) 857-862

Based on TPM/PTG limits, how many times a frying oil could be used successively?

III. Conclusions:

- It seems that domestic frying is “quite safe” only when repeated twice or thrice in the same oil, while at the 8th succesive frying in the same oil, safety is rather questionable, according to TPM limits
- It seems that the samples over the rejection limits comprise a relatively high part of the samples examined, indicating a possible health risk for the consumers of fast-foods and restaurants, according to TPM and PTG limits

- **Are the limits of TPM/PTG protective for public health?**

- **Is the defense system of the body adequate to neutralize the undesirable effect of TPM from a batch of French-fries?**

PART TWO

POTENTIAL ROLE FOR HEALTH

(1) LDL oxidation induced by TPM or MPM (atherogenicity). Inhibitory effect of oleuropein (main antioxidant of olive oil)

(2) Inhibitory effect of oleuropein on platelet aggregation induced by Platelet Activating Factor (PAF)

(3) Nutritional effect of frying for vit. A, C, E, trans-FA, MUFA, PUFA, minerals, polyphenols and squalene

(4) Feeding experimental animals with frying fats and oils (or fractions) and effects on : (a) native antioxidant systems, (b) native detoxification systems, respiratory chain systems (d) lipid metabolism (e) gene expression (f) blood cholesterol

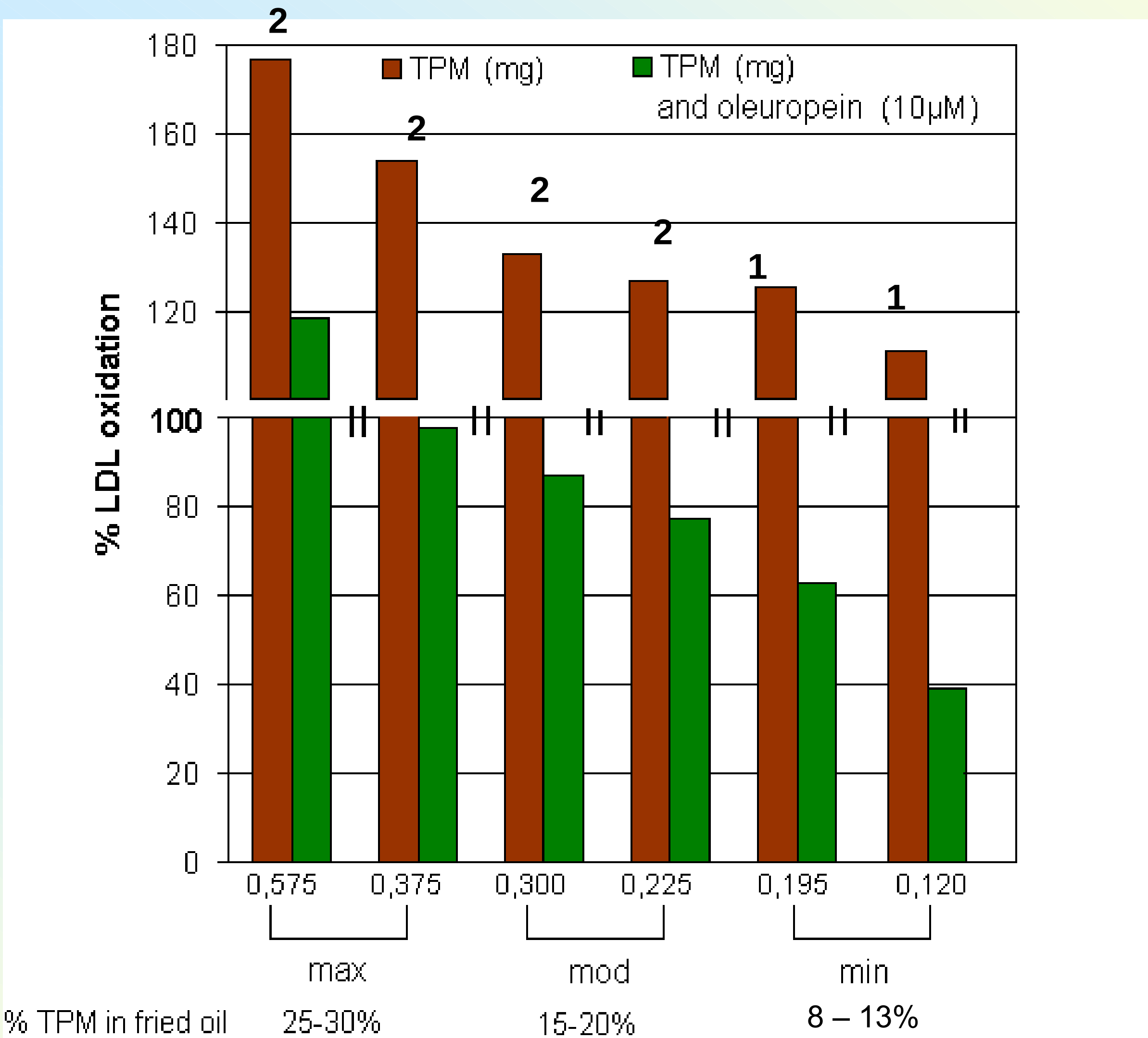
(5) Cosumption of frying by humans and effect on: (a) serum lipoprotein oxidation (b) atherogenesis

(6) Human exposure to fumes from frying fats (PAH)

(7) Aromatic heterocyclic amines in public health

(8) Acrylamide-hot off frying pan

TPM induced LDL oxidation (*in vitro*)



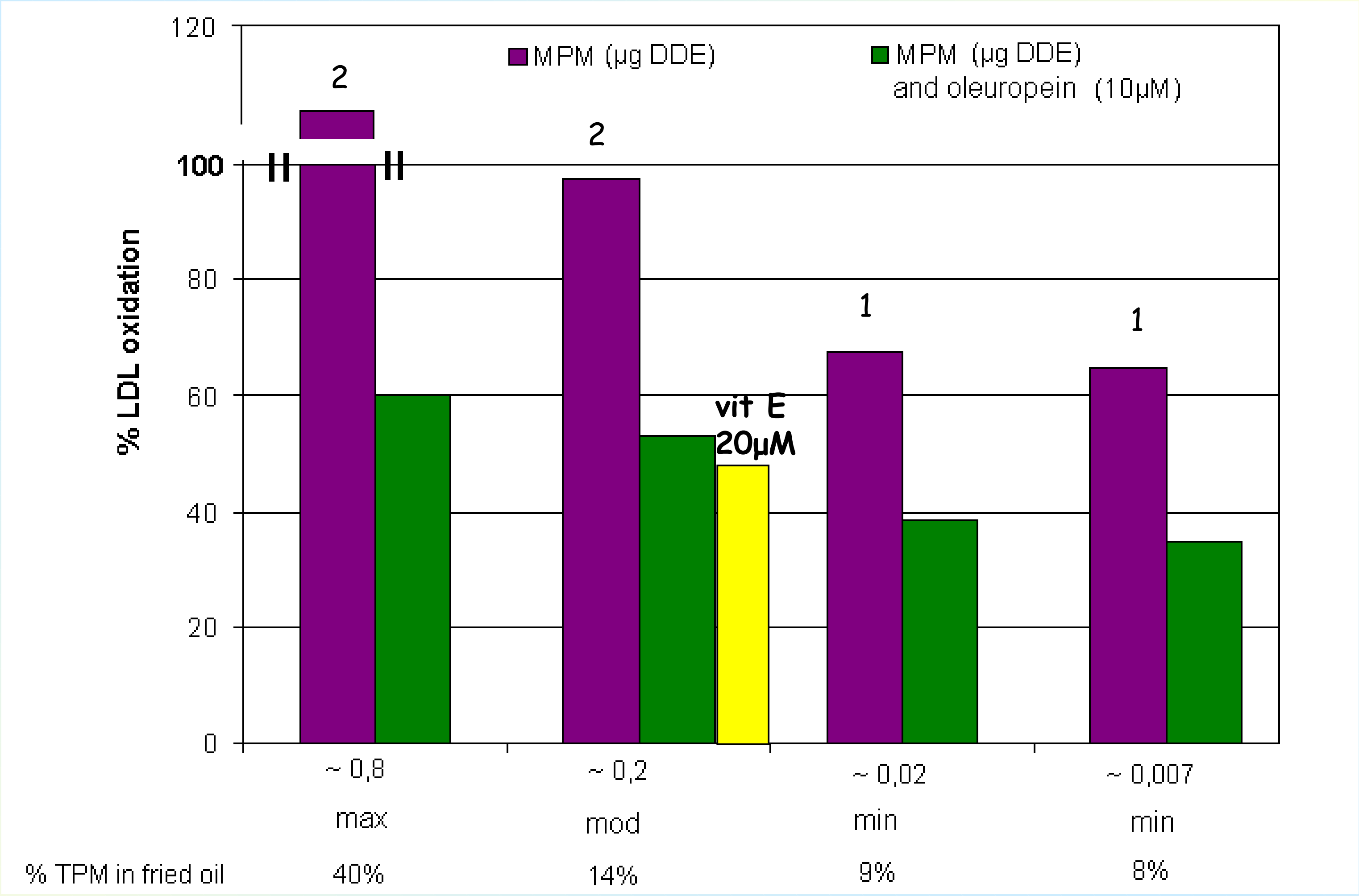
1. LDL + TPM from fried oils in domestic use

2. LDL + TPM from fried oils in fast foods & restaurants

TPM induced LDL oxidation (*in vitro*)

- 5 µM $CuSO_4$ induced LDL oxidation is defined as 100% oxidation
- TPM correspond to the quantity that it is distributed to blood LDL when eating a batch (150 g) of potatoes French-fried in oils of different deterioration (as indicated)
- NPM (Non Polar Materials) exhibit no oxidative effect on LDL
- Oleuropein = one of the main antioxidant polyphenols in olive oil
- 10µM Oleuropein in the test cuvette corresponds to approx. 5-8 olive pieces and a spoonful of salad olive oil

MPM induced LDL oxidation (*in vitro*)

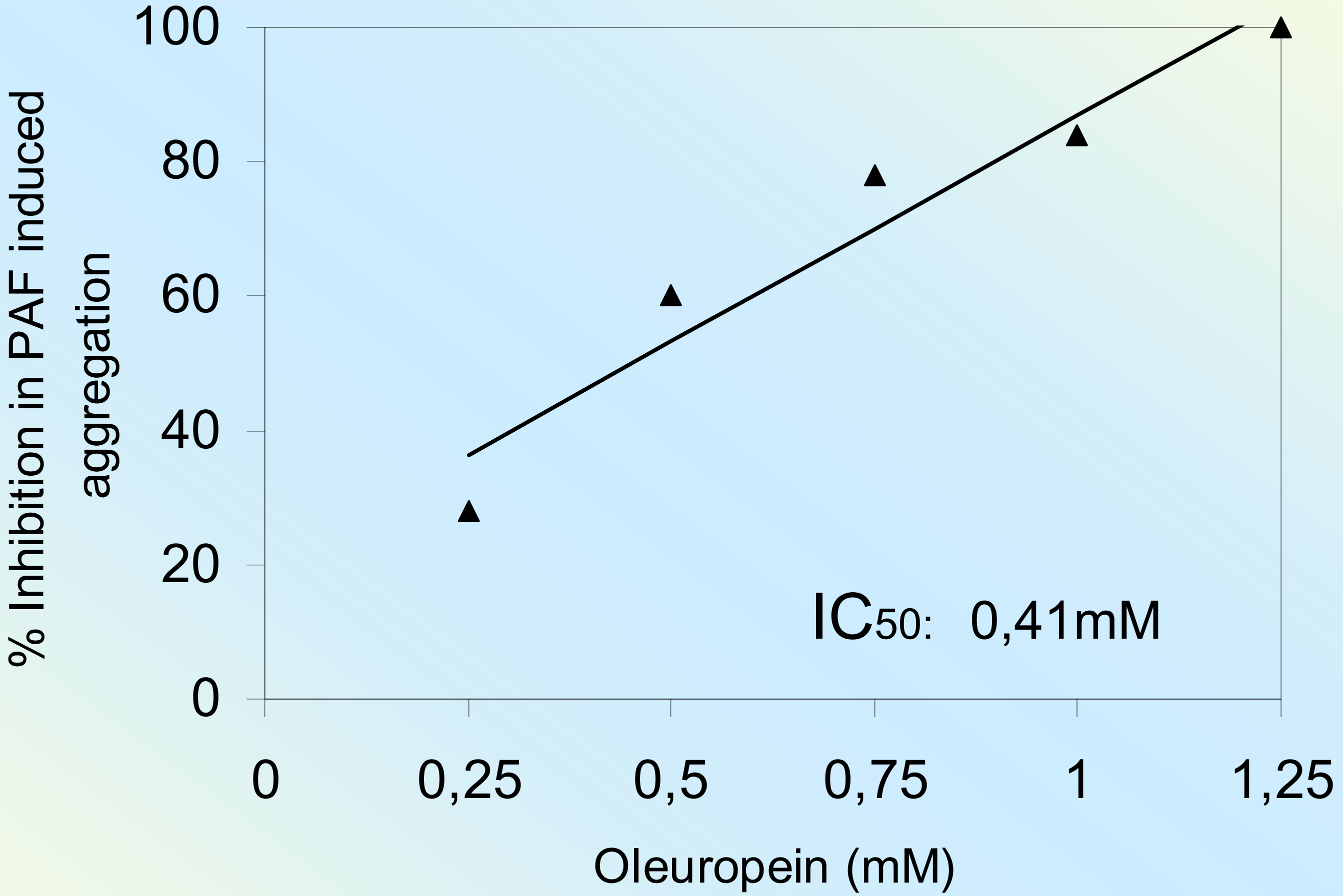


1. LDL + MPM from fried oils in domestic use

2. LDL + MPM from fried oils in fast foods & restaurants

MPM induced LDL oxidation (*in vitro*)

- MPM = Medium Polarity Materials isolated from fried oils (mainly 2,4-*trans*-*trans*-decadienal)
- MPM contained in a batch of potatoes French-fried in oil with 14% TPM exhibits approx. 100% LDL oxidation (same to that of 5 μM $CuSO_4$)
- Vitamin E contained in **30 ml** of salad oil (approx. 750 ppm Vit. E) results in **50%** inhibition of human body LDL oxidation



Andrikopoulos *et al*., Leb-Wiss Tech, 35, 479-484 (2002)

Nutritional effect of frying (I)

I. Vitamins

Vitamin	Cooked food	Cooking method	% Retention
Vitamin A	Vegetables	Boiling Frying	86 76
Vitamin C	Potatoes Potatoes Vegetables Meat, poultry	Baking Boiling Frying Various Various Frying Stewing Oven	80 – 85 60 – 80 25 – 80 57 – 96 57 – 77 80 80 80
Vitamin E (from frying oil)	Potatoes (fast food) Potatoes (home made)	Deep-frying Deep-frying (3-5 Frying cycles) Pan-frying (2-3 Frying cycles)	67 50 ~50

Nutritional effect of frying (II)

II. Fatty acids and micro-constituents

Constituents	Cooked food	Cooking method	Comments
Total *trans*-FA MUFA PUFA	Potatoes (in VOO, HOSO, SO) Potatoes (in VOO, SO, VS) -//- -//-	8 Frying cycles 20 Frying cycles 8 Frying cycles -//- -//-	20.8 – 45.2 mg/140g 86.4 – 129 mg/140g Negligible difference from fresh oils -//- -//-
Minerals	Potatoes Fish	Frying Boiling Baking	Minuscule decrease. Negligible losses.
Polyphenols	Potatoes (in VOO)	Deep-frying Pan-frying (4-5 Frying cycles)	Ref. 50% Ref. 50%
Squalene	(1) Potatoes (in VOO) (2) Small fishes (in VOO)	(1) Deep frying Pan frying (1-8 cycles) (2) Pan frying (1 cycle)	10-20% of the daily squalene intake

Nutritional effect of frying (III)

III. Conclusions

- High retention of Vitamin A and C ("approx. mean" 70%) in various foods
- Enrichment of foods with Vitamin E from the absorbed oil (over 50% of RDA)
- Enrichment of foods with the absorbed oil increases the caloric intake, which is important for the developing countries
- Enrichment of foods with squalene from the absorbed oil
- Low formation of total *trans*-FA (up to 5 mg/g oil) during frying. That corresponds to loading a batch of potatoes (150 g) with 0.138 mg which is much lower to the typical Western consumption (up to 4000 mg)

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Feeding exp. animals with frying fats and oils (I)

Exp. Animal	Feeding with frying fats	Period	Target	Affect	Ref
Albino wistar male rats	10% of the diet (SO)	3 w 5 w 7 w	Glutathione (G) G peroxidase G reductase	Significant increase	1
Rats	-	-	Mitochondrial respiratory chain components	Hydroperoxide TBARS Coenzyme Q Cyt (b, c, c1. a1, a3)	2
Rats	80g frying oil per kg (SO, VOO)	8 w	Liver microsomes	TBARS↑ Hydroperoxides↑ Ubiquinone ↓ (SO) Ubiquinone↑(VOO) PUFA↑ α-TOC ↓(VOO) SFA not affected	3

Feeding exp. animals with frying fats and oils (II)

Exp. Animal	Feeding with frying fats	Period	Target	Affect	Ref
Guinea pigs	15% of the diet (Soybean oil)	12 w	Liver microsomes	Growth rates ↓ Feed efficiency ↓ TBARS↑ UPD-glucuronyl-transferase↑ Cyt P-450↑ NADPH-CytC-reductase↑	4
Wistar rats	10% of the diet (Coconut oil)	12 w	Liver Heart, serum Kidney	Lipids total ↓ Cholesterol ↓ Damage start Lipids↑ Cholesterol↑ DHA /EPA↑↑ Damage start	5

Feeding exp. animals with frying fats and oils (III)

Exp. Animal	Feeding with frying fats	Period	Target	Affect	Ref
Rats	5 -20 % heated and fried oils	20 w	Activity of hepatic antioxidant enzymes	Catalase↑ GPX ↓ GST ↓ SOD ↓	6
Rabbits	10% VOO 1% Chol.	6 w	Liver,brain, heart, aorta, platelets	Lipid peroxidation ↓ Total / Oxidized glutathione↑ GP↑ GT↑	7
Guinea pig	15% (soyabean oil)	60 d	Liver	Liver weight↑ Microsomal protein↑ Cytochrome P450↑	8

Feeding exp. animals with frying fats and oils (IV)

Exp. Animal	Feeding with frying fats	Period	Target	Affect	Ref
Weaning long-trans male rats	15% (soyabeans)	6 w	Spleen	^{3}H –thymidine incorporation↑ PGE_2↑ α-tocopherol ↓	9
Albino male rats	EtOH EtOH + FF (SO)	45 d	Plasma Liver	Aspartate transam. (AST)↑ Alkal. Phosphatase (ALP) ↑ PL↑,Chol↑,TG↑ PL↓, Chol↑,TG↑ Phospholipases A, C↑ Hepatocytes degradation (↓)	10
Rats	5 and 20% (peanut oil, sesame oil and coconut oil)	20 w	Adipose tissue	Absorption rate of fats (ct.) Excretion↑↑ Deposition↑	11

Feeding exp. animals with frying fats and oils (V)

Exp. Animal	Feeding with frying fats	Period	Target	Affect	Ref
Rats	20g/100g 5g/100g (soyabean oil)	6 w	Plasma Liver tissue	Lipids↓, TG↓, Chol.↓ PPARα activated Gene expression↑ Lipid metabolism altered mRNA of acyl-CoA↑ Cyt P-450↑ CYP4A protein↑ TG↓	12
Rats	0-1 g/100g diet (cyclic FA)	2 w	Liver	Peroxisomal acyl CoA oxidase↑ Acyl- CoA oxidase↑ MUFA ↓ γ-linolenic↑ Lipid metabolism affected	13

Effect of frying fat fractions on exp. Animals (I)

Exp. Animal	Feeding with frying fats	Period	Target	Affect	Ref
1.Pups 2.Weaning rats	Cyclic FA from linseed and SO heated (in vitro)	-	Liver sub-cellular fractions incubation	Mitochondrial & peroxisomal oxidation	14
Albino wistar rats	100 mg/kg t-RP, t-2N (α,β unsaturated aldehydes)	24 h	Urine	Absorption & metabolism studies	15
Rabbits	Isoenergetic diet (VOO, SO, HOSO)	8 w	LDL-Plasma (FA, CoQ, TBARS, α TOC)	Peroxidation from SO > VOO	16

Effect of frying fat fractions on exp. Animals (II)

Exp. Animal	Feeding with frying fats	Period	Target	Affect	Ref
Rats	15% Shea oleine (nut of the tree *B. parkii*)	104 w	Clinical pathology & organs	Body weight ↓ Food intake ↓ Cholesterol ↓ Alkal. phosphatase↑ Heart weight↑ Incidence of pulmonary lipidosis↑	17
Rats	8% olive oil (48 & 69 potato frying operations) 8% SO (48 potato frying operations	8 d	Liver, spleen, skin.	Faecal iron (ct) Urinary iron excretion↑ Serum iron (ct) Iron content in liver & skin (ct)	18
Wistar Rats	1.Sardines fried in VOO 2. Fat from sardines	1): 3 w chol. loading. 2):2 w chol. free	Serum Liver	Chol. ↓ Lipids ↓	19

Effect of exp.frying fat consumption in humans (I)

Subject	Consumption	Period	Target	Affect	Ref.
Human (men) (8)	TG with labelled linoleic & linolenic cis & trans	48 h	CO_2 excretion in breath	Equal energy contribution from cis & trans Oxidation of linolenic > linoleic	20
Human (men) (16)	Native & heated safflower & olive oils	4 h	Dilute serum Isolated LDL	Susceptibility of oxidation of serum lipoproteins by Cu^{2+} in the postprandial period following meals rich in polyunsaturated vegetable oils (heated & native) and heated olive oil↑	21

Effect of exp.frying fat consumption in humans (II)

Subject	Consumption	Period	Target	Affect	Ref.
31 male, type II diabetic patients 22 poor glycemic control, 9 good glycemic control	Corn oil	2.5 h	Serum chylomicron	Conjugated dienes ↑ Oxidized lipids (ct.)	22
Men (10)	64.4 g fat that had been used for deep frying	4 h	Plasma	Atherogenesis accelerated	23

Human exposure to fumes from frying fats

Subject	Exposure	Target	Affect	Ref.
Women	Cooking oil fumes from frying fish	Lung (CL-3 cells)	DNA adduct formation	24
Chinese women	Carcinogenic chemicals emitted from not cooking oil	Lung	PAH concentration in fumes↑	25
Women (459)	Cooking fumes from rapeseed & linseed oils	Lung	Risk of lung cancer	26
Non-smoking females	Coal dust, frequent frying and deep frying food	Lung	Risk of lung adenocarcinoma	27
Chinese women (94 prof cooks, 43 house wives)	Cooking oil fumes: Benzo (a) pyrene, 1,3 butadiene (soyabean oil)	Lung	Cancer ho GG1 mRNA expression frequency↑	28

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Aromatic and heterocyclic amines in public health

I. The formation of HCAs during cooking can be decreased by:

1. natural and synthetic antioxidants

2. thrypthophan or proline

3. by removing the essential creatine through brief microwave cooking prior to frying or boiling

4. the amounts of HCAs in cooked foods are small, but other components in diet such as ω-6 polyunsaturated oils have powerful promoting effects in target organs of HCAs

Aromatic and heterocyclic amines in public health

II. Prevention of the formation of heterocyclic amines

Process	% Decrease of formation of mutagens during frying
Mixing 7-15% weight of soy protein concentrate with ground beef	90
Mixing 2,5 g pectin or textured procomm of 4.0 bontac (soy proteins products) to 50g beef patties	50-60
Mixing 1.3 mM chlorogenic acid or 10-20 mM BHA to 50 g beef patties	50-60
Applying 0, 0.5, 2.5, or 7% solution in water of a commercial green tea polyphenol to the two surfaces of 30g beef patties	Lowering
Using 159, 175, 521, 589mg of a black tea polyphenols to the both sides of 30g beef patties	70, 70, 90, 95
Applying L-tryptophane or L-proline to the surface of ground meat	Lowering

Weisburger . Mutation Research 506-507, (2002), 9-20

Acrylamide – hot off the frying pan

"There is currently no link
between acrylamide levels in food and cancer risk"

Dr. Colette Kelly, British Nutrition Foundation, Nutrition Bulletin 28 (2003) 5-6.

GENERAL COMMENTS

- The in vitro experiments on LDL oxidation gave evidence that TPM/ PTG limits could be considered as “quite safe” for humans for moderate consumption of fried foods.

- Nevertheless the possibility of consumption of TPM overloaded fried foods in fast food and restaurants are relatively high. Thus, more concern by the authorities should be taken.

- Consumption of foods rich in antioxidants together with fried foods has, in vitro, a remarkable protective effect from TPM undesirable properties.

- The detoxification system(s) is “moved on” indicating that some oxidation and toxic effects occur in the body after the consumption of fried food and their fractions.

Questions arising?

- How much rich-antioxidant foods should be consumed together with fried foods under real consumption conditions in order to neutralize the undesirable effects?

- What is the bioavailability of the antioxidants consumed?

- Is the capacity of the detoxification system of the body “good enough” to overcome the frying by-products under the daily real consumption of fried foods?

- Further research is essential in order to give answers to the above questions.

Thank you for your attention