Vol. 29 No. 5 CONTENTS

The Japan Oil Chemists' Society
YUSHI KOGYO KAIKAN, 13-11, Nihombashi 3-chome, Chuo-ku, Tokyo 103, JAPAN

総　説

過酸化脂質・最近の話題

金田尚志
東北大学農学部食糧化学科 (仙台市堤通雨宮町 1-1)

Recent Topics on Lipid Peroxides

Takashi KANEDA
Department of Food Chemistry, Faculty of Agriculture, Tohoku University (1-1, Amemiya-cho, Tsutsumi-dori, Sendai)

筆者に与えられた題は他の4人の方々の内容と重複しそうである。それは，現在の過酸化脂質の研究方向は生体内過酸化脂質，つまり，成人病との関連において多く論じられており，本企画も題名から察すると，ねらいは同じように思われる。そのため，私としては誠に書きにくいのだが，なるべく重複しないように心がけ，最近5年間ぐらいの過酸化脂質の研究について触れることにする。

1　脂質の自動酸化に関するもの

リノール酸メチルの自動酸化時には主として Table-I のような4種のヒドロペルオキシド (HPO) を生成するが，Chan ら[1]は高速液体クロマトグラフィー (HPLC) により各 HPO を単離後，40℃, 16h 保つと，HPO の再配列がおこり，それぞれの HPO から新たな HPO を生成するという。

Table-I　Major isomeric hydroperoxides formed from autoxidized methyl linoleate.

I	Methyl 9-hydroperoxy-*trans*-10,*cis*-12-octadecadienoate
II	Methyl 9-hydroperoxy-*trans*-10, *trans*-12-octadecadienoate
III	Methyl 13-hydroperoxy-*cis*-9, *trans*-11-octadecadienoate
IV	Methyl 13-hydroperoxy-*trans*-9, *trans*-11-octadecadienoate

例えば，I の HPO より II～IV の HPO も生ずる。このことはシス-トランス異性体でなく，トランス-トランス異性体でも認められる。Chan らは HPO の再配列機構をより明らかにするため，トマトのリポキシダーゼを用い，$^{18}O_2$ を標識した I をつくり，40℃ に 24h 保ったところ，異性体を生じ，その組成は I-37.5%, II-27.1%, III-6.7%, IV-28.7% となった。^{18}O- 異性体の割合は，I-81.8%, II-54.2%, III-56.4%, IV-53.9% で，残りは ^{16}O がついていた。この変化は再配列の際，空気中の三重項酸素と ^{18}O が入れ代わったものと想定している。

池田，福住ら[2]はシス及びトランス酸の酸化速度の比較を行っている。すなわち，オレイン酸及びエライジン酸の各メチルエステルの自動酸化速度を比較し，誘導期までは，オレイン酸はエライジン酸の 10.4 倍速く酸化すること，誘導期以降では 20 倍速やかであったとしている。また，リノール酸メチル及び *trans*-9, *trans*-12-オクタデカジエン酸メチルの場合は，前者が酸化しやすく，自動酸化に対する幾何異性体効果を認めている。オレイン酸，リノール酸，リノレン酸，$C_{18:3}+C_{21:6}$ 酸 (50 : 50) の各メチルの誘導期の比較からみた相対酸化速度は 1 : 8.0 : 21.7 : 39.10 であるという。また，*cis-trans* 及び *trans-trans* 共役オクデカジエン酸メチルの自動酸化においては，リノール酸メチルの自動酸化の場合のような明らかな誘導期が現れず，はじめから酸化が進行するが，誘導期後の酸化速度は小さいとした。しかし，酸化速度は小でもラジカルの生成速度は大で，また生成量も多いこと，共役ジエンの減少量が大で，孤立トランス二重結合形成が大なこと，HPO の形成は少ないこと，シス-トランス共役体はトランス-トランス共役体よりも自動酸化されやすいことなどを報告した。

2　ヒドロペルオキシドの分解

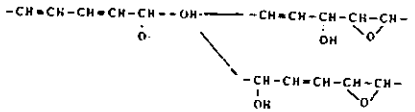
Selke, Frankel ら[3]はオレイン酸メチル HPO を加熱した際に生成する揮発性物質について検討している。この HPO は 8-HPO (27%), 9-HPO (23%), 10-HPO (23%), 11-HPO (27%) の混合物だが，これを GC-MS に直接注入し，19 余の主ピークを得ている。これらはヘプタン，オクタン，ヘプタナール，1-ヘプタノール，オクタナール，1-オクタノール，ノナナール，2-ノネナール，デカナール，2-デセナール，2-ウンデセナール，9-オキソノナン酸メチル，10-オクタデセン酸メチルなどを主とした。これら揮発物は，同氏らがさきに報

告した[9] トリオレイン酸化物の加熱により生成する揮発性物質と同様であった。

ヘム化合物による HPO の分解産物については従来，その詳細は不明だったが，ヘモグロビンによる分解について検討された。すなわち，Hamberg[10] は 13-L-ヒドロペルオキシ-9, 11-オクタデカジエン酸をエタノールに溶かし，ヘモグロビンを加えて 37℃ に 5 min 保ち，生成物を分離したところ，主成分は 13-ケト-9, 11-, 13-L-ヒドロキシ-9, 11-オクタデカジエン酸及び *erythro*-12, 13-エポキシ-11-ヒドロキシ-9, *threo*-12, 13-エポキシ-11-ヒドロキシ-9-, 9-DL-12, 13-エポキシ-ヒドロキシ-10-のオクタデセン酸をえた。このうち，13-オキソ-9, 11-オクタデカジエン酸は下記のような経過で 13-ペルオキシラジカルから OH· が離れ 2-オキソ酸になるとした。

$$-\overset{|}{C}H- \longrightarrow -\overset{|}{\underset{|}{C}}- \longrightarrow -\overset{|}{C}-\div OH\cdot$$
$$\quad\ O\cdot \qquad\quad OOH \qquad O$$

エポキシ-ヒドロキシ-オクタデセン酸は下記のように オキシラジカルをもったオクタデカジエン酸に対する OH· の付加と考えた。



エポキシドの生成については，Sevanian, Mead ら[11] も白ねずみの肺における生成を認めているが，これは 6.5 ppm の NO_2 をふくむ空気を白ねずみに 24 h 吸わせたとき肺組織に脂質エポキシドが生成したという。これは Hamberg の報告とは異なり，肺組織中のリノール酸にペルオキシラジカルを生じ，これが RO· に分解するとき隣接したリノール酸の二重結合がエポキシ化されるとしている。

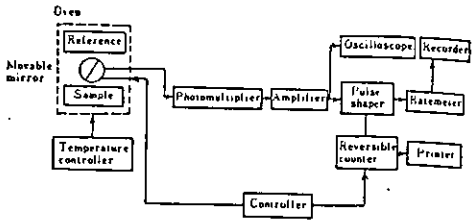


Fig.-1 Schematic diagram of the single photon counting system for the measurement of ultraweak chemiluminescence.

3 極微弱光計測による過酸化脂質の測定

ソ連の Vassilev ら[12] は 1962 年に，各種の有機化合物が酸化する際，極微弱光を発することを認めた。東北大電気通信研の稲葉教授らは 10^{-17} W という微弱光を検知する方法を開発した。すなわち，Fig.-1 に示すように，光電子増倍管 (photomultiplier) を用い，光電子計測方式により所定のゲートタイムにおける受光量を光電子パルス数として計測する方法である。

筆者らは本法を用いて各種食用油脂の劣化度と発光量の関係を検討した[13]。その結果，例えば，180℃ に加熱した大豆油の過酸化物価 (POV) 及びその他の化学特数と発光量の間には相関がみとめられた (Table-2)。また，室温で酸化した大豆油についても同様なことがいえた (Table-3)。即席ラーメン含有油脂の劣化程度もこの方法で求められるが，その際，ラーメンから油脂を抽出する必要はなく，砕いた試料粉末をそのまま使用できる。

筆者らはこの測定法を生体内過酸化脂質の測定に応用しようとしている。すなわち，老化に関する最近の研究によると，加令と過酸化脂質，あるいは生体内ラジカルとの関係が問題とされ，とくに一重項酸素やスーパーオ

Table-2 Chemiluminescence and chemical characteristics of thermally oxidized soybean oils with different heating times.

Heating time at 180℃ (h)	AV	POV	COV	IV	Conjugated unsaturated fatty acid (%)		Brown color[a]	Emission intensity (counts/32 s)
					Diene	Triene		
0	0.046	0.9	3	129	0.34	0.055	0.056	728
1	0.048	3.6	7	128	0.53	0.080	0.096	655
2	0.049	5.4	16	128	0.68	0.116	0.122	667
4	0.073	5.1	38	126	1.55	0.148	0.175	719
6	0.094	5.7	37	125	1.35	0.147	0.157	991
8	0.129	6.3	70	122	1.54	0.156	0.247	1361
14	0.309	8.1	87	120	1.90	0.128	0.404	2464
24	1.85	8.7	302	103	3.78	0.125	1.99	12193
32	2.01	9.6	304	96	4.45	0.116	4.41	22193

a Brown color was represented by the optical density of oils at 440 nm.

Table-3 Chemiluminescence of autoxidized soybean oils.

Period of autoxidation[a] (d)	POV	AV	Emission intensity (counts/32 s)
0	0.86	0.021	2,200
7	16.6	0.025	6,400
9	25.9	0.037	5,700
20	48.7	0.043	5,400
31	64.9	0.055	8,000
42	108.6	—	12,000

a At room temperature

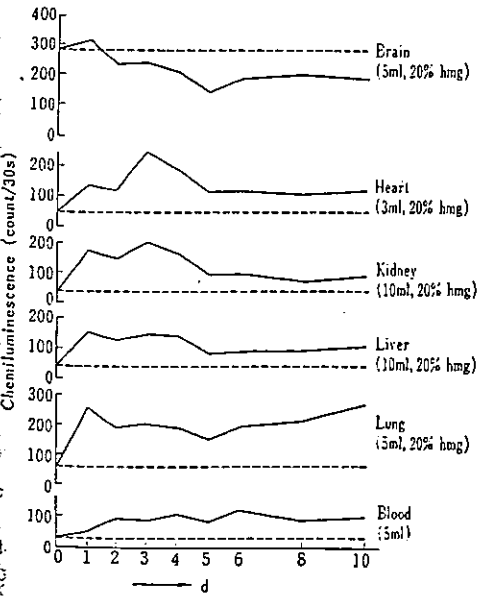
キシドアニオンなどの活性酸素が老化に強く関与しているとされる[7),14)]。従来，生体内過酸化脂質の定量や老化の判定には TBA 法が医学方面では多く用いられてきたが，定量結果は人により差を生じやすく，本法のみで生体老化度を判定するのは不充分といえる。そこで，生体に生成するであろう極微弱光を測定して老化度を判定しようとした。

まず，老化の際と同様，生体内の TBA 値が増加する自動酸化油 (POV 400) を白ねずみに 50% 致死量の 1/5 量，10 d 与え，体各部臓器の一般特数を測定するとともに発光量を測定した[15]。その結果，自動酸化油を与えると，白ねずみの各組織の TBA 値と POV は増加したが，とくに肺，脳，じん，心の TBA 値は顕著に増加した。また，POV は肺，じんでの増加がめだった。この際の発光量を示すと Fig.-2 のようである。すなわち，対照群に比べ，心，じん，肝，肺，血液などで発光量は増加した。一方，脳では減少が認められた。また，白ねずみに UV 照射を行ったところ，Table-4 のように，照射後の発光量は明らかに増加し，TBA 値との相関性もみとめられた。以上のように，生体内の発光量と過酸化脂質とは相関を有するように思われるので，老化と生体内発光の関係をより明らかにしようと目下研究中である。

Table-4 UV irradiation and the change of ultraweak chemiluminescence, TBA value in rat tissues.

Tissue	Irradiation	Chemiluminescence (count/30 s)	TBA value (O.D. 535 nm, ×10⁻²)
Plasma (5 ml)	Before	8	3.1
	After**	141	3.4
Liver*	Before	10	4.8
	After**	126	29.5
Lung*	Before	34	4.0
	After**	238	20.0
Heart*	Before	19	3.4
	After**	90	8.5
Kidney*	Before	64	4.7
	After**	87	21.0

* 5 ml, 10% homogenate.
** 90 h, 15 W.



——: Autoxidized oil fed rats. - - -: Standard meal fed rats.

Fig.-2 Autoxidized oil feeding and the change of ultraweak chemiluminescence in rat tissues.

4 脂質過酸化物と毒性

筆者らは脂質過酸化物が毒性を呈することを 1953 年に報告したが，その後，各種二次酸化物とくに炭素数 5~9 のヒドロペルオキシアルケナールの毒性が強いことを認めた[16]。また，リノール酸メチル HPO のマウスに対する 50% 致死量も求めた[17]。さらにリノール酸メチル HPO の分解により生ずる各種酸化物の 50% 致死量を求め Table-5 のような結果を得ている。本結果によれば，二次分解物は飽和アルデヒドを除き，いずれも，HPO に比べはるかに強い毒性を示し，とくにヒドロペルオキシアルケナールは HPO の 100 倍にも及ぶ強い毒性を示した。

筆者らは，また，リノール酸メチル HPO 及びその分解物による毒性発生機構を解明するため，マウスを用い，急性及び慢性毒性試験を行い，臓器の病理組織学的検討を行った[18]。その結果，同酸化物を与えて死亡したマウスの小腸，肝，肺，じんなどの組織には細胞壊死が共通してみられ，脂肪沈着がおこっていた。また，いずれの器官にも血管の拡張と充うっ血が認められ，その障害の程度はマウスに対する毒性の強さと平行していた。このことは各酸化物が小腸から吸収され，各組織に達し，障害をおこ

Table-5 Comparative LD_{50} of oxidation products formed in autoxidized methyl linoleate.

Compound	LD_{50} mmol/kg mice	LD_{50} mg/kg mice
n-Hexanal	82.79	8,292
trans-2-Hexenal	6.98	665.0
2-Hydoxyhexanal	5.15	598.2
4-Hydroperoxy-2-alkenals	0.45 as pentenal	52.5
	as nonenal	77.5
Methyl linoleate hydroperoxide	39.10	12,760

すと思われた。

柴田，衣巻ら[10]はマウスに自動酸化サフラワー油 (POV 2000) を9か月間与えた際の血中グルコース量，肝トリグリセリド及びコレステロール量を定量したところ，対照の新鮮サフラワー油給与群との間に差を認めなかった。また，肝におけるグルタミン酸オキザロ酢酸トランスアミラーゼ (GOT)，グルタミン酸ピルビン酸トランスアミラーゼ (GPT)，ATPase，α-グリセロリン酸脱水素酵素 (α-GPPH) などの活性も対照群と差がなかったという。

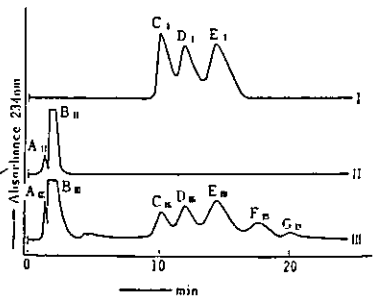
Budowski や Frankel ら[11]はサフラワー油を 145℃ で 24 h 加熱し鳥に与えると脳疾患 (Encephalopathy (NE)) を生じることを認めた。本油は新鮮油に比べリノール酸やビタミンEが少なかったが，このこと以外にも原因がありそうに思えた。合成オキソオクタデカン酸及びオキソデカジエン酸は鳥に NE をおこさせるが，加熱油より製した脂肪酸メチルの極性部分は NE の発生を促進した。このことより，加熱油中の共役ケトポリエン酸，または加熱により油中のビタミンEが少なくなり内因的に生成した同様ケト酸により NE を生ずるとしている。

5 不飽和酸ヒドロペルオキシドの腸管よりの吸収

脂質過酸化物は未変化の脂質に比べ栄養的に劣り，毒性さえも呈するが，この際の毒性発生機構については多くの報告が出されている。しかし，いちばんもとになる脂質過酸化物の腸における吸収形態については不明の点が多く，いろいろな仮説が出されている。すなわち，筆者らは 25 年も前から不飽和酸ヒドロペルオキシドの一部は分解されず，そのままの形で腸管を通過するとしていたが，他にも同様な見解が報告されている。しかし，脂質過酸化物を動物に与えてもリンパ中に過酸化物は見いだされず，過酸化物は腸壁で分解し，この分解物が腸管より吸収されて毒性を呈するとした報告も出された。ただし，この際，どのような形に分解され，吸収されるか知られていない。

Bergan ら[12]は 1-^{14}C-リノール酸メチル HPO をねずみに与え，リンパを集めて，脂質を検討したところ，ヒドロキシ酸を見いだしたが，ヒドロペルオキシドは存在しないとした。また，リンパトリグリセリドのトリエン酸に ^{14}C が取り込まれるとしている。そして，ヒドロキシ酸やトリエン酸は HPO の吸収の際，HPO の還元-脱水反応により生成すると述べている。

この報告が出されて以後，不飽和酸 HPO の吸収についてはあまり報告されていなかったが，筆者らはうさぎを用いて HPO の吸収形態を調べた[13]。すなわち，99.8% の純度のリノール酸メチル HPO を調製してうさぎに与え，リンパ液を集めて，含有脂質の高速液体クロマトグラフィーを行った。その結果，Fig.-3 に示すように，未変化の HPO が移行していることを突きとめた。また HPO はトリグリセリドにも取り込まれていた。この結果に基づき，筆者らは不飽和酸 HPO は吸収されにくいが，一部は未変化のままで吸収され，体各部において酵素の不活性化をおこし毒性を呈すると結論した。



Peak A and Peak B: Triglycerides and their related substance; Peak C: Methyl 13-hydroperoxy-*cis*-9, *trans*-11-octadecadienoate; Peak D: Methyl 13-hydroperoxy-*trans*-9, *trans*-11-octadecadienoate; Peak E: Methyl 9-hydroperoxy-*trans*-10, *trans*-12-octadecadienoate; Peak F and G: Derivatives of MLHPs which are not identified yet. MLHPs = methyl linoleate hydroperoxides.

Fig.-3 High performance liquid chromatography of original MLHPs (I), extracts from control lymph (II) and extracts from lymph of rabbit dosed MLHPs (III).

6 過酸化脂質と老化

生体内に生成する過酸化脂質と老化との関係について最近多くの研究が報告されるようになった[14)-22)]。

生体内過酸化脂質の老化への影響として各種酵素の不活性化が考えられるが，この反応機構が *in vivo* 及び *in vitro* で検討されている。また，組織中に生成する老化と関係のある色素の生成過程についても多くの報告がある。このほか，生体内脂質過酸化に関与する酸素の形

態についても報じられているので，そのうちのいくつかを紹介する。

生体に生じたスーパーオキシド $O_2\cdot^-$ は脂質過酸化に関与することが知られている。キサンチンのキサンチンオキシダーゼによる酸化により脂質は過酸化されるが，この際，Khan[23] によれば

$$2O_2\cdot^- + 2H^+ \longrightarrow {}^1O_2 + H_2O_2$$

のように一重項酸素を生じ，酸化されるという。また，Kellogy ら[24]は脂質過酸化は

$$O_2\cdot^- + H_2O_2 \longrightarrow OH^- + OH\cdot + {}^1O_2$$

のようにして生じた $^1O_2$ によるとしている。

Svingen ら[25]は脂質過酸化におけるスーパーオキシドや一重項酸素の役割について検討し，キサンチンオキシダーゼにより触媒される脂質過酸化の機構はスーパーオキシド，一重項酸素，アデノシン 5'-ジホスホキレートイオン (ADP-Fe^{3+}) 依存性であり，ADP-Fe^{3+} がないときやスーパーオキシドディスムターゼの存在において，酵素的脂質過酸化は完全に阻害されることを認めた。また，一重項酸素のトラップ剤として 2,5-ジフェニルフランを用いたところ，一重項酸素は過酸化の開始には関与せず，むしろ，過酸化脂質の連鎖生成に関与していた。

生体内に生成する lipofuscins やセロイドとよばれるけい光を有する色素は Tappel ら[26)-33)]により多くの研究が行われ，その基本構造は

$$-N\cdot\overset{|}{C}-C\cdot\overset{|}{C}-N-$$

とされている。すなわち，脂質の過酸化により生じたマロンアルデヒドが2個の第一級アミンと結びけし，共役シッフ塩を生じ，これがけい光発色団となるとされている[26]。Taubold ら[31]は人脳の lipofuscin を分け，その組成を検討し，重合脂質とアミノ酸または脂質またはタンパク質を含むリン脂質より成るとしている。

lipofuscin 色素はミトコンドリア，ミクロソーム，リソソームなどで脂質過酸化により生成する。本色素のけい光発光極大は 420 から 470 nm に，励起極大は 340 から 370 nm にある[32),33),27)]。lipofuscin の定量に際してはレチノールのような妨害物質をあらかじめ除く必要があるが，Csallany ら[32]はセファデックス LH-20 のカラムを使い，組織のクロロホルム，メタノール抽出液からレチノールやより低分子のけい光物質を除いたのち定量を行っている。

脂質過酸化物とアミノ酸，タンパク質のラジカル反応についてはいくつかの報告があるが，Schaich ら[34]は酸化リノール酸メチルからアミノ酸，タンパク質への遊離基の移動をモデル系で検討している。すなわち，リシン，アルギニン，トリプトファン，システイン，還元型グルタチオンなどをリノール酸メチルと混合し，37℃ に 20d 間保ち，ESR によるラジカルの測定をした結果，上記アミノ酸やグルタチオンにフリーラジカルのシグナルを生じた。本反応は次式のようにペルオキシラジカルやアルコキシルラジカルの連鎖生成の過程で，アミノ酸やタンパク質にラジカルが移行するためとしている。

$$LO_2\cdot + PH \longrightarrow P\cdot + LOOH$$
$$LO\cdot + PH \longrightarrow P\cdot + LOH$$
$$LOOH = \text{脂質 HPO},\ PH = \text{タンパク質}$$

タンパク質中のアミノ酸残基のうち含硫アミノ酸は酸化に対し最も不安定とされ，SH-酵素は脂質過酸化物により容易に不活性化される。SH 基をもつタンパク質を脂質過酸化物にさらすと thiyl ラジカルを生ずる[35]。また，システインを含めチオール類は脂質過酸化物によりジスルフィドにまで酸化される[36]。この際，遷移金属や合金属タンパク質は酸化を促進する[37),38)]。

リノール酸 HPO とシステインの鉄イオン触媒による反応生成物については多くの知見がえられているが，Gardner[39] は Fe^{2+}-Fe^{3+} の酸化還元系におけるラジカルによりシステインから thiyl ラジカルを生じ，RO・が HPO より生ずるとしている。また，80% エタノール中でリノール酸 HPO に *N*-アセチルシステインと鉄イオンを加えると，チオール結合の生成を認めている。すなわち，13-ヒドロペルオキシ-*trans*-11, -*cis* 9-オクタデカジエン酸と *N*-アセチルシステインより 9-*S*-(*N*-acetyl cysteine)-10-ethoxy-13-hydroxy-*trans*-11-octadecenoic acid などが生成するという。

生体内における過酸化脂質とタンパク質の反応を解明するため過酸化リン脂質を用いた実験もある。Nielsen[40] はカルジオリピンの懸濁液をワールブルグ検圧計フラスコに入れ，Cu^{2+} の存在下に 30℃，120 h 酸化させ，各段階の酸化物にアルブミンを加えてインキュベートしたところ両者間に共有結合がみられた。15 mol までのカルジオリピンは 1 mol のアルブミンに結合するが，重合反応はおこらなかった。また 20~25 mol 程度までカルジオリピンを増すとわずか重合がおこり，314 mol にすると重合がおこった。このことは，過酸化カルジオリピンとアルブミンの結合はタンパク質の分子内橋かけなしにおこることを示唆した。

Sundholm ら[41]は酸化脂質存在下でのコラーゲンの橋かけを検討するため，ゼラチン膜にとうもろこし油を加え，UV 及び日光照射したところ，コラーゲンの橋かけ反応を認めたが，本反応は添加脂質の酸化により生じたカルボニル化合物とアミノ酸の縮合によると推定している。

Matsushita らは脂質過酸化物及び二次分解物の酵素に対する作用をいくつか報告している。すなわち，リノール酸メチル HPO は RNase を不活性化するが，この際本 HPO は RNase 中へ取り込まれる。HPO の二次

酸化物による RNase の不活性化はリノール酸 HPO ほどではなかった。しかし，トリプシンに対しては不活性化を示した。ペプシンに対しては活性化の傾向を示した[18]。ペプシンに対する作用は筆者らも認めているが[19]，どうして，ペプシンの場合のみ，不活性化されぬばかりでなく，逆に活性化されるのかなお不明であり，今後のおもしろい研究題目となるだろう。

はじめに述べたように，過酸化脂質の研究方向は主として生体内脂質の過酸化に関する問題及び各種疾病との関係に向けられており，この分野の研究は今後，益々発展すると思われる。

（昭和 55 年 3 月 3 日受理）

文　献

1) H.S. Chan, G. Levett, J.A. Matthew, *Chemistry and Physics of lipids*, 24, 245 (1979)
2) 池田辰雄，福住一造，油化学，27，21，26，33 (1978)
3) E. Selke, E.N. Frankel, W.E. Neff, *Lipids*, 13, 511 (1978)
4) E. Selke, W.K. Rohwedder, H.J. Dutton, *J. Am. Oil Chem. Soc.*, 54, 62 (1977)
5) M. Hamberg, *Lipids*, 10, 87 (1975)
6) A. Sevanian, J.F. Mead, R.A. Stein, *Lipids*, 14, 634 (1979)
7) R.F. Vassilev, A.A. Vichutinskii, *Nature*, 194, 1276 (1962)
8) R. Usuki, T. Kaneda, A. Yamagishi, C. Takyu, H.Inaba, *J. Food Sci.*, 44, 1573 (1979)
9) 平井俊策，代謝，15，1329 (1978)
10) 八木国夫，老年医学，15，1327 (1978)
11) 宮沢陽夫，金田尚志，脂質生化学研究，21，366 (1979)
12) 吉岡啓子，鈴木勝久，金田尚志，油化学，21，881 (1972)
13) 吉岡啓子，金田尚志，油化学，23，321 (1974)
14) L.R. Tovar G, T. Kaneda, 油化学，26，169 (1977)
15) 白　台鴻，星野忠彦，金田尚志，栄養と食糧，29，85 (1976)；31，651 (1978)
16) 柴田宣和，衣巻貴穂，油化学，26，529 (1977)
17) P. Budowski, I. Bartov, Y. Dror, E.N. Frankel, *Lipids*, 14, 768 (1979)
18) G.M. Findlay, H.H. Draper, J.G. Bergan, *Lipids* 5, 970, 976 (1970)
19) K. Nakatsugawa and T. Kaneda, Joint Meeting of Am. Oil Chem. Soc. and Japan Oil Chem. Soc. April, 1979, San Francisco
20) 最新医学，特集，過酸化脂質障害の再検討，33，No. 5 (1978)
21) 老年医学，特集，老年者と過酸化脂質，16，No. 11 (1979)
22) ビタミン，特集，過酸化脂質とビタミン，53，No. 12 (1979)
23) A.U. Khan, *Science*, 168, 476 (1970)
24) E.W. Kellogy, I. Fridovich, *J. Biol. Chem.*, 250, 8812 (1975)
25) B.A. Svingen, F.O. O'Neal, S.D. Aust, *Photochemistry and Photobiology*, 28, 803 (1978)
26) C.J. Dillard, A.L. Tappel, *Lipids*, 6, 715 (1971)
27) C.J. Dillard, A.L. Tappel, *Lipids*, 8, 183 (1973)
28) V.G. Malshet, A.L. Tappel, *Lipids*, 8, 194 (1973)
29) U. Reiss, A.L. Tappel, *Lipids*, 8, 199 (1973)
30) W. Bidlack, A.L. Tappel, *Lipids*, 8, 203 (1973)
31) K.S. Chio, U. Reiss, B. Fletcher, A.L. Tappel, *Science*, 166, 1535 (1969)
32) B.L. Fletcher, C.J. Dillard, A.L. Tappel, *Anal. Biochem.*, 52, 1 (1973)
33) K.S. Chio, A.L. Tappel, *Biochemistry*, 8, 2821 (1969)
34) R. Trombly, A.L. Tappel, *Lipids*, 10, 441 (1975)
35) R.D. Taubold, A.N. Siakotos, E.G. Perkins, *Lipids*, 10, 383 (1975)
36) B.L. Fletcher, C.J. Dilland, A.L. Tappel, *Anal. Biochem.*, 52, 1 (1973)
37) K. Reddy, B. Fletcher, A.L. Tappel, *J. Nutrition*, 103, 908 (1973)
38) A.S. Csallany, K.L. Ayaz, *Lipids*, 11, 412(1976)
39) K.M. Schaich, M. Karel, *Lipids*, 11, 392 (1976)
40) M. Karel, K. Schaich, R.B. Roy, *J. Agri. Food Chem.*, 23, 159 (1975)
41) W.T. Roubal, A.L. Tappel, *Arch. Biochem. Biophys.*, 113, 5 (1966)
42) C. Little, P.J. O'Brien, *Biochem. J.*, 106, 419 (1968)
43) P.J. O'Brien, *Can. J. Biochem.* 47, 485 (1969)
44) H.W. Gardner, *J. Agri. Food Chemi.*, 23, 129 (1975)
45) H.W. Gardner, D. Weisleder, R. Kleiman, *Lipids*, 11, 127 (1976)
46) H. Nielsen, *Lipids*, 13, 253 (1978)
47) F. Sundholm, A. Visapää, J. Björksten, *Lipids*, 13, 755 (1978)
48) S. Matsushita, *J. Agri. Food Chem.*, 23, 151 (1975)

過酸化脂質の生成と分析

島　崎　弘　幸

帝京大学医学部第一生化学教室（東京都板橋区加賀2-11-1）

Formation and Determination of Lipid Peroxides

Hiroyuki SHIMASAKI

Department of Biochemistry, School of Medicine, Teikyo University (2-11-1, Kaga, Itabashi-ku, Tokyo)

1 はじめに

過酸化脂質の生体機序は，Farmer, Bolland, Gee らを中心とした，1940 年代の研究を Holman[1]，Uri[2]，Lundberg[3] らが総説にまとめ，一応の完成をみたものと思う。金田[4] にその理論も細部には不明りょうな問題点を残すと指摘しているが，今のところこれに代わる新しい理論はみられない。今日，過酸化脂質の生成が新たに注目されるのは，1969 年の Superoxide dismutase[5] の発見と，1974 年の血小板リポキシゲナーゼ[6] の発見で代表される，生体内の過酸化脂質の生成と消去に関するものであろう。本項では古典的な過酸化脂質の生成理論を基に，今日注目されている生体での過酸化脂質の生成と分析について，研究の現状を紹介する。

2 過酸化脂質の生成機序 I（自動酸化）

2-1 過酸化脂質の定義

過酸化脂質の定義は金田[7] に従い，「過酸化物（peroxide）を含む脂質」とする。また，ここでは特に断らないかぎり，過酸化脂質はヒドロペルオキシド（-OOH）の結合した脂肪酸，又は脂質に用いた。

主な略号は次のようにした。ROOH：過酸化脂質，LOOH：リノール酸ヒドロペルオキシド，MLH：リノール酸メチルヒドロペルオキシド，R·：脂肪酸から水素原子が離脱してできたラジカル，又は同脂質，PUFA：高度不飽和脂肪酸，PG：プロスタグランジン，PGG_2：プロスタグランジンG_2，ADP：アデノシンジホスフェート，NADPH：nicotinamide adenine dinucleotide phosphate（還元型），$NADP^+$：同（酸化型）。EDTA：エチレンジアミン四酢酸。

2-2 油脂の自動酸化

油脂の自動酸化（autoxidation）は自触媒で進行するラジカル連鎖反応であり，理想的な条件下での過酸化脂質の生成は 図-1a のように表される[8]。反応の初期は誘導期（induction period）と呼ばれ，過酸化脂質の生成は非常にゆっくり進む。誘導期を過ぎると過酸化脂質は急激に増加し，その過程は自触媒（autocatalysis）で進行するラジカル連鎖反応（propagation）と呼ばれる。一般に油脂の自動酸化は次のように説明される[9,10]。

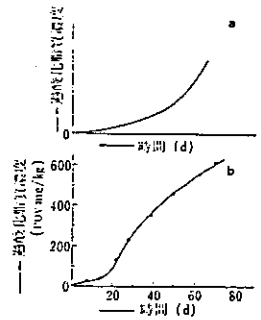


図-1　a. 典型的な油脂の自動酸化[11] と b. 実測例（著者ら，未発表）

① Initiation（反応開始）

$$RH \longrightarrow R\cdot + H\cdot$$

②Propagation（反応連鎖）

$$R\cdot + O_2 \longrightarrow ROO\cdot$$

$$ROO\cdot + RH \longrightarrow ROOH + R\cdot$$

③ Termination（反応停止）

$$R\cdot + R\cdot \longrightarrow RR$$

$$R\cdot + ROO\cdot \longrightarrow ROOR$$

$$ROO\cdot + ROO\cdot \longrightarrow ROOR + O_2$$

①不飽和脂肪酸から水素原子が引き抜かれ，ラジカルが生成する。②R· ラジカルは酸素と結合してペルオキシラジカル（ROO·）となり，他の脂肪酸から水素を引き抜いてヒドロペルオキシド（ROOH）となる。他方，水素を失った脂肪酸は，新しい R· ラジカルとして再び

qualities of crackers produced with good hydrogenated shortenings.

Oat flour and oat flour extracts were found to have but a slight favorable effect on the keeping qualities of crackers (Triebold, 1938). The oat flour and extracts were added to the cracker sponge, in doughing up the sponge, or sprayed or dusted upon the baked crackers. A protective factor from 0 to 2, which for all practical purposes is negligible, was exerted by the various treatments.

Lundberg, Halvorson, and Burr (1944) found that NDGA (nordihydroguaiaretic acid) when added to a lard used in making pie crusts and soda crackers exerted some stabilizing effect on the resulting product (protective factor of approximately 2 in crackers and 10 in pie crusts). The lesser effectiveness of the NDGA in crackers was thought to be due to the alkalinity imparted by the baking soda since alkaline solutions of NDGA oxidize rapidly when exposed to air.

Higgins and Black (1944) studied the effect of several antioxidants added to lard used in the preparation of crackers. They found gum guaiac to be an effective antioxidant for lard with the stability carrying over into the baked product (protective factors of 2.5-7, depending upon concentration). Propylgallate exerted a stabilizing effect on the lard but practically none on the resulting crackers. The same was also true for the tocopherols and for a wheat germ oil derivative (an ethylene dichloride extract of wheat germ oil combined with citric acid).

Mixing, Fermentation and Baking. These manipulative procedures involved in the manufacture of baked goods likely play a role in the keeping quality of the resulting product. This has been referred to previously in the destruction of pro-oxidants and antioxidants present in a fat when baked into crackers. Mixing spreads the fat over a greater area and also in the presence of salt may cause the solution of certain fat components into the aqueous phase, thereby facilitating their oxidation later. Fermentation produces sugars and organic acids, and these may have an effect upon the stability of the shortening.

The temperature and length of baking time might be anticipated to have an effect on the stability of the fat in baked products. Apparently as long as there is sufficient moisture present so that the product does not scorch, the effect is not great. However, crackers with scorched spots or crackers that have been crisped by successive reheatings show a marked decrease in keeping quality.

Packaging. The possibility of the absorption of fat from a baked product by the package must not be overlooked. The lining of cracker packages with grease-proof paper has helped greatly. However, some baked products are packaged in cardboard boxes. In such packages the fat may be absorbed rapidly by the cardboard, thereby spread over a large surface, and consequently undergoes rapid oxidation. This emphasizes the need for proper packaging to insure a good keeping quality product.

Several patents have been taken out on the impregnation of cardboard packages and wrappers with antioxidants and these are used to some extent. In certain instances these have proved helpful in retarding spoilage of the products contained, while in other instances they have been ineffective.

The use of colored glass to cut out the blue and ultraviolet rays of light was suggested by Burr (1907) as a means of protecting dairy products from oxidative deterioration. This has led to the development and effective use of colored cellophanes for packaging food products that will be exposed to light when merchandized.

Summary. In summarizing, it would appear that effects on the stability of cereal products to oxidative deterioration by formula components as well as methods of processing, are not understood to the degree that they should be and that there is a great need for studies along these lines.

REFERENCES

1. Bohn, R. M., and Olson, R. S., Oil and Soap *11*, 210 (1934).
2. Burr, A., Illust. Landw. Ztg. *27*, 274 (Abst. in Chem. Abs. 1, 1879, 1907).
3. Carlin, G. T., and Lannerud, E., Oil and Soap *18*, 60 (1941).
4. Elder, L. W., Jr., Oil and Soap *18*, 38 (1941).
5. Fine, M. S., and Olsen, A. G., Ind. Eng. Chem. *20*, 652 (1928).
6. Higgins, J. W., and Black, H. C., Oil and Soap *21*, 277 (1944).
7. Holm, G. E., and Greenbank, G. R., Ind. Eng. Chem. *16*, 595 (1924).
8. Lundberg, W. O., Halvorson, H. O., and Burr, G. O., Oil and Soap *21*, 33 (1944).
9. McKinney, R. H., and Bailey, A. E., Oil and Soap *18*, 147 (1941).
10. Triebold, H. O., Cereal Chem. *8*, 518 (1931).
11. Triebold, H. O., and Bailey, C. H., Cereal Chem. *9*, 50 (1932-a).
12. Triebold, H. O., and Bailey, C. H., Cereal Chem. *9*, 91 (1932-b).
13. Triebold, H. O., Webb, R. E., and Rudy, W. J., Cereal Chem. *10*, 263 (1933).
14. Triebold, H. O., Food Ind. *10*, 71 (1938).
15. Wilder, H. K., and Lindow, C. W., U. S. Patent 2,093,260 (Sept. 14, 1937).

Toxicity of Rancid Fats*

F. W. QUACKENBUSH
Purdue University, Lafayette, Ind.

THE scientific literature contains a number of reports of malnutrition resulting from rancid dietary fat. The symptoms include ophthalmia, gastric papillomatosis, and other digestive disturbances, reproductive failure, anemia, dermatitis, and cancer. In some cases the symptoms have been attributable to known deficiencies. Some others are not so readily explained even in terms of present knowledge. Whether rancid fats exert a direct toxic action is also uncertain.

First, I should like to discuss briefly some effects which oxidized fats are known to produce through the oxidation of dietary essentials, especially the vitamins. The five fat-soluble dietary essentials, linoleic acid, tocopherol, vitamins A, D and K, are susceptible, though not equally so, to oxidation. Tocopherol and vitamin A are the least resistant, and their oxidation is accelerated by the peroxides of linoleic acid. It has long been recognized that dry foods or food materials containing them lose their potency on continued exposure to air. Recently it has been shown (1, 2) that even when these materials are fed in fresh condition, they may lose potency in the digestive tract before absorption. Carotene fed in linoleic ester was found ineffective unless fed with tocopherol. Undoubtedly, the tocopherol itself was partially inactivated in protecting the carotene from oxidation. Water-soluble antioxidants such as hydroquinone were ineffective in the tract.

The loss of dietary essentials through oxidation by rancid fat is not confined to those which are fat-soluble. Some of the water-soluble vitamins are known to be inactivated as well. Biotin has been shown to be destroyed by oxidized fat (3), and ascorbic acid may be oxidized to some extent in performing its synergistic role with antioxidants of the phenolic type (4). Not nearly enough work has been done to establish the effects of rancid fat on other B-vitamins, either directly or through an influence on the microflora which produce vitamins in the intestinal tract.

So much for effects on known dietary essentials. The symptoms which result from the individual deficiencies are well known, and many of them have been observed in animals on rancid diets.

Secondly, I should like to review briefly some of the symptoms which have been observed on rancid diets but which were not readily attributable to known deficiencies. One of these is anemia. Gyorgi et al. (5) fed rats a synthetic diet containing thiamin, riboflavin, pyridoxine, pantothenic acid, and 16% of crude linoleic acid. The rats lost weight, developed an anemia of the secondary type, and a leucopenia. The condition was prevented by the addition of yeast to the diet.

Burr and Barnes (6) fed a synthetic diet containing lard, cod liver oil, and wheat germ oil and found that rats lost weight and died. Even when the cod liver oil was fed separately from the rancid lard diet the animals lost weight and became anemic, and their white and red blood cell count fell. Vitamin A deficiency was ruled out since the livers of these animals contained normal amounts. Yeast prevented the anemia. Since yeast also prevented the development of rancidity in the diet, its effect was attributed to its antioxidant activity. It was suggested that the rancid fat exerted a direct toxic action. A study of the relationship to certain B-vitamins might be worthwhile in this connection.

Dermatitis is another symptom sometimes reported to occur on rancid diets. Whipple (7) fed four dogs a diet containing 25% rancid lard and produced an "oxidized fat syndrome." The symptoms were loss of hair, skin lesions, emaciation, intestinal hemorrhage, and death. Three dogs on fresh fat all remained normal. Since the dermal symptoms resembled those of linoleic acid deficiency in rats, the probable cause was believed to be destruction of the unsaturated fat linkages. Similar symptoms were subsequently (8) produced in rats on a low fat diet. Ether-extracted yeast was fed as a separate supplement. The rats which were fed .10 cc. daily of oxidized lard (per. no. 15 to 20) lost weight, lost hair, and developed scaly legs and later edematous swelling of lips and paws. The symptoms were similar to those later described as acrodynia. Increasing the lard supplement to .20 cc. did not cure but hastened death. On the other hand, increasing the yeast supplement caused the animals to resume their previous rate of growth.

In our studies (9) on acrodynia, several highly oxidized fats (per. no. 300 to 400) were tested for curative properties. The only B-vitamins contained in the basal diet were thiamin and riboflavin. Ten mg. daily of linoleic ester effected permanent cures. Twenty mg. of oxidized wheat germ oil or corn oil likewise effected prompt cures; however, many of these were followed by relapse and death two or three weeks later. Increased supplements of rancid fat intensified the symptoms and hastened death. In view of these observations and the comparatively low degree of oxidation of the lard used by Whipple, it seems doubtful that linoleic deficiency caused the death of her dogs and rats. Apparently the rancid lard exerted some other effects.

Some attention has been given to reproductive failure as one of the symptoms caused by rancid dietary fat. Kudrjashov (10) reported a direct toxic effect on the fetus during pregnancy and in some cases the prevention of implantation of the fertilized egg. Pregnant rats fed rancid fats resorbed their fetuses on the 6th to 9th day, not because of vitamin E deficiency but because of toxic decomposition products in the fat. These were believed to be higher aldehydes and ketones. Others (11) have reported degenerative changes in the testicles of rats which were fed 10 to 25% rancid fat in addition to a diet containing ample vitamin E. On the other hand, Mattill and his coworkers (12) found that rancid fats and their degradation products did not interrupt pregnancy in rats. Litters were born normally unless the doses were large enough to produce systemic intoxication in the mother. However, mortality of the young was high.

Finally, mention should be made of the alleged carcinogenic properties of rancid fats. Roffo (13) has reported that olive oil or animal fats which had been oxidized by heating tended to produce cancer when fed to rats. He pointed out that the spectral characteristics of the heated fats resembled those of carcinogenic phenathrene derivatives. Lavik and Bauman (14) found that high fat diets increased tumor formation on the skin of rats which had been painted with methyl cholanthrene. The action of fat was increased by heating one hour at 300° C. Prolonged heating had no further effect. The carcinogenic effect of fats was found to be at least partly due to an increased caloric intake. Apparently it was not related to rancidity since fats rancidified by treatment with ultraviolet light or copper oleate were not more carcinogenic than fresh fats.

In summary, it is evident that one of the chief adverse effects of rancid fat is the destruction of vitamins and other dietary essentials. However, some symptoms which have been observed and confirmed are not readily explained in such terms. These include certain types of anemia, dermatitis, and reproductive failure. It is probable that further work will provide additional specific instances in which rancid fat exerts its effect through inactivation of dietary essentials. However, until such experimental evidence provides the full explanation, it must be assumed that rancid fats are able to exert a direct toxic effect.

REFERENCES

1. Quackenbush, F. W., Cox, R. P., and Steenbock, H.—J. Biol. Chem. *145*, 169 (1942); *140*, civ (1941).
2. Sherman, W. C.—J. Nutrition *22*, 153 (1941).
3. Pavcek, P. L., and Shull, G. M.—J. Biol. Chem. *146*, 351 (1942).
4. Columbic, C., and Mattill, H. A.—J. Am. Chem. Soc. *63*, 1279 (1941).
5. Gyorgi, P., Tomarelli, R., Ostergard, R. P., and Brown, J. B.—J. Exp. Med. *76*, 413 (1942).
6. Burr, G. O., and Barnes, R. H.—Physiol. Rev. *23*, 256 (1943).
7. Whipple, D. V.—Proc. Soc. Exp. Biol. N. Y. *30*, 319 (1932).
8. Whipple, D. V.—Oil and Soap *10*, 228 (1933).

* Presented at the Conference on Problems Related to Fat Deterioration in Foods, under the auspices of Committee on Food Research, Research and Development Branch, Military Training Division, Office of the Quartermaster General, in Washington, June 1945.

9. Quackenbush, F. W., Steenbock, H., Kummerow, F. A., and Platz, B. R.—J. Nutrition *24*, 225 (1943).
Quackenbush, F. W. and Cox, R. P.—Unpublished.
10. Rudriashov, B. A.—Arch. Exp. Path. Pharm. *169*, 275 (1933).
11. Kolodziejska, Z., and Duszynska, J.—Acta Biol. Exptl. (Warsaw) *13*, 10 (1939). Chem. Abs. *36*, 7085.
12. Deatherage, F. E., McConnell, K. P., and Mattill, H. A.—Proc. Soc. Exp. Biol. Med. *46*, 399 (1941).
13. Roffo, A. H.—Prensa méd. argent. *26*, 619 (1939). Chem. Abs. *37*, 2055.
14. Lavik, P. S., and Baumann, C. A.—Cancer Research *1*, 161 (1941), *ibid. 3*, 749 (1943).

A Volumetric and a Weighing Method for Measuring Semi-Micro Oil Samples*

RUSSELL O. SINNHUBER
Seafoods Laboratory, Oregon Agricultural Experiment Station
and
J. G. RUGGLES
Bioproducts, Oregon Ltd.
Astoria, Oregon

DURING several years of spectrophotometric vitamin A assay and plant testing of the liver reduction process two methods for measuring semi-micro oil samples have been developed which have served almost all laboratory and plant requirements. The volumetric procedure described below enables the analyst rapidly to test vitamin A bearing oils for routine control work. The volumetric method also has been of value in conducting vitamin A stability tests on oils during storage. The weighing procedure described has been used as an accurate and rapid method in the quantitative estimation of vitamin A in fish liver oils and concentrates.

Volumetric Method: This procedure is suitable for routine use in vitamin A liver processing plants. Blood pipettes commonly in use for red and white cell counts are employed. The red cell type holds approximately 9 mg. of oil and the white cell type approximately 26 mg. of oil. The pipettes are standardized by weighing them filled to the mark below the bulb with the oil commonly being analyzed. In the assaying procedure the pipette is filled to the mark with the oil, and the tip is wiped carefully with a cleansing tissue or towel. The filled pipette is then attached to a siphon containing the desired solvent, and the sample is washed thoroughly into a volumetric flask. The pipette may then be cleaned with petroleum ether and dried with suction. Acetone should not be used since it may dissolve the mixing bead.

Weighing Method: In vitamin A assay, a simple method of weighing small oil samples is that of using a micro cover glass. The square cover slip, which has been cut into halves or thirds by using the edge of a carborundum pencil or stone, is suitable as long as it will easily slip through the neck of the volumetric flask. The cut cover slip will weigh approximately 100 mg. With the aid of a small glass rod, samples of oil from 10 to 30 mgs. may be transferred to the slip and weighed. With forceps the slip with oil is dropped into a volumetric flask, which contains a small amount of suitable solvent. The flask is swirled for a few seconds until all visible oil is dissolved then made up to volume and mixed. This technique eliminates the washing error that may be inherent in some oil weighing methods since fish liver oils frequently vary as regards their solubility, e.g. in isopropanol. The error involved in using the cover slip procedure is roughly plus 0.04% in 100 ml. and is of little concern in the present vitamin A assay.

* * *

THE foregoing note by Sinnhuber and Ruggles was shown before publication to the members of the Vitamin Committee of the American Oil Chemists' Society, and two of them made further suggestions.

For field use, when samples of 100 to 500 mg. are needed, we have satisfactorily employed a calibrated 0.5 cc. tuberculin syringe. The conventional weighing of a sample of a vitamin A oil in a volumetric flask can be somewhat simplified by adjusting the length of brass jack chain (used to secure the stopper to the volumetric flask used for the primary dilution) so that the tare is always within + 100 mg. of an even gram. This permits the tare to be obtained on a chainomatic balance without the use of the weight on the notched beam and simplifies somewhat the manipulation and arithmetic involved. A small card in the balance case giving flask numbers and tare weights also facilitates the weighing of the sample.

T. D. SANFORD
F. E. Booth Company, Inc.
San Francisco 11, California

* * *

A useful and accurate method for weighing semi-micro samples is the following, which was suggested by Dr. K. C. D. Hickman in 1935, and has since been used for rapid tests as well as exact control work:

The carrier for the oil is a light gauge nichrome wire about three inches long bearing a loop on one end and on the other a helix consisting of five or six turns 1/8" in diameter and spaced about 1/64" apart. The coil is dipped into the oil sample to pick up a 20 to 40 mg. drop. To be weighed, the carrier is then hung by the loop on a balance. The drop of oil is rinsed off into the volumetric flask, or the whole carrier may be dropped in before the flask is filled with solvent. Very rapid weighings can be made with the use of a Roller-Smith 125 mg. torsion balance with counterweight.

NORRIS EMBREE
Distillation Products, Inc.
Rochester 13, New York

*Published as Technical Paper No. 467 with the approval of the Director of the Oregon Agricultural Experiment Station. Contribution of the Department of Food Industries through the Seafoods Laboratory.

Abstracts

Oils and Fats

Edited by
M. M. PISHUR and SARAH HICKS

SOLVENT EXTRACTION OF COTTONSEED AND PEANUT OILS. BOILING POINT-VAPOR PRESSURE-COMPOSITION RELATIONS FOR MISCELLAS OF OILS IN HEXANE. E. F. Pollard, H. L. E. Vix and E. A. Gastrock. *Ind. Eng. Chem. 37*, 1022-6 (1945). The boiling points and densities of mixtures of cottonseed and peanut oils with commercial hexane are reported. They are useful in the design of vacuum evaporators and strippers and for control operations involving temperature, time of heating, and concentration of oil-solvent mixtures of various compositions, to prevent or minimize fixation of objectionable coloring matter or other deteriorative heat effects.

THE DETERMINATION OF FAT IN MIXTURES CONTAINING FATTY ACIDS, AND THE DETERMINATION OF UNSAPONIFIABLE MATTER IN OILS AND FATS. N. D. Sylvester, A. N. Ainsworth and E. B. Hughes. *Analyst 70*, 295-8 (1945). The method depends on adsorbing the free fat acids on Al_2O_3 in an adsorption column. With oils free fat acids are removed from glycerides and unsaponifiable. When the glycerides are saponified, and the acid freed with acid, the acids can be removed from the unsaponifiable by the process.

SPECTROPHOTOMETRIC STUDIES OF THE OXIDATION OF FATS. V. THE COLOR OF OXIDIZED FATS IN ALCOHOLIC ALKALI. R. T. Holman, W. O. Lundberg and G. O. Burr. *J. Am. Chem. Soc. 67*, 1669-72 (1945). The ultraviolet absorption spectra of diacetyl, acetylpropionyl, 9,10-diketostearic acid, *p*-xyloquinone, duroquinone, diduroquinone, chroman-5,6-quinone, and rancid lard have been determined and compared with their spectra in alkaline solution. Diketostearic acid in alkali may give rise to a quinone homologous to duroquinone. The alcoholic alkali color of rancid fats is probably not due to the formation of *p*-quinones from α-dicarbonyl compounds formed during the oxidation of the fat. The alcoholic alkali color is only to a very small extent due to chroman-5,6-quinone derived from tocopherol or its degradation products in alkali. The alcoholic alkali color may to an appreciable extent be due to compounds derived from the unsaturated fatty acids which are closely related to the compounds obtained from chroman-5,6-quinone by treatment with alkali. The alcoholic alkali color may largely result from other unsaturated carbonyl compounds which are oxidation products of unsaturated fatty acids.

FLAVOR REVERSION IN HYDROGENATED LINSEED OIL. II. EFFECT OF VARIATIONS IN PROCESSING PROCEDURES. H. W. Lemon, A. Lips and W. H. White. *Can. J. Res. 23F*, 295-303 (1945). Shortenings prepared from various linseed oils by different methods were stored at 43.3° and sampled at 8-week intervals for 56 weeks. Storage life in terms of flavor reversion was not highly correlated with mean peroxide value or unsaturation. All shortenings were resistant to normal oxidation, but unstable to reversion unless hydrogenated to a very low I number. Hot and cold pressed oils yielded products equally susceptible to flavor reversion. Changes in stability attributable to variations in methods of alkali refining, bleaching, and hydrogenation were only minor. High alkali concentrations (30-40° Bé) were beneficial, while hydrogenation at 190° was preferable to hardening at lower temperatures (140° and 115°). Blending with other vegetable oils, or the use of hydroquinone or a wheat-germ oil preparation as antioxidants, slightly retarded the onset of reversion. None of the laboratory or commercially prepared samples examined was considered to be a satisfactory stable product.

CORROSION OF LEAD BY OXIDIZING AGENTS AND LAURIC ACID IN HYDROCARBON SOLVENTS. C. F. Prutton, D. Turnbull and D. R. Frey. *Ind. Eng. Chem. 37*, 917-24 (1945). Corrosion of lead by an acyl peroxide is independent of acid concentration down to concentrations as low as 10^{-3} mole of acid per liter and is determined only by peroxide concentration. Detailed studies of the mechanism of reaction of quinone and lauric acid with Pb in benzene at 70° showed that at reactant concentrations below 0.05 mole per liter the reaction is second order, and the rate is proportional to the concentration of both quinone and lauric acid. Further, Pb laurate had a pronounced catalytic effect upon the rate of this reaction. For hydroperoxides and O_2 it is probable that $Pb(OH)_2$ or PbO is intermediate in the corrosion process. At low temperatures insoluble soap films formed on the Pb surface are very effective in slowing the corrosion rate. A film formed slowly appears to be more protective than one formed rapidly.

HYDROXYLATION OF MONOUNSATURATED FATTY MATERIALS WITH HYDROGEN PEROXIDE. D. Swern, G. N. Billen, T. W. Findley and J. T. Scanlan. *J. Am. Chem. Soc. 67*, 1786-9 (1945). A new and rapid general reaction for the quantitative hydroxylation of long-chain, monounsaturated, aliphatic compounds has been described. The oxidizing agent, performic acid, is not isolated but is prepared and utilized in situ. This is accomplished by dissolving the unsaturated compounds in HCOOH and adding H_2O_2. Because of the rapidity of the reaction and the mild conditions, only one mole of H_2O_2 is required for each mole of monounsaturated compound. This reaction has been applied to pure oleic, elaidic, and hendecenoic (undecylenic) acids, oleyl alcohol, and Me ricinoleate to give excellent yields of the corresponding hydroxy derivatives. In addition, it has been shown that substantially identical results are obtained when HOAc-containing catalytic quantities of H_2SO_4 is substituted for HCOOH in the mixture with H_2O_2. The oxidizing agent in the case is peracetic acid. Either of the 2 hydroxylation methods described should be suitable for the industrial production of hydroxylated fatty acids and related compounds.

TALL OIL ESTERS AS PLASTICIZERS FOR GR-S. W. I. Harber and C. S. Yoran. *Ind. Eng. Chem. 37*, 953-6 (1945). Tall oil is a rich source of resin and fatty acids. Previous work has shown that this material exerts a plasticizing effect on GR-S. The structure of tall oil was modified by esterification with alcohols. Most interesting were those esters derived from hydroxy compounds related to GR-S unit structure. They were superior to tall oil in rate of incorporation

SUMMARY

Experiments were conducted with young male albino rats to determine: (1) whether lamb protein is deficient in cystine, methionine, leucine, isoleucine, phenylalanine, tryptophan or valine at the 7.5% protein level; and (2) the biological value of the protein in different cuts of lamb at 4 levels of intake.

The results of these experiments indicate that the protein from the entire carcass of lamb is deficient only in cystine or methionine. The addition of the same quantity of either amino acid to the diet was equally effective in promoting much more rapid growth and better utilization of the lamb protein.

When the protein in the leg, shoulder, and entire carcass of lamb was fed at the 7.5, 10.0, 12.5, and 15.0% levels of intake, it was found in one experiment, at the first three protein levels, that the protein in the entire carcass was superior in biological value to that in the leg. In a second experiment with another lot of lambs, when protein constituted 12.5 and 15.0% of the diets, practically the same biological values were obtained for the three cuts of lamb at each protein level.

LITERATURE CITED

HOAGLAND, R., N. R. ELLIS, O. G. HANKINS AND G. G. SNIDER 1948 Supplemental value of certain amino acids for beef protein. J. Nutrition, *35:* 167.

HOAGLAND, R., AND G. G. SNIDER 1926 Nutritive value of the protein in voluntary muscle, heart, liver and kidney from cattle, sheep and hogs. J. Agr. Res., *32:* 1025.

———— 1946 Nutritive value of protein in dehydrated meat. Food Res., *11:* 494.

SCHWEIGERT, B. S., B. T. GUTHNECK, H. R. KRAYBILL AND D. A. GREENWOOD 1949 The amino acid composition of pork and lamb cuts. J. Biol. Chem., *180:* 1077.

U. S. DEPARTMENT OF AGRICULTURE, BUREAU OF AGRICULTURAL ECONOMICS 1950 The National Food Situation. N. F. S. 53, July-September.

THE EFFECT OF HEAT TREATMENT ON THE NUTRITIONAL VALUE OF SOME VEGETABLE OILS

EARLE W. CRAMPTON, FLORENCE A. FARMER AND F. MARION BERRYHILL[1]

Department of Nutrition, Macdonald College, McGill University, Province of Quebec, Canada

(Received for publication October 16, 1950)

INTRODUCTION

Oil-seed crops are becoming increasingly important to Canadian economy. While the meal plays a significant role in livestock feeding, the oils pressed from seeds have been adapted to many uses. Of current concern is the manufacture of vegetable oils into edible products such as margarine and salad oils.

Because certain of the more highly unsaturated vegetable oils develop undesirable odors and flavors on exposure to heat or light, their use as edible products was hindered. In 1945 Privett and his co-workers showed thermal polymerization to be a simple and yet satisfactory method for rendering such vegetable oils less susceptible to this "flavor reversion." Polymerization has reportedly been used in the processing of herring oil for human consumption in the United States (Lassen et al., '49), and in Norway (Schwitzer, '48) to replace olive, cotton seed and groundnut oils with herring oil for canning, cooking and to some extent as a table and salad oil.

However, when linseed oil prepared by the method of Privett et al. ('45) was fed in 28-day tests to rats in this laboratory (Crampton and Millar, '46) a high incidence of death occurred.

[1] Contribution from the Faculty of Agriculture, McGill University, Macdonald College, Que., Canada. Journal Series No. 276.

Issued as paper no. 257 of the Canadian Committee on Food Preservation.

Because unheated linseed oil is known to be wholesome and nutritious (Molotkow, '32), it was suspected that heat polymerization had somehow deleteriously affected the nutritional value of that oil.

Reports in the literature of adverse affects on fats of severe heat are scanty. Roffo ('44) found that rats fed sunflower seed and olive oils heated to 350°C. for 30 minutes developed stomach tumors which became cancerous over a period of 12 to 30 months. Although no other published reports confirm these observations, Morris et al. ('43) noted growth failure and weight loss in rats fed diets containing 50% lard which had been heated at 300°C. for 120 minutes. Accompanying these effects on growth, Morris observed in some animals a paralysis similar to that produced in rats fed a vitamin E-deficient diet by Mackenzie et al. ('40); also some cirrhosis of the liver and chronic gastric ulcers. However, both these groups of workers used fat heated in air, where oxidation may have been an important factor, whereas Privett's polymerization is conducted in an atmosphere of inert gas.

In 1947, Harris fed rats fish oils heated at 280°C. under a vacuum of 10 mm of mercury for 8 to 12 hours and observed symptoms of retarded growth and general ill-health.

Subsequent to our original findings, a series of experiments was conducted, the most pertinent of which are reported herein, to investigate further the influence of heat on the nutritiousness of herring oil, linseed oil and other vegetable oils which are potentially important to the food industry.

EXPERIMENTAL PROCEDURE

The general plan of each of the tests in this study involved feeding to young growing rats basal diets in which one or the other of the several fats, subjected to heats of varying duration, was incorporated at levels of 10% or 20% by weight. Fats examined in the tests reported include linseed, soybean, rapeseed, corn, peanut, and herring oils.

The vegetable oils were heated in a salt bath at 275°C. for the desired time in all-glass apparatus. Carbon dioxide was blown through the oil continuously to exclude air and to remove volatile decomposition products thoroughly. The herring oil was a caustic soda-refined, bleached, deodorized product of a process reportedly used in Norway (Schwitzer, '48), involving heating while passing through steam at 280°C. under a vacuum of 10 mm of mercury for 10 hours.

The effect of the heat treatment on the nutritive value of the oils was measured by the growth rates of the rats, their feed consumption, and their general conditions of health and thrift as compared to those of animals fed identical basal diets but containing the unheated oil. Post mortem examination of the viscera was performed on all rats failing to survive the planned feeding period and on representatives of the groups at the close of the test.

Equal numbers of male and female white rats of approximately 28 days of age were allotted at random within sexes to the test groups, which ranged in size from 6 to 16 animals per lot. Thus each lot contained equal numbers of male and female animals. They were housed in individual wire-bottom cages, and were allowed feed and water ad libitum throughout each 28-day test period. Weekly weight gain and feed consumption for each rat were recorded.

Diets

The percentage compositions of the three basal diets used are shown in table 1.

It will be noted that diets I and III differed only in the level of fat, which varied by weight at the expense of the flour. Diet II was a modification of diet I through the addition of yeast in place of the thiamine, riboflavin and niacin. The level of protein in the basal diets was such that it supplied between 20% and 24% of the Calories.

In preparation for feeding, the oils were added to the thoroughly mixed dry ingredients and the whole baked at 375°F.

for approximately 20 minutes. The resulting biscuit was then granulated and air-dried for 24 hours, after which the supplements were added. Diets were refrigerator-stored until the beginning of the test period, during which time they were held at room temperature.

In addition to the diet, weekly doses of cod liver oil were administered orally to supply 175 I.U. of vitamin A and 35 I.U. of vitamin D.

TABLE 1

Percentage compostion of the basal diets

INGREDIENTS	I	II	III
White flour	47.0	44.0	57.0
Casein	11.5	11.5	11.5
Milk powder	19.0	19.0	19.0
Oil	20.0	20.0	10.0
Bone meal	2.0	2.0	2.0
Salt	0.5	0.5	0.5
Yeast [1]	..	3.0	..
Thiamine [1]	10 p.p.m.	..	10 p.p.m.
Niacin [1]	230 p.p.m.	..	230 p.p.m.
Riboflavin [1]	5 p.p.m.	..	5 p.p.m.
Protein in the diet (%)	20.4	20.4	24.3

[1] Added after baking.

RESULTS OF FEEDING TRIALS

Clinical observations

Symptoms of defective diet were most pronounced in those animals fed polymerized linseed oil, and the severity of the symptoms varied with the amount of oil incorporated in the diet. However, animals fed any of the polymerized oils, and whose weight gains were significantly lower than those of their controls, excreted dark, sticky feces. Lassen et al. ('49) noted abnormal feces from adult rats fed polymerized sardine oil as 5% of the diet. In our tests, in addition to the sticky fecal residue, animals fed polymerized oil as 20% of the diet showed oily, matted coats.

Growth and feed consumption

In examination of the results which follow it must be understood that the data presented were collected from a series of individual tests. Control groups of animals fed the appropriate unheated oils were carried in each experiment, and quantitative comparisons of the data shown in these tables are strictly valid only within tests.

TABLE 2

Summary of data showing the effects of heat treatment of oils as measured by daily gains of rats, feed intakes, and gains per 1,000 Calories eaten

TEST AND OIL USED	DIET NO.	OIL IN DIET	DURATION OF HEAT TREATMENT AT 275°C.	NO. OF ANIMALS	AVE. DAILY GAIN	AVE. DAILY FEED INTAKE	GAIN/1,000 CALORIES
		%	hrs.		gm	gm	gm
Linseed	III	10	nil	10	3.9	9.6	91
		10	2	10	3.8	9.6	91
		10	4	10	3.4 [1]	9.5	80
		10	8	10	2.5 [2]	8.1	69
Rapeseed	III	10	nil	10	3.4	10.0	76
		10	15	10	2.9	9.5	69
		10	30	10	2.3 [1]	7.8	64
Corn	III	10	nil	12	3.6	10.4	78
		10	15	12	2.8	8.7	71
		10	30	12	2.3 [1]	8.0	64
Peanut	III	10	nil	12	3.6	9.3	87
		10	15	12	3.6	10.0	80
		10	30	12	0.8 [1]	5.2	30
Soybean	II	20	nil	10	4.6	10.2	90
		20	3	10	4.0	9.4	85
		20	6	10	3.3 [1]	8.3	80
		20	9	10	2.9 [1]	7.7	76
Herring	I	20	nil	16	2.6	8.1	64
		20	10	16	0.89 [1]	6.3	28

[1] Weight gains significantly lower than those of control animals fed the unheated oil.

[2] Weight gains significantly lower than those of animals fed linseed oil heated only 4 hours.

In table 2 is presented a summary of the average daily gains and feed intakes of animals fed in 6 different tests covering a series of potentially edible oils.

It should be noted that the duration of heating stated is not necessarily the earliest point at which the nutritional value of the oil is damaged. Our experiments, for example, have shown linseed oil heated two hours at polymerizing temperatures, and soybean oil heated three hours, to be as nutritious as the unheated control oils. The intermediate periods between 15 and 30 hours for rapeseed, corn, and peanut oils have not been tested, nor the periods up to 10 hours with herring oil.

TABLE 3

Effect of 10% vs. 20% of heated oil in the diets as measured by daily gains and feed intake ratios of rats

TEST AND OIL FED	OIL IN DIET	DURATION OF HEAT TREATMENT	NO. OF ANIMALS	AVE. DAILY GAIN	AVE. DAILY FEED INTAKE	GAIN/1,000 CALORIES
	%	hrs.		gm	gm	gm
Linseed	20	nil	10	3.7	10.1	80
	10	12	10	2.3[1]	8.2	72
	20	12	10	−0.1[2]	6.0	4
Soybean	10	nil	10	3.7	10.3	92
	10	6	10	3.5	10.4	87
Soybean	20	nil	10	4.6	10.2	90
	20	6	10	3.3[1]	8.3	76

[1] Weight gain significantly lower than that of group fed unheated oil.
[2] Weight gain significantly inferior to that of group fed heated oil as 10% of the diet.

That the duration of heating is a factor in the extent of the damage in linseed and soybean oils is clearly indicated by the progressive decrease in weight gains as the heating period was prolonged.

The data presented in table 3 indicate that the diet becomes progressively poorer as the proportion of heated oil in it increases.

When linseed oil, heated 12 hours, was fed as 10% of the diet, the growth resulting over a 4-week period was significantly lower than that of animals fed unheated oil. When the heated oil was included in the diet as 20% by weight, the final

weight of the group was even lower than the starting weight, showing extreme growth inhibition.

When soybean oil was fed as 10% of the diet, oil heated 6 hours appeared as nutritious as the unheated oil. However, when oil treated in the same manner was fed as 20% of the diet, significantly lower gains resulted.

While the results from this series of trials leave no doubt that heat polymerization does adversely affect the nutritive value of the oils examined, the mechanism of this damage was not elucidated. Digestion studies rule out incomplete absorption as a causal factor. It can be postulated that growth inhibition was either the result of some factor in the heated oil toxic to the animals, or that it was a consequence of the destruction by abnormal products of fat polymerization of some necessary dietary component other than fat. To differentiate between these two possibilities as a guide to further investigations, the effect of feeding polymerized linseed oil by dropper was compared to that of feeding the oil mixed into the diet.

Animals fed the oil by dropper were given free access for 16 hours daily to diet III minus the fat. Subsequently, and following three hours of fasting, an amount of oil was administered equal to 10% of the fat-free diet consumed during the preceding 16-hour feeding period. Five hours then elapsed before access to the diet was again permitted.

The results of a 28-day feeding trial conducted in this manner are summarized in table 4.

The method of oil feeding had no effect on the growth of animals receiving unheated oil. However, the growth of animals fed the heated oil by dropper appeared superior to that of animals receiving the heated oil as part of the diet mixture, perhaps because the oil-free diet was more acceptable than the mixture containing the heated fat. However, comparison of gains made in relation to equal caloric intake shows that feed utilization was equally poor in the diets in which heated oil was included, regardless of the method of oil feeding.

We are not prepared at this point to explain why appetite was depressed only when the heated oil was a diet component. However, the observation that growth was depressed even when the oil was fed apart from the rest of the diet indicates that the deleterious effects exerted by polymerized oils were not incurred through the destruction of some essential non-lipid diet component, but through some oil constituent that acted in a toxic manner directly upon the animals' metabolic pools. This is in accord with the previous observations that the growth depression was intensified as the level of polymerized oil in the diet was increased, and also as the time of heating was lengthened.

TABLE 4

Summary of daily gains, feed intake and gains per 1,000 Calories eaten of animals fed polymerized linseed oil by dropper and as a dietary component

TREATMENT OF OIL	METHOD OF OIL FEEDING	NO. OF ANIMALS	AVE. DAILY GAIN	AVE. DAILY FEED INTAKE	GAIN/1,000 CALORIES
			gm	*gm*	*gm*
Unheated	As a diet component	8	4.2	12.4	87
Unheated	by dropper	8	4.1	12.5	85
Heated 12 hrs.	As a diet component	8	2.7	9.7	71
Heated 12 hrs.	by dropper	8	3.4	12.4	71

It appears logical at this time, therefore, to conclude that the toxic material is some product of polymerization and that its development is related to the unsaturation of the oil. Linseed is the most highly unsaturated of all the oils tested and was the most rapidly susceptible to nutritive damage by heating.

A series of nutritional studies of various fractions of polymerized oils designed to identify the toxic substance or substances will be reported in a subsequent paper.

CONCLUSIONS

From the data reported herein, the following conclusions are warranted:

1. Ingestion of certain oils heat-polymerized at 275°C. has been shown to depress rat growth and the efficiency of feed utilization. This reflects a lowered nutritive value of those oils which varies in severity with the degree of unsaturation of the oil, the length of time of heating each oil, and the level at which the heated oil is incorporated in the diet.

2. The factor or factors responsible for these adverse nutritional effects appear to be present in the oil itself, and act in a manner directly toxic to the animal.

ACKNOWLEDGMENTS

The authors are indebted to a number of persons not connected with this Department, whose collaboration facilitated the performance of this series of experiments.

Financial assistance was received from the Committee on Food Preservation of the National Research Council, Ottawa, Canada, through their Sub-Committee on Edible Fats and Oils. Thanks are due to Dr. N. H. Grace and Dr. H. J. Lips of this latter Committee and to Mr. H. W. Lemon of the Ontario Research Foundation for providing and processing certain of the oils used.

The soybean oil was prepared and contributed through the courtesy of Dr. W. D. McFarlane, Canadian Breweries, whose personal interest in these studies has been of much help.

Special chemical facilities were made available by Dr. R. H. Common, Department of Chemistry, Macdonald College, who also assisted in the planning and interpretation of these studies.

LITERATURE CITED

CRAMPTON, E. W., AND J. MILLAR 1946 Studies on the utilization of different types of shortening—linseed oil, rapeseed oil, lard, and a commercially prepared peanut oil. Unpublished data.

HARRIS, P. 1947 Unpublished information by letter from the author.

LASSEN, S., E. K. BACON AND H. J. DUNN 1949 The digestibility of polymerized oils. Arch. Biochem., *23:* 1-7.

MACKENZIE, C. G., J. B. MACKENZIE AND E. V. MCCOLLUM 1940 Occurrence of tremors and incoordination in vitamin E-deficient adult rats. Proc. Soc. Exp. Biol. Med., *44:* 95.

MOLOTKOW, G. W. 1932 Experimental morphology with single and repeated doses of linseed oil. Frankfurter Zschr. Path., *44:* 292. Cited in: Nutrition Abstr. and Rev., *2:* 797.

MORRIS, H. P., C. D. LARSEN AND J. W. LIPPINCOTT 1943 Effects of feeding heated lard to rats. Histological description of lesions produced. J. Natl. Cancer Inst., *4:* 285. Cited in: Nutr. Abstr. and Rev., *15:* 718.

PRIVETT, A. S., R. B. PRINGLE AND W. D. MCFARLANE 1945 Elimination of flavor in linseed shortening by heat polymerization and solvent segregation of the oil. Oil and Soap, *22:* 287.

ROFFO, A. H. 1944 The carcinogenic action of oxidized vegetable oils. Bol. Inst. med. exp. (Buenos Aires) *21:* (64): 1-134. Cited in: Biol. Abstr., *19:* 922.

SCHWITZER, M. K. 1948 Oleagineux, *3:* 243. Information received by letter from H. J. Lips, Div. App. Biology, National Research Council, Ottawa.

THE NUTRITION OF THE MOUSE

X. STUDIES ON THE UTILIZATION OF HIGH AND MODERATELY LOW PROTEIN DIETS FOR GROWTH IN FOUR STRAINS OF MICE [1].

PAUL F. FENTON AND CLAIRE J. CARR
WITH THE TECHNICAL ASSISTANCE OF LOUISE REILLY
Department of Biology, Brown University, Providence, Rhode Island

FOUR FIGURES

(Received for publication October 20, 1950)

Tumor growth, whatever its cause, involves the deposition of new tissue and the synthesis of new protein. It may be expected to give rise to measurable alterations in the pattern of nitrogen metabolism of the host. This immediately raises the question of whether the nitrogen metabolism of a tumor-susceptible animal during the period before tumors appear differs from that of a non-susceptible one.

Mice of the highly inbred strains C_{57}, I, A and C_3H maintained in our colony seemed to be particularly suited to the investigation of this question since the first two strains show a low incidence and the last two a high incidence of spontaneous tumors. Previous observations (Fenton and Cowgill, '47; Fenton, Cowgill, Stone and Justice, '50) have shown significant strain differences to exist with respect to the requirements of certain vitamins for growth. It seemed possible that there might also be differences with respect to

[1] This investigation was supported by research grants from the American Cancer Society on the recommendation of the Committee on Growth of the National Research Council; the National Cancer Institute, U. S. Public Health Service; and the Anna Fuller Fund.

Vitamins of the B complex were obtained through the courtesy of Hoffmann-LaRoche, Inc. Folic acid was furnished by the Lederle Laboratories Division, American Cyanamid Company.

STUDIES TO DETERMINE THE NATURE OF THE DAMAGE TO THE NUTRITIVE VALUE OF SOME VEGETABLE OILS FROM HEAT TREATMENT[1, 2]

II. INVESTIGATION OF THE NUTRITIOUSNESS OF THE PRODUCTS OF THERMAL POLYMERIZATION OF LINSEED OIL

EARLE W. CRAMPTON, R. H. COMMON, FLORENCE A. FARMER, F. MARION BERRYHILL AND L. WISEBLATT

Departments of Nutrition and Chemistry, Macdonald College, McGill University, Province of Quebec, Canada

FOUR FIGURES

(Received for publication January 6, 1951)

INTRODUCTION

Heating unsaturated oils to polymerization temperatures has been advocated as a practical method of reducing or eliminating the flavor reversion of shortening produced from such oils (Privett et al., '45). However, if oils treated in this manner are included in the diets of rats, then the rate of gain, the feed intake and the feed efficiency are decreased. These effects may be due to the action of some toxic material (Crampton et al., '51).

Of the oils which have been tested in this laboratory, linseed oil has proved to be the one most susceptible to nutritive damage by heating. Accordingly, linseed oil was selected for further studies on the nature of the material responsible for the toxic effects. The results of these experiments are reported in the present paper.

[1] Contribution from the Faculty of Agriculture, McGill University, Macdonald College, Quebec, Canada. Journal Series No. 280.

[2] Issued as paper 263, Canadian Committee on Food Preservation.

Unsaturated fats yield dimeric, trimeric and higher polymeric glycerides when heated at 275°C. in the absence of oxygen. It is rather generally accepted that polymerization does not proceed to any appreciable extent beyond dimerization so far as the fatty acid radicals themselves are concerned (Privett et al., '47). The extent to which such dimerization of the fatty acid radicals may result in the presence of intrapolymeric glycerides remains controversial. Indeed, Bernstein ('48) has dismissed the possibility on theoretical grounds. Adams and Powers ('44), on the other hand, produced considerable experimental evidence in support of the view that such intrapolymers are formed in the early stages of polymerization, and that they may in turn give rise to dimeric and higher polymeric glycerides by transesterification. Experimental evidence obtained in our laboratories supports the contention that thermally polymerized linseed oil may contain considerable amounts of intrapolymeric material (see appendix, table 1). Kass ('47) studied the thermal polymerization of eleostearates, and, as well as observing normal polymerization products, described side reactions leading to production of free fatty acids, aldehdyes, hydrocarbons and acids and esters of reduced molecular weight. He found that some of the latter fragments also polymerize, and produced evidence of cyclization leading to the production of cyclic aliphatic monomers and mono- and polycyclic aromatic compounds. We have essayed to indicate in plate 1 the nature of the substances which are thought to be the main products of thermal polymerization.

At the outset we suspected that polymerized triglycerides might be mainly responsible for the lower nutritive value of heated linseed oil. Consequently we sought to obtain for feeding tests a fraction which would consist essentially of non-polymeric glycerides. Privett et al. ('47) have described the preparation from heated linseed oil of an acetone-soluble segregate which, they reported, was free from "dibasic acids and was, therefore, non-polymeric in nature." Bernstein ('48) has described the use of propanol for the segregation of monomeric from higher polymeric glycerides, such monomeric fractions being regarded by him as free from dimeric acids. In view of these reports, segregates were prepared by the use of acetone and also by the use of propanol. Our chemical data (see appendix, table 2) indicate that propanol was more effective than acetone for recovery of a non-polymeric glyceride fraction, thus supporting the findings of Bernstein in this respect. We have shown, however, that the acetone-soluble segregate of polymerized linseed oil appears to contain appreciable amounts of intrapolymeric material (see appendix, table 1). The propanol segregate does not contain dimeric or higher polymeric glycerides, but we have not examined this fraction for the presence of intrapolymers. Presumably the principal polymerized material, if any, in the propanol segregate is intrapolymeric. The acetone-soluble fraction is probably composed mainly of the molecular types indicated in plate 1, figure 2, while the propanol-soluble fraction is probably composed of those indicated in plate 1, figure 3.

It was also thought possible that the dimeric fatty acid radicals themselves might be implicated in the nutritional defects of polymerized linseed oil. Since solvent segregations offered little promise for the separation of true monomeric glycerides from intrapolymeric glycerides, it was decided to esterify the heated oil and then to separate monomeric esters from dimeric esters by distillation under vacuum. The esterification yielded the fractions indicated in plate 1, figure 4, and the succeeding distillation under vacuum achieved an essentially complete separation of polymeric material (see appendix, table 3). The distillate consisted of monomeric esters, but it may possibly have included some cyclic monomeric material. The undistillable residue consisted essentially of esters of dimeric acids, together with such sterols as were present and presumably some non-volatile products of thermal decomposition which were soluble in acetone.

EXPERIMENTAL PROCEDURE

The materials prepared for feeding comprised fractions of heated linseed oil composed largely of (a) monomeric esters, and (b) dimeric esters, as well as several fractions which contained various mixtures of monomeric and dimeric glycerides and of intrapolymers. In addition to examining biologically the fractions of the oil segregated by solvents (acetone and propanol) and by distillation of the esters of the fatty acids, crude and refined heated oils were compared to discover some indication as to whether or not the non-glyceride constituents of the oil were concerned in growth inhibition.

Polymerization and acetone segregation were carried out by the technique of Privett, McFarlane and Gass ('47). The polymerizations were based on a treatment of 12 hours at 275°C. in an all-glass apparatus while passing through it a current of carbon dioxide. A sufficient flow of carbon dioxide was maintained to keep the oil surging vigorously; attention to this detail resulted in products of excellent color and blandness and with remarkably low acid values (of the order of 0.6 to 1.0% F.F.A.). Propanol segregations were performed according to the directions of Bernstein ('48). Esterifications were carried out by interesterification in the presence of the appropriate anhydrous alcohol, using sodium hydroxide as catalyst; the oil or fraction was alkali-refined to remove F.F.A. prior to esterification. Esters were distilled at about 0.5 mm Hg absolute pressure (see appendix).

The several fractions of linseed oil, indicated in table 1, were fed to albino rats as the source of dietary fat for 28-day periods. The animals ranged from 21 to 28 days of age at the outset of the tests.

The basal diets had the composition described in table 2. Diets were baked for 20 minutes at 375°F. and the resulting biscuit granulated and air-dried for 24 hours. Vitamin B supplements, in the case of diet II; or yeast, in the case of diet III, were then added. Weekly doses of cod liver oil were

administered orally to supply 175 I.U. of vitamin A and 35 I.U. of vitamin D.

FEEDING TRIAL RESULTS

Table 1 is a summary of the weight gains and feed intakes of animals fed the various fractions of linseed oil. It is recognized that negative gains are unsatisfactory as quantitative measures of the nutritional values of rations, nor are

TABLE 1

Summary of average daily gains and average feed intakes of animals fed diets containing 20%[1] of various fractions of linseed oil

TEST NO.	OIL FRACTION	TREATMENT AT 275°C.	PRINCIPAL COMPONENTS OF FRACTION	NO. ANIMALS	AVE. DAILY GAINS	AVE. DAILY FEED INTAKE
		hrs.			*gm*	*gm*
1. A	Whole	none	Monomeric glycerides	5	3.0	6.8
B	Whole	12	Monomeric glycerides Intrapolymers Dimeric and polymeric glycerides	5	−3.0	4.6
C	Acetone-soluble	12	Monomeric glycerides Intrapolymers Dimeric glycerides	5	−3.0	5.3
2. A	Acetone-soluble, alkali-refined	12	Monomeric glycerides Intrapolymers Dimeric glycerides	6	−2.1	4.5
B	Acetone-soluble, crude	12	Monomeric glycerides Intrapolymers Dimeric glycerides Non-glyceride constituents	6	−0.7	3.5
3. A	Whole	none	Monomeric glycerides	10	3.8	10.0
B	Acetone-soluble	12	Monomeric glycerides Intrapolymers Dimeric glycerides	10	−1.4	6.0
C	Propanol-soluble	12	Monomeric glycerides Intrapolymers	10	0.2	5.5
4. A	Whole	none	Monomeric glycerides	8	3.4	9.7
B	Whole ethyl esters	none	Monomeric esters	8	2.3	7.5
C	Acetone-soluble ethyl esters	12	Monomeric and dimeric	8	0.8	5.8
D	Distillate of 4C	12	Monomeric esters	8	0.7	4.5
E	Residue of 4C	12	Dimeric esters	8	[2]	3.5

[1] Excepting test 2, where 36% fat was used.
[2] No gain could be calculated, as 6 out of 8 animals died within the first week. The remaining two survived three and 4 weeks, respectively.

statistical analyses of weight losses justified. In these trials such data are nevertheless indicative of the differences in nutritive quality of the diets studied. Where weight losses were encountered (tests 1, 2, and 3), the diets may be judged unwholesome without the benefit of statistics. However, it was possible to make an analysis of the variance of both gain and feed intake in test 4. Differences significant at the 5% point were apparent between A and B, B and C, and B and D. C and D were not different.

TABLE 2

Composition of diets

INGREDIENTS	DIET I (test 2)	DIET II (test 1)	DIET III (tests 3 and 4)
White flour (%)	26.5	47.0	44.0
Casein (%)	15.0	11.5	11.5
Milk powder (%)	20.0	19.0	19.0
Linseed oil (%)	36.0	20.0	20.0
Bone meal (%)	2.0	2.0	2.0
Salt (%)	0.5	0.5	0.5
Yeast (%)	..	..	3.0
Thiamine	[1]	10 p.p.m.	..
Niacin	[1]	230 p.p.m.	..
Riboflavin	[1]	5 p.p.m.	..

[1] Where diet I was fed (test 2), thiamine, niacin and riboflavin were administered orally to provide daily intakes of 0.1 mg, 2.3 mg, and 0.5 mg, respectively, per rat.

It is realized that purified esters are of uncertain feeding value because of their susceptibility to oxidative changes (Quackenbush et al., '42). For this reason, the effects on rat growth of esters vs. the triglycerides were compared. Animals fed esterified unheated oil gained significantly less weight than animals fed the unheated triglyceride; the feed intake of ester-containing diets decreased progressively during storage at room temperatures, indicating decreasing palatability of those diets. However, the utilization of feed for producing weight gain was not significantly different between the ester- and triglyceride-containing diets, nor could any clinical indication of the toxicity of the esters be found. Esters of heated linseed oil showed the same adverse clinical effects as did the heated triglycerides, as will be seen in table 1.

DISCUSSION

The data shown in table 1 confirm that 12 hours' heating at 275°C. impairs the nutritive value of linseed oil.

A comparison of heated, whole oil with the acetone-soluble fraction (test 1) shows both of them to be equally incapable of supporting normal growth in rats. One may conclude, therefore, that acetone does not segregate toxic from non-toxic material.

There was no difference between the crude and refined oils (test 2), suggesting that the deleterious material in polymerized oil is derived from the glyceride component.

The propanol-soluble fraction was not significantly better nutritionally than the acetone-soluble fraction (test 3). It thus follows that the glyceride polymerization is not responsible for growth inhibition by heated linseed oil. Since neither acetone nor propanol is effective in segregating out the toxic material in heated linseed oil, one naturally turns to a consideration of the intrapolymers as the offending material. An interpolymer is a triglyceride within which two fatty acid chains are dimerized, and one might suspect the dimeric fatty acid structure of being the deleterious factor in heated oil. It is the only quantitatively important component of the propanol-soluble fraction of heated oil that is not present in unheated oil. How far this surmise is correct is evident from test 4, in which the "dimeric" fraction separated from the ethyl esters of the acetone segregate of polymerized oil was fed.

This undistillable "dimeric" fraction, which may also contain minor amounts of non-fatty material, proved lethal to rats in approximately 5 days.

The animals fed the distillable fraction (i.e., monomeric esters), although they did not grow, were thrifty in appear-

ance and excreted well-formed feces. Those fed the undistillable residue had oily, matted coats and excreted dark, sticky feces. This condition was also noted, but to a lesser degree, with the rats fed the mixed monomeric plus dimeric esters, and had also been recorded in previous tests in which the acetone-soluble fraction of heated oil had been fed. It appears possible, therefore, that the explanation of the failure to gain in weight on a diet containing the monomeric esters may be the absence of an essential unsaturated fatty acid, presumably because these more reactive acids had become polymerized or isomerized during heating. Thus dimerization may not only remove essential fatty acids, but produce toxic radicals as well. The strong adverse reaction of the rats to the dimeric esters may be the consequence of the absence of a needed growth factor plus the presence of a toxic agent.

CONCLUSIONS

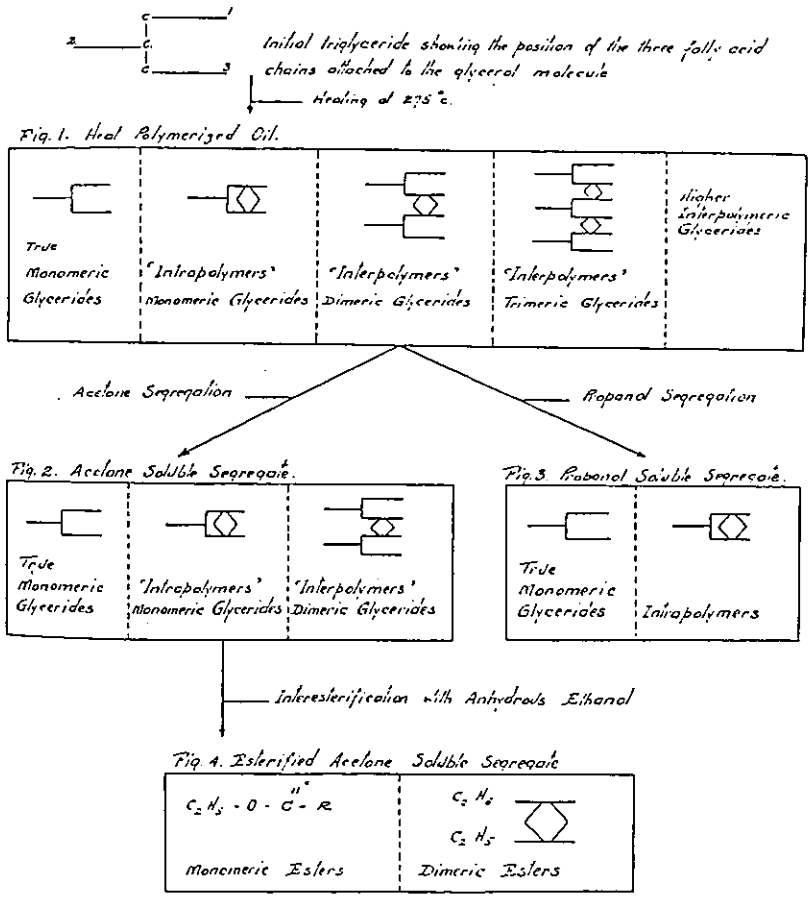
On the basis of these results one might tentatively conclude that the primary cause of the lowered nutritive value of diets containing thermally polymerized linseed oil is the presence of one or more dimeric fatty acid radicals which are in some way inimical to the well-being of the animals. Their deleterious effects could well be aggravated since they are produced at the expense of unsaturated fatty acids, some of which may be those essential to the animal for growth.

ACKNOWLEDGMENTS

The authors are indebted for financial aid and advice to the National Research Council, Committee on Food Preservation, through its Subcommittee on Edible Fats and Oils.

Thanks are also due to Mr. D. Mills, Department of Chemistry, Macdonald College, for his assistance in the preparation and analyses of the oils.

DIAGRAMMATIC REPRESENTATION OF THE MAIN CONSTITUENTS OF LINSEED OIL FOLLOWING THERMAL POLYMERIZATION, SOLVENT SEGREGATION AND ESTERIFICATION.



APPENDIX

Distillation of ethyl esters of the acetone-soluble fraction from polymerized linseed oil

The distillation of the esters was carried out using simple distilling bulbs and receivers and a glycerol bath. The distilling bulb was filled with clean glass wool to reduce bumping and frothing. Distillation temperature limits were established on esters of alkali-refined whole linseed oil, and these were observed closely in all distillations of esters from polymerized oils. The distillation pressures were of the order of 0.5 mm Hg. The distillable esters usually distilled completely between 155° and 175°C. When the vapor temperature had passed its maximum, the bath temperature was taken steadily up to 240°C. If no further rise in vapor temperature occurred, the bath was removed and the residue allowed to cool rapidly in the bulb without breaking the vacuum.

Demonstration of the presence of intrapolymers in the acetone-soluble fraction of polymerized linseed oil

A series of linseed oils were polymerized in a current of CO_2 for varying periods at 275°C. The methyl esters of their acetone-soluble segregates were prepared by interesterification. Cryoscopic determinations of mean molecular weight were carried out on the acetone-soluble fractions themselves and on the methyl ester fractions. The cyclohexane (British Drug Houses) used as cryoscopic solvent was carefully purified by successive treatment with fuming sulfuric acid and barium hydroxide, followed by distillation in a Stedman column. The purified solvent had M.P. 6.4°C., refractive index (25°C.) = 1.4237, values which are close to those recorded for very pure cyclohexane (Glasgow et al., '46). The observed mean molecular weights were corrected for the presence of free fatty acids by the method of Bernstein ('48), which assigns to the free fatty acids a dimeric average mean molecular weight of 558; i.e., assumes that association of the free fatty acids is practically complete. From the cryoscopic mean molecular weights of the acetone-soluble fraction, and assuming the presence of monomeric and dimeric glycerides of theoretical mean molecular weights of 878 and 1756, it was possible to calculate the percentages of monomeric and dimeric glycerides. From these figures, and assuming that polymerization of fatty acid radicals had not proceeded beyond the dimeric stage (Privett et al., '47), a theoretical yield of monomeric and dimeric esters could be calculated. The difference between these values and the observed values for monomeric and dimeric esters obtained by distillation may be reasonably ascribed to the presence of intrapolymers, which would behave as monomeric glycerides in the mean molecular weight determinations. The percentage differences can be converted to a triglyceride basis by multiplying by 3/2. The results are set out in appendix table 1, and provide support for the belief that the acetone-soluble fractions of heated linseed oil contain considerable amounts of dimeric fatty acid in the form of intrapolymers.

Even after 13 hours of heating, the acetone-soluble fraction contained approximately 30% of its total fatty acids in the form of dimeric acids, but only approximately 12% could be accounted for as dimeric glyceride and some 19% of dimeric acid was probably present as intrapolymers. The amount of intrapolymers (soluble in acetone) would be expected to decline as polymerization progresses. It seems quite clear from these results that solvent segregation methods are unlikely to be effective in differentiating between intrapolymers and true monomeric glycerides.

Superiority of propanol over acetone in segregating a non-polymeric glyceride fraction from thermally polymerized linseed oil

The analytical data in table 2 show that both the viscosity and the refractive index of the propanol-soluble fraction were lower than those of the acetone-soluble fraction. The data confirm that propanol is the more effective solvent in segregating a non-polymeric fraction from thermally polymerized linseed oil.

Effectiveness of vacuum distillation in separating monomeric from dimeric ethyl esters of the acetone-soluble segregate of polymerized linseed oil

The analytical data presented in appendix table 3 relate to two samples of methyl esters prepared from acetone-soluble fractions of heated linseed oil. The data show that the "distillable" fraction consisted essentially of methyl esters of monomeric fatty acids, the mean molecular weights being 296 and 295. The "non-distillable" fraction consisted essentially of methyl esters of dimeric fatty acids, the mean molecular weights being 587 and 596.

The fact that refractive indices and iodine values for the recombined samples were practically the same as the values for the charge before distillation is evidence that the heating during distillation had little, if any, effect on the constitution of the esters.

APPENDIX TABLE 1

Calculation of the intrapolymer content of the acetone-soluble fractions of thermally polymerized linseed oil

HOURS HEATED	MEAN MOLECULAR WEIGHT OF GLYCERIDES OF HEATED OIL BY CRYOSCOPY	CALC. % DIMERIC GLYCERIDE	CALC. % DIMERIC METHYL ESTERS	OBSERVED % DIMERIC METHYL ESTER BY DISTILLATION	DIFFERENCE TO BE ASCRIBED TO INTRA-POLYMERS
5	1,010	36.1	8.7	21.7	13.0
9	1,030	29.6	9.7	23.1	13.2
13	1,060	24.4	11.5	30.7	19.2
17	970	19.0	6.3	14.8	8.5

APPENDIX TABLE 2

Analyses of acetone and propanol fractions from heated alkali-refined linseed oil

FRACTION	YIELD	REF. IND 25°C.	VISCOSITY AT 250°C. CP.	IODINE VALUE
1. Unheated, recovered from acetone	5			
2. Heated, acetone-soluble	45	1.4789	50	183
3. Heated, propanol-soluble	28	1.4811	180	124
4. Heated, propanol-insoluble	72	1.4791	127	135
		1.4851	890	121

APPENDIX TABLE 3

Properties of methyl esters and fractions obtained in distillation

SAMPLE	FRACTION	PER CENT YIELD	MEAN MOLECULAR WEIGHT	REF. IND. AT 250°C.	IODINE VALUE
1	Charge (acetone-soluble)		334	1.4618	128.0
	Distillate	82.0	296	1.4600	137.2
	Residue	17.5	587	1.4896	105.5
	Recombined sample[1]			1.4647	127.6
2	Charge (acetone-soluble)		336	1.4647	129.0
	Distillate	81.9	295	1.4600	135.6
	Residue	17.6	598	1.4901	102.8
	Recombined sample[1]			1.4647	128.4

[1] Distillate and residue blended in the exact residue of their yields, to approximate original sample.

LITERATURE CITED

ADAMS, H. E., AND P. O. POWERS 1944 Mechanism of heat bodying linseed oil. Ind. Eng. Chem., *36:* 1124.

BERNSTEIN, I. N. 1948 Polymer fractionization of heat polymerized non-conjugated vegetable oils. J. Phys. Colloid. Chem., *52:* 613.

CRAMPTON, E. W., F. A. FARMER AND F. M. BERRYHILL 1951 The effect of heat treatment on the nutritional value of some vegetable oils. J. Nutrition, *43:* 431.

GLASGOW, A. R., JR., E. T. MURPHY, C. B. WILLINGHAM AND F. D. ROSSINI 1946 Purification, purity and freezing points of 31 hydrocarbons of the API-NES series. J. Res., Natl. Bur. Standards, RP 1734. Cited in Chem. Abstr., *41:* 17g (1947).

KASS, J. P. 1947 Polymerization of the unsaturated fatty acids. Biological antioxidants. Trans., Second Conf., Josiah Macy, Jr., Foundation, p. 27.

PRIVETT, O. S., W. D. MCFARLANE AND J. H. GASS 1947 Studies on heat polymerization and solvent segregation of vegetable oils. J. Am. Oil Chem. Soc., 24: 204.

PRIVETT, O. S., R. B. PRINGLE AND W. D. MCFARLANE 1945 Elimination of flavor in linseed shortening by heat polymerization and solvent segregation of the oil. Oil and Soap, *22:* 287.

QUACKENBUSH, F. W., H. STEENBOCK, F. A. KUMMEROW AND D. R. PLATZ 1942 Linoleic acid, pyridoxine and pantothenic acid in rat dermatitis. J. Nutrition, *24:* 225.

STUDIES TO DETERMINE THE NATURE OF THE DAMAGE TO THE NUTRITIVE VALUE OF SOME VEGETABLE OILS FROM HEAT POLYMERIZATION [1, 2]

I. THE RELATION OF AUTOXIDATION TO DECREASE IN THE NUTRITIONAL VALUE OF HEATED LINSEED OIL

EARLE W. CRAMPTON, E. H. COMMON, FLORENCE A. FARMER, F. MARION BERRYHILL AND L. WISEBLATT

Departments of Nutrition and Chemistry, Macdonald College, McGill University, Province of Quebec, Canada

(Received for publication January 6, 1951)

Ingestion of heat polymerized linseed oil was followed by lowered growth rates in rats, this growth depression becoming more severe with increased lengths of heating time of the oils and with increased amounts of oil in the diet (Crampton et al., '51). Our earlier experiments indicated that the low nutritive value of heated linseed oil diets is attributable to the oil itself and not to the effects of the heated oil upon non-lipid diet constituents.

As thermal treatment causes oil to be less stable to oxidation, it has been proposed that biologically deleterious products may accumulate through progressive autoxidation during storage of the oil-containing diets at room temperature. Oven temperatures used during the baking of the diets may also promote oxidative changes.

The extent of autoxidation can be controlled by the addition of an anti-oxidant. A mixture of nordihydroguaiaretic acid (NDGA) with citric acid has been shown to be an effective

[1] Contribution from the Faculty of Agriculture, McGill University, Macdonald College, Quebec, Canada. Journal Series No. 281.

[2] Issued as paper No. 260 of the Canadian Committee on Food Preservation.