Attachment 1a

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245    10$aFood research international.
260    ##$aOttawa, Ontario, Canada : $bPublished on behalf of the
Canadian Institute of Food Science and Technology by Elsevier
Applied Science, $cc1992-
300    ##$av. : $bill. ; $c30 cm.
310    ##$aSix issues per volume, one volume [per year]
362    0#$aVol. 25, no. 1-
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OCLC 25848747 **No holdings in XXX - 142 other holdings**

Continuing Resources

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082 00 664/.005
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130 0 Food research international (Ottawa, Ont.)
210 0 Food res. int.
222 0 Food research international
245 10 Food research international.
260 Ottawa, Ont. : $b Published on behalf of the Canadian Institute of Food Science and Technology by Elsevier Applied Science, $c ©1992-
300 volumes : $b illustrations ; $c 30 cm
310 Six issues per year
336 text $b txt $2 rdacontent
337 unmediated $b n $2 rdamedia
338 volume $b nc $2 rdacarrier
362 0 Vol. 25, no. 1-
588 Latest issue consulted: Vol. 29, no. 7 (Oct. 1996).

650 0 Food industry and trade $v Periodicals.
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650 0 Food.
650 2 Food $0 (DNLM)D005502
650 2 Food Technology $0 (DNLM)D005524
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710 2 Canadian Institute of Food Science and Technology.
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Attachment 1c

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024 8_ |**0** http://eurovoc.europa.eu/5100

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667 __ |**a** Includes the Old catalog headings: Canada and Canada (Province)
667 __ |**a** Non-Latin script references not evaluated.
670 __ |**a** López Portillo, J. En Suecia y Canadá, 1980: |**b** t.p. (Canadá)
670 __ |**a** Medding, P. Ma'amadah shel Medinat Yiśra'el be-shalosh ḳehilot dovrot Anglit, 1983: |**b** t.p. (Ḳanadah) added t.p. (Canada [in rom.])
670 __ |**a** LC data base, 02-28-95 |**b** (hdgs.: Canada and Canada (Province))
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670 __ |**a** Ukraïns′ko-anhliĭs′kyĭ slovnyk biznesovykh terminiv, 1992: |**b** cover, p. 1 (Kanada, Canada)
670 __ |**a** NLC 06-22-94 |**b** (Canada: In 1841 Upper Canada and Lower Canada united to become the Province of Canada. With confederation of July 1, 1867 the Dominion of Canada was created and the former Province of Canada was superceded by the Provinces of Ontario and Quebec; French forms: Haut-Canada, Bas-Canada, Province du Canada, Dominion du Canada)
670 __ |**a** Wikipedia, July 16, 2008 |**b** (Canada. The French colony of Canada referred to the part of New France along the Saint Lawrence River and the northern shores of the Great Lakes. Later, it was split into two British colonies, called Upper Canada and Lower Canada until their union as the British Province of Canada in 1841. Upon Confederation in 1867, the name Canada was adopted for the entire country, and Dominion was conferred as the country's title. It was frequently referred to as the Dominion of Canada until the 1950s. The Canada Act 1982 refers only to "Canada" and, as such, it is currently the only legal (and bilingual) name) Afrikaans site (Kanada) Alemannisch site (Chanada) Arabic site (كندا = Kanadā) Belarusian site (Канада = Kanada) Catalan site (Canadà) Greek site (Καναδάς = Kanadas) Spanish site (Canadá; Republica de Canadá;

Dominio del Canadá) Esperanto site (Kanado) Farsi site (کانادا = Kānādā) Irish site (Ceanada) Manx Gaelic site (Yn Chanadey) Korean site (캐나다 = K‘aenada) Hawaiian site (Kanakā) Hebrew site (קנדה = Ḳanadah) Japanese site (カナダ = Kanada) Scots Gaelic site (Canadae) Albanian site (Kanadaja) Chinese site (加拿大 = Jianada) Yiddish site (קאנאדע = Ḳanade) Yoruba site (Kánádà)

670 __ |**a** Wikipedia, July 6, 2018 |**b** (Canada is a federal parliamentary democracy and a constitutional monarchy, with Queen Elizabeth II being the head of state)

670 __ |**a** Sapanoṃ kā ākāśa, 2021: |**b** t.p. (Kaineḍā)

751 _6 |**a** Canada |**0** (CaOONL)ncf10034654

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Library of Congress Authorities
URL: http://authorities.loc.gov/
Library of Congress Online Catalog
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Attachment 2a

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Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

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245 10 $aFood chemistry$h[e-journal].

260 $a[Amsterdam] :$bElsevier Science

310 $a16 no. a year,$b1999-

321 $aFrequency varies,$b<1995>-1998

362 1_ $aPrint began with vol. 1 (July 1976).

538 $aMode of access: World Wide Web.

588 $aDescription based on: Vol. 52, issue 1 (1995); title from contents screen (ScienceDirect, viewed Oct. 13, 2003).

588 $aLatest issue consulted: Vol. 83, issue 4 (Dec. 2003), viewed Oct. 13, 2003.

500 $aRefereed/Peer-reviewed

650 _0 $aFood$xAnalysis$vPeriodicals.

650 _0 $aFood$xComposition$vPeriodicals.

655 _4 $aElectronic journals.

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776 1_ $x0308-8146

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Attachment 2b

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906 __ |**t** 8888 |**u** te00 |**v** 0
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Attachment 2c

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

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The Library's Main Reading Room will close to researchers earlier than usual, at 5:00pm, on Thurs., Nov. 3. All other reading rooms will be open according to their daily schedules.

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LCCN Permalink: https://lccn.loc.gov/sh85050191
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Attachment 2d

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550 __ |**w** g |**a** Serial publications

550 __ |**a** Newspapers

550 __ |**a** Press

670 __ |**a** UMI business vocab. |**b** (Journals)

680 __ |**i** Here are entered works on the periodicals of the world and, with appropriate subdivisions, works on special aspects or sections of periodicals. Works on periodicals in a specific language, or in a specific country or larger area, are entered under the adjectival form of the language or area, e.g. |**a** Arabic periodicals; Canadian periodicals.

906 __ |**t** 0420 |**u** te03 |**v** 0

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Attachment 2e

OCLC 2728297 **No holdings in XXX - 186 other holdings**

Continuing Resources

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Iron Bioavailability of Hemoglobin from Soy Root Nodules Using a Caco-2 Cell Culture Model



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Reddy, Manju
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Abstract

Heme iron has been identified in many plant sourcesmost commonly in the root nodules of leguminous plants, such as soy. Our objective was to test the effectiveness of soy root nodule (SRN) and purifi

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Abstract

Heme iron has been identified in many plant sources—most commonly in the root nodules of leguminous plants, such as soy. Our objective was to test the effectiveness of soy root nodule (SRN) and purified soy hemoglobin (LHb) in improving iron bioavailability using an in vitro Caco-2 cell model, with ferritin response as the bioavailability index. We assessed bioavailability of iron from LHb (either partially purified (LHb_A) or purified (LHb_D)) with and without food matrix and compared it with that from bovine hemoglobin (BHb), ferrous sulfate ($FeSO_4$), or SRN. Bioavailability of each treatment was normalized to 100% of the $FeSO_4$ treatment. When iron sources were tested alone (100 ug iron/mL), ferritin synthesis by LHb_D and BHb were 19% ($P > 0.05$) and 113% ($P < 0.001$) higher than $FeSO_4$, respectively. However, when iron sources were used for fortification of maize tortillas (50 ppm), LHb_A and BHb showed similar bioavailability, being 27% ($P < 0.05$) and 33% ($P < 0.05$) higher than $FeSO_4$. Heat treatment had no effect on heme iron but had a significant reduction on $FeSO_4$ bioavailability. Adding heme (LHb_A) iron with nonheme ($FeSO_4$) had no enhancement on nonheme iron absorption. Our data suggest that heme iron from plant sources may be a novel value-added product that can provide highly bioavailable iron as a food fortificant.

Keywords: Iron bioavailability; heme iron; soy root nodules; leghemoglobin

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Library of Congress Online Catalog
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Attachment 5c

LIBRARY OF CONGRESS AUTHORITIES

LC control no.: sh2008108541
LCCN Permalink: https://lccn.loc.gov/sh2008108541
HEADING: Nutrition Periodicals

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001 7460925
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150 __ |**a** Nutrition |**v** Periodicals
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953 __ |**a** te00

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Attachment 5d

OCLC 12330732 **No holdings in XXX - 237 other holdings**

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246 3 Chemical Rubber Company critical reviews in food science and nutrition
246 14 CRC critical reviews in food science and nutrition
260 Boca Raton, Fla. : $b CRC Press, $c ©1980-
260 3 $3 2004- : $a Philadelphia, PA : $b Taylor & Francis Group, LLC
300 volumes : $b illustrations ; $c 26 cm
310 Twenty-two issues a year, $b 2019-
321 Frequency varies
336 text $b txt $2 rdacontent
337 unmediated $b n $2 rdamedia
338 volume $b nc $2 rdacarrier
362 0 Vol. 12, issue 3 ([June] 1980)-

510 2 Chemical abstracts $x 0009-2258
525 Supplements accompany some vols.
530 Also issued via the World Wide Web to subscribers of OCLC FirstSearch Electronic Collections Online and CRCnetBASE.
588 Latest issue consulted: Vol. 58, issue 17 (2018) (surrogate).
650 0 Food industry and trade $v Periodicals.
650 0 Nutrition $v Periodicals.
650 0 Food.
650 0 Nutrition.
650 2 Food $0 (DNLM)D005502
650 2 Food-Processing Industry $0 (DNLM)D005525
650 2 Nutritional Physiological Phenomena $0 (DNLM)D009747
650 6 Aliments $0 (CaQQLa)201-0001300 $x Industrie et commerce $0 (CaQQLa)201-0001300 $v Périodiques. $0 (CaQQLa)201-0378307
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856 4 $u http://firstsearch.oclc.org $z Address for accessing the journal using authorization number and password through OCLC FirstSearch Electronic Collections Online. Subscription to online journal required for access to abstracts and full text
856 4 $u http://firstsearch.oclc.org/journal=1040-8398;screen=info;ECOIP $z Address for accessing the journal from an authorized IP address through OCLC FirstSearch Electronic Collections Online. Subscription to online journal required for access to abstracts and full text

Attachment 5e

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LCCN Permalink: https://lccn.loc.gov/sh85050184

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053 _0 |**a** RA601 |**b** RA602 |**c** Public health

053 _0 |**a** TX341 |**b** TX641 |**c** Home economics

150 __ |**a** Food

360 __ |**i** subdivision |**a** Food |**i** under ethnic groups, and individual animals and groups of animals, e.g. |**a** Fishes--Food; |**i** also headings beginning with the word |**a** Food; |**i** and individual foods and beverages, e.g. |**a** Bread; Milk

450 __ |**a** Foods

450 __ |**w** nne |**a** Primitive societies |**x** Food

550 __ |**w** g |**a** Dinners and dining

550 __ |**w** g |**a** Home economics

550 __ |**w** g |**a** Table

550 __ |**a** Cooking

550 __ |**a** Diet

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953 __ |**a** xx00 |**b** td04

906 __ |**t** 2206 |**v** 0

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Attachment 5f

LC control no.: sh 85093451

LCCN Permalink: https://lccn.loc.gov/sh85093451

HEADING: Nutrition

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040 __ |**a** DLC |**c** DLC |**d** DLC

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053 _0 |**a** RA784 |**c** Personal health

053 _0 |**a** TX341 |**b** TX641 |**c** Home economics

150 __ |**a** Nutrition

360 __ |**i** subdivision |**a** Nutrition |**i** under classes of persons and ethnic groups; and subdivision |**a** Nutritional aspects |**i** under diseases and types of diseases, e.g. |**a** Cancer--Nutritional aspects

450 __ |**a** Alimentation

450 __ |**a** Food |**x** Health aspects

450 __ |**a** Nutrition |**x** Health aspects

550 __ |**w** g |**a** Health

550 __ |**w** g |**a** Physiology

550 __ |**a** Diet

550 __ |**a** Dietetics

550 __ |**a** Digestion

550 __ |**a** Food

550 __ |**a** Food habits

550 __ |**a** Malnutrition

680 __ |**i** Here are entered works on the sum of the physiological processes involved in the assimilation and utilization of nutrients for proper body functioning and health. This heading may be subdivided by place to indicate the nutritional status of a group of

people in the place. Works on the food and drink regularly consumed by an individual or group of people are entered under |**a** Diet.

681 __ |**i** Note under |**a** Diet

906 __ |**t** 0334 |**u** te04 |**v** 0

953 __ |**a** xx00 |**b** ta25

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URL: http://authorities.loc.gov/
Library of Congress Online Catalog
URL: https://catalog.loc.gov/

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Attachment 6a

MARC Bibliographic Record

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337          $aunmediated$bn$2rdamedia
338          $avolume$bnc$2rdacarrier
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650    _0    $aFood industry and trade$vPeriodicals.
785    00    $tCRC critical reviews in food science and nutrition$w(OCoLC)02243202
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Attachment 6b

OCLC 1553990 **No holdings in XXX - 175 other holdings**

Continuing Resources

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060 00 W1 $b C555G
082 00 664/.005
090 $b
110 2 Chemical Rubber Company.
210 0 CRC crit. rev. food technol.
222 0 CRC critical reviews in food technology
245 10 CRC critical reviews in food technology.
246 3 Chemical Rubber Company critical reviews in food technology
246 10 Critical reviews in food technology
246 10 C.R.C. critical reviews in food technology
260 Cleveland, $b CRC Press.
300 5 volumes $c 27 cm
310 Quarterly
336 text $b txt $2 rdacontent
337 unmediated $b n $2 rdamedia
338 volume $b nc $2 rdacarrier
362 0 v. 1-5; Feb. 1970-Apr. 1975.
530 Also issued online.
650 0 Food industry and trade $v Periodicals.
650 2 Food-Processing Industry. $0 https://id.nlm.nih.gov/mesh/D005525
650 7 Food industry and trade. $2 fast $0 (OCoLC)fst00930843

650 07 Lebensmitteltechnologie. $0 (DE-588)4034901-9 $2 gnd
650 07 Zeitschrift. $0 (DE-588)4067488-5 $2 gnd
650 07 Lebensmitteltechnologie. $2 swd
650 07 Zeitschrift. $2 swd
655 2 Periodicals. $0 https://id.nlm.nih.gov/mesh/D020492
655 7 Periodicals. $2 fast $0 (OCoLC)fst01411641
655 7 Periodicals. $2 lcgft $0 http://id.loc.gov/authorities/genreForms/gf2014026139 $0 (uri) http://id.loc.gov/authorities/genreForms/gf2014026139
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785 00 $t CRC critical reviews in food science and nutrition $x 0099-0248 $w (DLC)   75646640 $w (OCoLC)2243202
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Attachment 7a

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245	10	$aProcessed meats /$cA.M. Pearson, T.A. Gillett.
250		$aThird edition.
264	_1	$aNew York :$bChapman & Hall,$c[1996]

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

264 _4 $c©1996

300 $axi, 448 pages :$billustrations ;$c24 cm

336 $atext$btxt$2rdacontent

337 $aunmediated$bn$2rdamedia

338 $avolume$bnc$2rdacarrier

504 $aIncludes bibliographical references and index.

505 0_ $a1. Introduction to Meat Processing -- 2. Composition and Nutritive Value of Raw Materials and Processed Meats -- 3. Curing -- 4. Smoking -- 5. Meat Cookery and Cooked Meat Products -- 6. Raw Materials -- 7. Sectioned and Formed Meat Products -- 8. Least Cost Formulation and Preblending of Sausage -- 9. Sausages -- 10. Sausage Formulations -- 11. Casings, Extenders, and Additives -- 12. Herbs, Spices, and Condiments -- 13. Cured and/or Smoked Meats -- 14. Reduced and Low Fat Meat Products -- 15. The Canning Process -- 16. Canned Meat Formulations -- 17. Restructured Meat Products.

520 1_ $a"Reflecting the changes taking place in the meat processing industry, this updated edition provides a comprehensive introduction to the principles and practices involved in processing meat and poultry into consumer products. The volume covers a range of topics, from the economics of processing to the industry's recent trends and new developments, including new chapters on spices and low fat processed meat." "Processed Meats is a unique and valuable text for undergraduate and graduate students. In addition, professionals in the meat and poultry industries will benefit from the current information found in this new, single-source guide."--BOOK JACKET.

650 _0 $aPacking-houses.

650 _0 $aPacking-house products.

700 1_ $aGillett, Tedford A.,$d1935-

997 $aMARCIVE

MMS IDs

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Attachment 7b

OCLC 463356391 **No holdings in XXX - 12 other holdings**

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050 00 TS1970 $b .P43 1999
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090 $b
100 1 Pearson, A. M. $q (Albert Marchant), $d 1916-
245 10 Processed meats / $c A.M. Pearson, T.A. Gillett.
250 3rd ed.
260 Gaithersburg, MA : $b Aspen Publishers, Inc., $c 1999.
300 xi, 448 pages : $b illustrations ; $c 24 cm
336 text $b txt $2 rdacontent
337 unmediated $b n $2 rdamedia
338 volume $b nc $2 rdacarrier
504 Includes bibliographical references and index.
505 0 1. Introduction to Meat Processing -- 2. Composition and Nutritive Value of Raw Materials and Processed Meats -- 3. Curing -- 4. Smoking -- 5. Meat Cookery and Cooked Meat Products -- 6. Raw Materials -- 7. Sectioned and Formed Meat Products -- 8. Least Cost Formulation and Preblending of Sausage -- 9. Sausages -- 10. Sausage Formulations -- 11. Casings, Extenders, and Additives -- 12. Herbs, Spices, and Condiments -- 13. Cured and/or Smoked Meats -- 14. Reduced and Low Fat Meat Products -- 15. The Canning Process -- 16. Canned Meat Formulations -- 17. Restructured Meat Products.
520 Reflecting the changes taking place in the meat processing industry, this updated edition provides a comprehensive introduction to the principles and practices involved in processing meat and poultry into consumer products. The volume covers a range of topics, from the economics of processing to the industry's recent trends and new developments, including new chapters on spices and low fat processed meat. Processed Meats is a unique and valuable text for undergraduate and graduate students. In addition, professionals in the meat and poultry industries will benefit from the current information found in this new, single-source guide.
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700 1 Gillett, Tedford A., $d 1935-

Attachment 7c

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450 __ |**a** Meat packing industry
450 __ |**a** Packing industry
450 __ |**a** Red meat processing plants
550 __ |**w** g |**a** Food processing plants
550 __ |**w** g |**a** Meat industry and trade
680 __ |**i** Here are entered works on meat packing, i.e. the wholesale meat industry. Works on the packing of meat for shipment are entered under |**a** Meat--Packing.
681 __ |**i** Note under |**a** Meat--Packing
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910 __ |**a** Proposal saved by tc03 on 05/23/2016 at 12:03:50
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Case No.: IPR2023-00207
U.S. Patent No. 11,224,241



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Library of Congress Online Catalog
URL: https://catalog.loc.gov/

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Attachment 7d

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

LC control no.: sh 85096529
LCCN Permalink: https://lccn.loc.gov/sh85096529
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906 __ |**t** 9499 |**u** ---- |**v** 0
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953 __ |**a** xx00 |**b** ta21

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Public Catalog

Copyright Catalog (1978 to present)
Search Request: Left Anchored Title = processed meats
Search Results: Displaying 2 of 7 entries

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Labeled View

Processed meats / A. M. Pearson, T. A. Gillett.

Type of Work: Text
Registration Number / Date: TX0004188034 / 1996-02-13
Title: Processed meats / A. M. Pearson, T. A. Gillett.
Edition: 3rd ed.
Imprint: New York : Chapman & Hall, c1996.
Description: 448 p.
Copyright Claimant: Chapman & Hall
Date of Creation: 1995
Date of Publication: 1996-01-02
Names: Gillett, T. A.
Pearson, Albert Marchant, 1916-
Chapman & Hall

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362 1  Began with v. 1, issue 1 (Jan. 1977).
506    Available only to authorized users.
515    Some issues combined.
525    Supplements accompany some issues.
538    Mode of access: World Wide Web.
550    The official journal of the American Meat Science
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588 0  Vol. 39, issue 1 (1995); title from contents screen
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588 1  Vol. 67, issue 1 (May 2004), viewed January 8, 2004.
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710 2  American Meat Science Association.
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Attachment 9a

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

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Red meat in the diet

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Abstract

Summary The aim of this paper is to provide an overview of the existing scientific literature on the role of red meat in the diet. It discusses current levels of red meat intake, the nutritional benefits of consuming red meat, dietary and lifestyle factors associated with meat consumption and the effects of red meat intake on health and chronic disease outcomes.

Definitions

Much of the evidence presented in this paper is based on epidemiological studies investigating associations between meat intake and health/disease outcomes. Not all studies define what is meant by meat and, where definitions are offered, they are not always the same, which can make comparisons between studies difficult. Some studies include poultry under the definition of meat, while others exclude it; some look at total meat consumption (including red meat and processed meat), while others analyse red meat and processed meat separately. In general, red meat refers to beef, pork and lamb in main dishes, and processed meat refers to meat products, such as sausages, burgers and smoked, cured and tinned meats. Offal is also a form of meat, but there is little epidemiological evidence specific to this category of meat.

For the purposes of this paper, the following definitions for red and processed meat will be used, which are based on definitions currently used in epidemiological studies looking at the health effects of meat consumption, such as the European Prospective Investigation into

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

Cancer and Nutrition (EPIC) study. Where studies have used different definitions, this is highlighted.

Red meat includes beef, veal, pork and lamb (fresh, minced and frozen).

Processed meat includes meat that has been preserved by methods other than freezing, such as salting, smoking, marinating, air-drying or heating *e.g.* ham, bacon, sausages, hamburgers, salami, corned beef and tinned meat.

Source: Linseisen *et al.* (2002).

Red meat consumption

Trends in consumption of individual meats vary widely. In developed countries there has been a general decrease in the amount of red meat consumed, which can primarily be attributed to a reduction in beef consumption since the mid- to late 1990s, as the variation in consumption of lamb/mutton has been minimal (Red Meat and Health Expert Advisory Committee 2001; Fowler 2004; Meat and Wool New Zealand 2004). Pig meat is the most widely consumed meat in the EU and consumption has been steadily increasing. While it is less commonly consumed in New Zealand and Australia, consumption has also been increasing in these areas.

Data quality

Data from food balance sheets (which indicate meat available for consumption) have primarily been used to follow trends in the supply of meat. Food balance sheets are based on statistical data on the production, import and export of carcases, and eventual shifts in stock. Due to the large quantity of material discarded prior to meat reaching the table for consumption (*e.g.* bones and cartilage) and at the table (*e.g.* trimmed fat, wastage), the apparent supply from this source will always be an overestimation of the true meat intake in a population. Detailed analysis of food consumption is often available from dietary surveys undertaken within a country. Such surveys take into account the food intake of individuals, and provide more reliable information about the frequency, amount and type of foods consumed. However, it is difficult to use these data to make international comparisons due to the different methodologies used to collect and analyse the data. However, the recent EPIC study has provided comparable information on meat intakes across a range of European countries, using a standardised methodology to collect the dietary data.

Consumption of red and processed meat

Despite the numerous issues with data quality, as described above, the quantity of data available presents a useful picture about meat consumption. Data from EPIC (Linseisen *et al.* 2002) and national dietary surveys indicate gender differences in meat intake. For example, a recent survey of dietary habits in Denmark shows that men consume more meat and meat

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

products than women; on average 141 g per day compared with 89 g per day (excluding poultry) (Danish Institute for Food and Veterinary Research 2005a). However, this is not just a European phenomenon. Data from the 1995/6 Australian National Nutrition Survey on red meat intakes (including beef, veal and lamb, as cuts or as products in mixed dishes) also found a notable difference. The survey found that, on average, women consumed 45 g of red meat per day, while men consumed 88 g per day, and that only 28% of men and 12% of women who consumed red meat ate more than 120 g per day (Red Meat and Health Expert Advisory Committee 2001).

When considering the health effects of red meat consumption, it is useful to consider how meat is consumed as part of the diet (*e.g.* portion sizes), and the type of meat consumed. Data from the recent North/South Food Consumption Survey for Ireland found that, compared with all meats, portions of beef and veal were largest (on average 60 g), and consumed by 55% of the sample (Irish Universities Nutrition Alliance 2001). According to the survey, the most commonly consumed meats in Ireland were bacon and ham (consumed by 91% of the sample). Average meat intake increased from 33 g to 134 g per day when intakes of all processed and carcase meats were included. In the UK, bacon and ham are also the most commonly consumed meats (with 77% of men and 64% of women consuming these products) (Henderson *et al.* 2002). Overall, carcase meat consumption in the UK has been recorded at just 32 g per person per day (Department for Food and Rural Affairs 2005). However, this does not take into account ready meals and convenience meat products which have become very popular in the UK. The popularity of these foods, along with other meat products such as meat pies, makes it difficult to accurately measure the average amount of red meat consumed. For example, data from the North/South Ireland Food Consumption Survey found that there was a 43% overestimation of meat intake, without disaggregation of composite foods (such as lasagne, sausage rolls) (Cosgrove *et al.* 2005a).

Although consumer attitudes to meat are influenced by a number of factors, such as price and availability, the major differences in the volume and type of meat consumed between countries are thought to be primarily due to differences in culture and traditional eating habits (Fowler 2004). From the EPIC data it is evident that different European countries favour certain meats; for example, Germany shows the highest consumption of pork, while mutton/lamb seems to be the most frequently consumed meat in France and Spain. With regard to processed meat intake, Sweden, Norway and Germany have the highest intakes, primarily due to the amount of sausages they consume (Linseisen *et al.* 2002). Table 1 shows average daily intakes of red and processed meat in a range of European countries, as determined by the EPIC calibration study.

Table 1. Mean daily intake (g per day) of total meat, red meat, processed meat and red + processed meat in selected countries participating in the European Prospective Investigation into Cancer and Nutrition (EPIC) calibration study

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

	Total meat*		Red meat		Processed meat		Red meat + processed meat	
	Men	Women	Men	Women	Men	Women	Men	Women
Greece	78.8	47.1	45.3	25.5	10.0	5.8	55.3	31.3
Spain	170.4	99.2	74.0	37.8	52.8	29.6	126.8	67.4
Italy	140.1	86.1	57.8	40.8	33.5	19.6	91.3	60.4
Germany	154.6	84.3	52.2	28.6	83.2	40.9	135.4	69.5
Netherlands	155.6	92.7	63.8	41.0	72.4	37.9	136.2	78.9
UK	108.1	72.3	40.0	24.6	38.4	22.3	78.4	46.9
Denmark	141.1	88.3	69.6	44.1	51.9	25.3	121.5	69.4

Source: Linseisen *et al*. (2002).

* Total meat includes pork, beef, veal, lamb/mutton, poultry, game, rabbit, horse, goat and offal.

Nutrient composition of red meat and contribution to nutrient intakes

The main health benefits associated with eating red meat relate to its nutritional composition. Red meat contains high biological value protein and important micronutrients, all of which are essential for good health throughout life. Most healthy balanced diets will include lean meat in moderate amounts, together with starchy carbohydrates (including wholegrain foods), plenty of fruit and vegetables, and moderate amounts of milk and dairy foods.

Data on the nutrient composition of red meat are available in food composition tables and databases. There are over 150 food composition tables and electronic databases worldwide and undoubtedly meat and meat products will be listed in all of these, although slightly different nutrient values are likely to be found in different versions. Table 2 shows the nutrient composition of 100 g of lean raw beef, lamb and pork according to the food composition tables of four countries, and illustrates how these figures can vary. This variation may be due to a number of reasons. For example, there may have been some differences in the nutrient composition of the meats selected for sampling, perhaps due to differences in the animals' feeding regime. Also, different sampling techniques may have been used (*e.g.* values for beef may be based on one particular cut of meat from one breed of cattle while others may be based on a variety of cuts and breeds). Furthermore, different technologies or methods may

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

have been used to analyse the products, or there may be differences in classification of the various cuts of meat.

Table 2. Comparison of selected nutrients in beef, lamb and pork (per 100 g) according to food composition databases from four countries

	Denmark	UK	Australia	USA
Beef, lean, raw				
Energy (kJ)	470	571	520	531
Protein (g)	22.3	22.5	23.0	22.3
Fat (g)	2.5	5.1	3.6	3.5
Niacin (mg)	10.1	5.0	3.0	6.5
Vitamin B_{12} (μg)	1.4	2.0	1.1	0.9
Iron (mg)	2.4	1.8	2.0	1.6
Zinc (mg)	4.7	4.1	4.2	4.0
Selenium (μg)	6.5	7.0	10.0	30.8
Lamb (leg), lean, raw				
Energy (kJ)	545	651	501	561
Protein (g)	20.2	20.2	20.4	20.5
Fat (g)	5.5	8.3	4.2	5.1
Niacin (mg)	7.5	5.4	5.6	6.3
Vitamin B_{12} (μg)	1.2	2.0	0.9	2.8

Sources: Chan *et al*. (1995); Red Meat and Health Expert Advisory Committee (2001); Danish Institute for Food and Veterinary Research (2005b); USDA (2005).

N/A: data were not available.

Energy

The amount of energy provided by meat is variable. Meat contains virtually no carbohydrate, and is principally composed of protein (which provides 17 kJ/4 kcal of energy per gram).

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

Meat also contains fat in varying amounts (providing 37 kJ/9 kcal of energy per gram). The more fat that meat contains, the higher the energy content will be, as shown in Table 3.

Table 3. Energy, fat and protein content of lean and untrimmed cuts of red meat (per 100 g; UK figures)

Meat (barbecued or grilled)	Energy kJ (kcal)	Fat (g)	Protein (g)
Rump steak – lean	741 (176)	5.7	31.2
Rump steak – lean and fat	849 (203)	9.4	29.5
Leg joint of lamb – lean	879 (210)	9.6	30.8
Leg joint of lamb – lean and fat	986 (236)	13.0	29.7
Loin chops of pork – lean	780 (186)	6.8	31.1
Loin chops of pork – lean and fat	1066 (255)	15.8	28.3

Source: Chan *et al*. (1995).

Protein

Dietary protein is needed for growth, maintenance and repair of the body, and can also provide energy. Protein from foods consists of chains of hundreds to thousands of amino acids. Some amino acids can be synthesised in the body, while others – essential (or indispensable) amino acids – cannot, and therefore essential amino acids need to be consumed in the diet to maintain good health. There are eight essential amino acids and red meat (and in some cases meat products) are important sources of these. In comparison, nearly all plant proteins have low levels of at least one essential amino acid, known as the 'limiting amino acid', for example in the case of wheat, this is lysine. Eating different plant foods, in combination, can provide the right balance of essential amino acids, although less efficiently than meat.

Red meat contains, on average, 20–24 g of protein per 100 g (when raw). Cooked red meat contains 27–35 g of protein per 100 g (cooked weight); as meat is cooked the water content decreases and the nutrients become more concentrated, therefore the protein content increases. Leaner meat also contains a higher proportion of protein, as shown in Table 3.

In most developed countries, average protein intakes for all age groups are in excess of the minimum protein requirements needed for good health, provided energy intakes are sufficient. Any excess protein in the diet is used to provide energy. Meat and meat products (including

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

poultry) contribute 36% of total protein intake and are the major dietary source of protein in the UK (Henderson *et al.* 2002). Slightly lower figures have been recorded elsewhere in Europe, with meat and meat products contributing 30.7% in France (including poultry) and 26% in Denmark (excluding poultry) (Credoc 2003; Danish Institute for Food and Veterinary Research 2005a). The contribution meat makes to protein intakes is within a similar range in other developed countries; for example, in New Zealand meat and meat products (including poultry) contribute 33% of protein intake, and 22% in Australia (excluding poultry) (Russell *et al.* 1999; Baghurst *et al.* 2000).

Fat

Fat is the richest dietary source of energy and supplies essential nutrients such as fat-soluble vitamins and essential fatty acids, but must be consumed in moderation. Fat also provides palatability and flavour to foods. Fat is made up of different types of fatty acids: saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). The fatty acid profile of a food is determined by the proportions in which each of the fatty acids are present. It is now well recognised that different fatty acids have different effects on blood cholesterol levels, some beneficial and some adverse, and therefore it is important to consider the fatty acid profile of a food (see *Cardiovascular disease* section).

Fat in meat is present as intermuscular fat (between the muscles), intramuscular fat (or marbling, *i.e.* within the muscles) and subcutaneous fat (below the skin). The fat content of red meat varies widely, depending on the type of red meat, the cut and the degree of trimming (BNF 1999a; Higgs 2000). In some countries, meat with a low fat content is classified as 'lean meat'. There is no international definition of lean meat, however, standards seem to be similar in different countries. For example, in Australia and New Zealand, meat containing less than 10% fat meets the Heart Foundation's 'tick of approval', and in Denmark, meat containing between 5 and 10% fat is classified as 'lean' (Red Meat and Health Expert Advisory Committee 2001; Ovesen 2002).

Fatty acid composition of meat

Overall, lean red meat contains similar proportions of MUFAs to SFAs, although as illustrated in Table 4, the exact proportions vary depending on the type of meat (Chan *et al.* 1995).

Table 4. Typical fatty acid composition (g/100 g) of different types of red meat (lean only, cooked) (UK figures)

	Beef	Lamb	Pork	Bacon (grilled)
Total SFA	3.26	5.36	2.31	7.91
Total MUFA	3.41	4.06	2.56	8.85

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

Total PUFA	0.38	0.59	1.15	2.71
n-6 PUFA	0.36	0.48	1.02	2.41
n-3 PUFA	0.09	0.23	0.12	0.31

Source: MAFF (1995).

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

The fatty acid profile of meat will also vary depending on the proportions of lean and fat present. For example, lean meat is higher in PUFAs and lower in SFAs (*e.g.* less than 2 g of SFAs per 100 g of meat), compared with untrimmed meat. Trimming the fat off meat will affect the proportions of fatty acids, as visible fat is higher in SFAs, containing around 37 g of SFAs per 100 g of meat (Li *et al.* 2005).

The main SFAs present in red meat are palmitic acid and stearic acid (Fink-Gremmels 1993; MAFF 1998). There are also minor amounts of myristic acid which is thought to increase cholesterol levels more potently than palmitic acid, while stearic acid appears to have no effect on cholesterol levels, despite being a SFA (Ulbricht & Southgate 1991; Fink-Gremmels 1993; MAFF 1998; Higgs 2000). The principal MUFA in meat is oleic acid, and typically between 30 and 40% of the fat in meat is composed of MUFAs (Fink-Gremmels 1993; MAFF 1998).

Red meat also contains PUFAs. The predominant PUFAs in meat are linoleic (*n*-6) and α-linolenic acid (*n*-3), which are known as essential fatty acids because they cannot be made in the body. Although meat contains low levels of PUFAs, in the UK meat and meat products (including poultry) contribute substantially to intakes, providing 18% of *n*-6 PUFAs and 17% of *n*-3 PUFAs, while contributing to 23% of overall fat intake (Henderson *et al.* 2003a). The contribution that meat and meat products make to fatty acid intakes varies between countries. For example, in Denmark meat and meat products contribute 26% of MUFAs, 11% of PUFAs, and only 18% of SFAs in the diet (Danish Institute for Food and Veterinary Research 2005a). Similar figures have been recorded for other European countries, such as Ireland and the UK (Henderson *et al.* 2003a; Cosgrove 2005a). However, the contribution of red meat (from cuts, dishes and products, excluding the pastry, potatoes, etc.) to total fatty acid intake in Australia is much lower, providing up to 11% of MUFAs, 3% of PUFAs and 10% of SFAs (Baghurst *et al.* 2000).

Meat also contains small amounts of the long-chain *n*-3 PUFAs eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) (Fink-Gremmels 1993; Wood *et al.* 1999). As outlined later, studies have demonstrated that long-chain *n*-3 PUFAs, *e.g.* EPA and DHA, have potential benefits in relation to heart health, especially in those who have already had a heart attack. As a result of this, the UK Department of Health recommends that

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

intake of long-chain *n*-3 PUFAs should be at least 450 mg per day (SACN & COT 2004). Only a small amount of these long-chain *n*-3 PUFAs are found in meat, but there are few rich sources apart from oily fish and therefore meat can usefully contribute to intakes of these important fatty acids for those who consume little or no oily fish (Higgs 2000; Red Meat and Health Expert Advisory Committee 2001).

The content of PUFAs in meat is strongly influenced by the feeding regime of the animal (Wood *et al.* 1999). For example, meat from ruminant animals fed on grass throughout the year (as in northern Europe, Australia and New Zealand) has higher levels of PUFAs. This is because a small proportion of the major fatty acid in grass, α-linolenic acid, can escape hydrogenation in the rumen, and is absorbed into the tissue lipids. Meat will also provide long-chain *n*-3 PUFAs as a result of the transformation of dietary α-linolenic acid to EPA and DHA. Oil seeds, such as linseed and rapeseed are also high in α-linolenic acid, and therefore the meat of animals reared on feeds containing these will also contain higher levels of *n*-3 PUFAs (primarily α-linolenic acid) (Givens 2005).

Meat from ruminant animals is also a source of another naturally occurring fatty acid – conjugated linoleic acid (CLA). CLA is a collective term used to describe a mixture of positional and geometric isomers of linoleic acid. CLA isomers are intermediates in the bio-hydrogenation of linoleic acid and the majority of CLA is produced within the peripheral tissues from the rumen-derived fatty acid vaccenic acid. CLA is naturally found in small amounts in products from ruminant animals, *e.g.* lamb, beef, cheese and milk. Feeding practices influence the CLA content of meat. For example, concentrations of CLA in Irish and Australian beef can be 2–3 times higher than those in beef from the USA, reflecting the greater consumption of pasture throughout the year (Moloney, in press). Based largely on animal studies in rodents, there is interest in the potential health benefits of CLA in humans in connection with cancer, blood lipids and an influence on the lean : fat tissue ratio (in favour of lean tissue) (Calder 2002); however, no clear conclusions have yet been made with respect to human health. It is of interest that one of the two double bonds in most forms of CLA is in the *trans* configuration (Kelley & Erickson 2003; Stanner 2005).

The double bonds between carbon atoms in unsaturated fatty acids exist in a *trans* form or a *cis* form, which affects the way in which the fatty acid behaves. The usual form in nature is *cis*. *Trans* fatty acids (including CLA) are present in small amounts in ruminant meat. They are produced through the natural biological hydrogenation of *cis*-fatty acids, which occurs through bacterial action in the intestine of ruminant animals; vaccenic acid is recognised as the most common *trans* fatty acid produced. *Trans* fatty acids can also be formed from industrial processing (hydrogenation) of *cis*-fatty acids in oils and fats during the manufacture of products such as traditional margarine (many margarines and spreads are now processed differently to be virtually *trans* fat free), and are found in bakery and confectionery products which contain these partially hydrogenated oils. High intakes of *trans* fatty acids have been shown to

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

adversely influence the ratio of low density lipoprotein (LDL)-cholesterol to high density lipoprotein (HDL)-cholesterol, a recognised risk factor for cardiovascular disease (CVD) (see later). Therefore, dietary guidelines in the UK recommend an upper limit in the consumption of *trans* fatty acids of 2% of dietary energy intake (Department of Health 1994). It should be noted that the natural *trans* fats found in meat and milk from ruminant animals may not have such an adverse effect on blood lipid concentrations, although further research in this area is needed (BNF 1995a).

Changes in the fat content and fatty acid composition of meat

Advances in food processing technologies and breeding programmes, as well as modification of animal feeds and modern butchery techniques have all led to a reduction in the fat content of carcase meat over the past 15 years. For example, the fat content of carcase meat in the UK has been reduced by over 30% for pork, 15% for beef and 10% for lamb (Lee *et al.* 1995; Higgs 2000). New Zealand has seen similar changes, and in particular has worked with butchers to encourage the trimming of fat from red meat before sale, through the introduction of a 'Quality Mark'. These efforts have resulted in a 48% reduction of fat in beef, and a 43% reduction in lamb (Laugesen 2005).

Successful modification of the fatty acid profile of some carcase meat has also taken place by alterations in feeding practices. The fatty acid profile of non-ruminant meat is essentially a reflection of that in the diet (Givens & Shingfield 2004). For example, in mono-gastric animals such as pigs, inclusion of vegetable and fish oils in feeds has resulted in significant increases in *n*-3 PUFAs, particularly when compared with a traditional cereal-based diet, with the meat fat principally comprising SFAs and MUFAs. In ruminants, the composition of fat is less variable and it has been more difficult to modify the fatty acid profile of the meat, owing to the influence of gut bacteria (Higgs 2000).

A reduction in fat content has not been seen for all cuts of meat, but this may be because some fat is added back to the meat at retail level (*e.g.* some cuts of topside of beef for roasting) in some countries, such as the UK. There remains further potential to make greater fat savings in the meat sector (*e.g.* in meat products such as pies), as well as in other food sectors. Meanwhile, many consumers now recognise that by trimming the fat off meat, they can reduce the amount of fat they consume from a cut of meat (Leeds *et al.* 1997). Consumers can also modify the fat content of meat through preparation methods and cooking. For example, fat gains can occur as a result of the meat sitting in the fat used for frying, and deep frying with breadcrumb coating. Other methods of cooking, such as grilling or dry frying, can result in fat losses for meat products (Clausen & Ovesen 2005).

Micronutrient composition of red meat

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

Red meat contains an array of micronutrients, some in substantial amounts, which are required for general health and well-being. According to EU legislation, when a serving of a food contributes a sixth of the EU reference intake, it can be classified as a *source*. If a serving provides half of the recommended daily amount (RDA), it can be classified as a *rich source* (BNF 2002). It should be noted that the definition of the term *'source'* is different across the world, for example, in Canada a food is a *source* if it provides 5% of the recommended nutrient intake. In the EU, a 100 g serving of beef, pork or lamb can be classified as a *rich source* of vitamin B_{12}, and a *source* of niacin, vitamin B_6, zinc and phosphorus. The content of other B vitamins varies between the different meats. In this paper, particular attention has been paid to those nutrients for which red meat is regarded as a *source* according to EU regulations, and other micronutrients such as iron, of which red meat is an important dietary contributor.

Vitamins

The B vitamins work as cofactors in different enzyme systems in the body. In developed countries, intakes of B vitamins meet dietary requirements for the majority of individuals. However, as meat and animal-derived foods are the only foods that naturally provide vitamin B_{12}, some individuals who exclude such foods from their diet are at risk of inadequate intakes. Dietary intakes of vitamin B_{12} are consistently reported as being lower in vegetarian diets, and particularly low in vegan diets (Herbert 1988, 1994), thus indicating the important contribution of meat and animal-derived products in the diet to vitamin B_{12} intake.

Red meat contains a number of B vitamins: thiamin, riboflavin, pantothenic acid, folate, niacin, B_6 and B_{12} (Chan *et al.* 1995). In particular, in the EU red meat can be termed a *rich source* of vitamin B_{12} and makes an important contribution to B_{12} intake in a number of countries. For example, 35% of vitamin B_{12} in the Danish diet comes from meat and meat products and similar figures have been reported in the UK and New Zealand (Russell *et al.* 1999; Henderson *et al.* 2003b; Danish Institute for Food and Veterinary Research 2005a).

Vitamin D is essential for the development and maintenance of bone. Only a few foodstuffs contribute to intake of vitamin D, however, dietary requirements for most people are thought to be low as the majority of vitamin D comes from the action of sunlight on 7-dehydrocholesterol in the skin. Nevertheless, housebound people and those who wear concealing clothing are particularly reliant on a dietary supply of vitamin D, and meat may usefully contribute to dietary intake. It is thought that the vitamin in meat is derived from the action of sunlight on the skin of animals, or from the animals' feed (MAFF 1995). In particular, the vitamin D metabolite 25-hydroxycholecalciferol [$25(OH)D_3$] is found in significant quantities in meat and liver, and is assumed to have a high biological activity, resulting in better and faster absorption from the diet, compared with its parent compound (Groff *et al.* 1995). Furthermore, it has been suggested that components of meat protein may enhance the utilisation of vitamin D in humans, particularly where exposure to sunshine is limited. However, there is a need for more research in this area (Dunnigan & Henderson 1997).

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

Although the contribution that meat makes to vitamin D intake is well recognised, it is difficult to accurately assess intakes of vitamin D from the diet, as food composition tables differ in the way vitamin D is expressed (Gibson & Ashwell 1997; Clausen *et al.* 2003). According to the most recent UK National Diet and Nutrition Survey, meat and meat products (including poultry, processed meats and meat products) contribute 22% of daily intake of vitamin D (Henderson *et al.* 2003b). This is similar to reports elsewhere in Europe. For example, in Denmark meat and meat products (excluding poultry) contribute 26% of vitamin D intake (Danish Institute for Food and Veterinary Research 2005a). It is also difficult to assess the contribution that meat makes to *requirements* of vitamin D, owing to a substantial amount being derived from the action of sunlight on the skin.

There are small amounts of vitamin E present in meat. As this is a fat-soluble vitamin, concentrations of vitamin E will be higher in fattier cuts of meat. Vegetable oils are particularly high in vitamin E, and therefore the recent trend to include seed oils in animals' diets will have contributed to an increase in the vitamin E content of meat. Offal, particularly liver, is a good source of vitamin A, in the form of retinol. However, the amount present in liver can be variable and indeed very high, and will depend on the age of the animal and the composition of the feed consumed. As fat-soluble vitamins are not excreted by the body, very high doses can have adverse health effects. For example, there has been concern about high vitamin A intakes in relation to bone health (SACN 2005a).

Minerals

Meat is an important dietary source of bioavailable minerals and trace elements, in particular iron and zinc. Iron plays a major role as an oxygen carrier in haemoglobin in blood, or myoglobin in muscle, and it is also required for many metabolic processes. Dietary iron exists in two forms, haem and non-haem iron, with haem iron being more readily absorbed and utilised by the body. Most of the iron present in meat is in the haem form. Approximately 20–30% of haem iron is absorbed in the intestine, compared with only 7% of non-haem iron (BNF 1995b). In terms of the overall diet, meat and meat products provide 17% of total dietary iron intake in the UK (Henderson *et al.* 2003b) and up to 22% in New Zealand (Russell *et al.* 1999).

Iron deficiency is a worldwide problem, even in developed countries. For example, in New Zealand 45% of teenage girls and 42% of women aged 25–44 years are not achieving recommended iron intakes. In the UK, 25% of women (aged 19–64 years) are not reaching the lower reference nutrient intake (LRNI – the amount of nutrient that is sufficient to meet the needs of only 3% of the population) (Henderson *et al.* 2003b). A number of studies have confirmed the positive effect of including meat in the diet, on intakes of dietary iron. For example, a study by Gibson & Ashwell (2003) found an increased risk of low iron intake (below the UK LRNI) in those who ate the least meat and processed meat (≤90 g per day), compared with high consumers (≥140 g per day). Other studies have found similar positive effects

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

among different population groups, such as children (Nathan *et al.* 1996) and older men (Wells *et al.* 2003). However, this type of investigation is particularly difficult for a variety of reasons. For example, the iron content of the same type of meat may vary, this variation being due to the age of the animal at the time of slaughter, the diet of the animal and husbandry practices. The bioavailability of iron from a food is also of utmost importance, and can be influenced by other dietary factors, particularly in the case of non-haem iron (Hambraeus 1999). For example, phytate is recognised to inhibit the bioavailability of non-haem iron, and ascorbic acid enhances the bioavailability. Small amounts of meat, as it is one of the few foods that contribute to haem iron intake, are also recognised to enhance the absorption of non-haem iron from plant foods; it is understood that this is the effect of an active component in meat referred to as 'meat factor'.

Zinc is primarily associated with the activity of a wide variety of enzymes. Meat contains substantial amounts of zinc, and can be classified as a *source* of zinc, in line with EU regulations (BNF 2002). Importantly, the zinc contained in meat is present in a highly bioavailable form. In Ireland, meat and meat products provide approximately 41% of total zinc intake (Cosgrove 2005a). Although the contribution that meat makes to intakes of zinc is slightly lower in other countries, contributing 17% of zinc intake in France, and 31–34% of zinc intake in Denmark, UK and New Zealand (Russell *et al.* 1999; Credoc 2003; Henderson *et al.* 2003b; Danish Institute for Food and Veterinary Research 2005a). It is, therefore, not surprising that in a small-scale observational study ($n = 50$), zinc intake was significantly lower among female vegetarians, compared with meat-eaters (Ball & Ackland 2000). Similar findings were recorded in a large UK cohort study (Davey *et al.* 2003). Zinc intake among adult meat-eaters in this study was above the RNI level, in line with findings from the most recent UK National Diet and Nutrition Survey (Henderson *et al.* 2003b). However, there are some concerns regarding low intakes of zinc among some subgroups of the UK population. For example, in the most recent dietary survey of young people, 10% of girls and 5% of boys aged 7–10 years had zinc intakes below the LRNI. A low intake of zinc is also recognised as a problem among adolescents in the UK, with zinc intakes below the LRNI for 10% of girls aged 15–18 years (Gregory *et al.* 2000). Older adults have also been found to be at risk of low zinc intakes. In the most recent UK dietary survey of older adults, 15% of men and 10% of women aged 85 and over had an intake of zinc below the LRNI (Finch *et al.* 1998). As with dietary iron, a number of factors affect the bioavailability and absorption of dietary zinc, including the composition of the diet. For example, a small amount of lean beef (75 g per day) has been found to enhance iron and zinc utilisation in young women (Johnson & Walker 1992), whereas phytate is a well know inhibitor of zinc bioavailability.

Meat and meat products also contain useful amounts of magnesium, copper, cobalt, phosphorus, chromium and nickel. In particular, red meat usefully contains selenium, although the concentration will depend on the diet of the livestock and the soil in which the animal feed was grown. In New Zealand, meat (including chicken) and meat products contribute 23% of

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

selenium intake. Similarly in Denmark, red meat contributes 21% of intake (Russell *et al.* 1999; Danish Institute for Food and Veterinary Research 2005a). The proportion of selenium that meat contributes to the diet has not been measured in the UK since 1997 (when it contributed 23% of dietary intake). However, it is recognised that intakes of selenium in the UK have been decreasing over the past 20 years, as European wheat has replaced selenium-rich wheat from Canada and the USA and therefore meat may now contribute a larger proportion of selenium in the diet (BNF 2001; SACN 2005b). Low intakes of selenium have also been reported in France and Germany (SACN 2005b).

Processed meats and meat products that contain lower amounts of lean meat are likely to have a lower micronutrient content per 100 g, but may provide other nutrients not usually found in meat (*e.g.* carbohydrate and fibre). Also, the addition of some ingredients (*e.g.* soy, fibre) can be used to add functional properties to meat products that could offer potential benefits for health (Fernandez-Gines *et al.* 2005). It should be noted that the composition of different processed meats varies widely between countries. Overall, meat products and processed meats are more likely to have a higher content of sodium than lean meat. Sodium is added to meat products to enhance and modify the flavour, the physical properties and sensory attributes of the food, and to contribute to the preservation of the product (Matthews & Strong 2005). Owing to the adverse health effects associated with a high intake of sodium (see *Cardiovascular disease* section), work is underway within the UK to reduce the amount of salt in processed meat products and, in particular, products that contain the highest levels of sodium (Matthews & Strong 2005).

Summary

Meat and meat products can make an important contribution to nutrient intakes in the diet. They provide a number of essential nutrients, including protein, long-chain *n*-3 fatty acids, iron, zinc, selenium, vitamin D and vitamin B_{12}. In particular, some of these nutrients are more bioavailable in meat than alternative food sources, and intake is also recognised to be below recommendations for some population subgroups across Europe and in other developed countries.

Dietary and lifestyle factors associated with red meat consumption

There are relatively few large-scale studies that have looked at the dietary and lifestyle habits of meat-eaters compared with other dietary groups, such as vegetarians. However, this topic has recently been examined in two large UK cohorts: the EPIC-Oxford cohort (Davey *et al.* 2003) and the UK Women's Cohort Study (Cade *et al.* 2004).

The EPIC-Oxford (UK) study

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

The EPIC-Oxford study comprises a large cohort of 33 883 meat-eaters and 31 546 non meat-eaters. The participants were divided into four dietary groups: meat-eaters, fish-eaters, lacto-ovo-vegetarians and vegans, which were then compared for differences in nutrient intake and other lifestyle factors (Davey *et al*. 2003). The cohort comprises 22% men and 78% women within the age range 20–97 years (median age of 46 for men, 43 for women). Meat-eaters had the highest median age, followed by fish-eaters, then vegetarians and finally vegans (this pattern was found in both men and women). The difference in age between dietary groups may also partly explain some of the other differences in diet and lifestyle observed (see below).

With regard to other lifestyle characteristics, such as smoking, this was found to be a fairly health-conscious cohort, with 56% of men and 65% of women having never smoked; however, a smaller proportion of meat-eaters (51% of men and 63% of women) had never smoked compared with the other dietary groups. Age-adjusted mean body mass index (BMI) was found to be significantly different between the four groups. Meat-eaters had the highest mean BMI (24.9 kg/m^2 in men and 24.3 kg/m^2 in women) and vegans had the lowest mean BMI (22.5 kg/m^2 in men and 21.9 kg/m^2 in women) across all age groups, for both men and women, while fish-eaters and vegetarians had a similar, intermediate BMI.

As expected, large variations in nutrient intakes were found between the dietary groups. The nutrient intakes of meat-eaters tended to differ most markedly from vegans, with fish-eaters and vegetarians tending to fall in-between. These patterns were similar for both men and women (Davey *et al*. 2003). Table 5 summarises the main differences in nutrient intakes between the dietary groups (data shown for men only). Overall, mean energy intake was found to be 14% higher in meat-eaters than vegans, with fish-eaters and vegetarians having an energy intake in-between the other groups. Carbohydrate was found to provide approximately 50% of energy in both men and women, with mean intakes highest in vegans and lowest in meat-eaters. Conversely, mean protein intake was found to be highest in meat-eaters and lowest in vegans. Mean total fat intake was also highest in meat-eaters and lowest in vegans, although the differences between groups were small. The SFA intake (% energy) of meat-eaters was found to be more than twice that of vegans. On the other hand, vegans had a higher PUFA intake than meat-eaters, again with the other groups in-between.

Table 5. Mean daily nutrient intakes by diet group for men from the EPIC-Oxford study (Davey *et al*. 2003)

	Meat-eaters *n* = 6951	Fish-eaters *n* = 1500	Vegetarians *n* = 3748	Vegans *n* = 770	All *n* = 12 969
Energy (MJ)	9.18	8.9	8.78	8.01	8.96
% energy from	46.9	49.8	51.2	54.9	49.0

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

carbohydrate					
% energy from protein	16.0	13.9	13.1	12.9	14.7
% energy from fat	31.9	31.1	31.1	28.2	31.4
% energy from SFA	10.7	9.36	9.37	4.99	9.8
% energy from PUFA	5.21	5.64	5.67	7.53	5.53
Fibre (NSP) (g)	18.7	22.1	22.7	27.7	20.8
Retinol (μg)	740.0	337.0	306.0	74.2	529.0
Thiamin (mg)	1.69	1.8	1.9	2.29	1.8
Vitamin B_{12} (μg)	7.25	5.01	2.57	0.41	5.23
Folate (μg)	329.0	358.0	367.0	431.0	350.0
Vitamin C (mg)	119.0	130.0	123.0	155.0	125.0

NSP, Non-starch polysaccharides; EPIC, European Prospective Investigation into Cancer and Nutrition; SFA, saturated fatty acids; PUFA, polyunsaturated fatty acids.

Mean fibre intakes were substantially higher in vegans than meat-eaters (41% higher in vegan men and 36% higher in vegan women). Fibre intakes in fish-eaters and vegetarians were similar, with intermediate values. With regard to vitamin intake, meat-eaters were found to have the lowest intakes of thiamin, folate, vitamins C and E, but the highest intakes of retinol, vitamin B_{12} and vitamin D. Meat-eaters were also found to have the highest intake of zinc, but the lowest intake of magnesium. Intakes of iron were found to be similar across all diet groups, but with the highest intake in vegans. However, it is important to consider that the bioavailability of iron from plant sources (non-haem iron) is much lower than that from animal sources (haem iron).

There may have been some degree of under-reporting of energy and nutrient intake, however, owing to the methodology used for measuring dietary intake [food frequency questionnaire (FFQ) with standard portion sizes] and this may partly explain the lower energy intake observed in vegetarians and vegans compared with meat-eaters. Non-meat-eaters may eat larger portions of staple foods, such as bread and potatoes, which would not have been taken into account using standard portion sizes. Data on the use of supplements were not incorporated

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

into this analysis, however, 45% of men and 61% of women in this cohort reported taking dietary supplements, and this would clearly have an impact on nutrient intakes.

The UK Women's Cohort Study

The UK Women's Cohort Study (UKWCS) comprises a total of 35 372 women aged 35–69 years at recruitment who were selected to ensure a wide range of dietary intakes, in order to optimise exposure to dietary factors of interest. The cohort was divided into four dietary groups, defined as follows (Cade *et al*. 2004):

Meat-eaters – eat meat once per week or more.

Oily fish-eaters – eat oily fish 2–4 times per week and meat less than once per week.

Other fish-eaters – eat fish once per week or more and meat less than once per week (and eat oily fish less than 2–4 times per week).

Vegetarians – eat meat or fish less than once per week (includes vegans).

Table 6 shows the baseline characteristics of the cohort by diet group. This cohort was not representative of the British middle-aged female population, with the majority of the cohort being white, middle-class and generally well-educated. They were also found to be fairly health-conscious with only 11% of the cohort being current smokers (8% smoking daily) and 58% taking dietary supplements.

Table 6. Characteristics of the UKWCS cohort by diet group (Cade *et al*. 2004)

	Meat-eaters(*n* = 24 738)	Oily fish-eaters(*n* = 870)	Other fish-eaters(*n* = 3286)	Vegetarians(*n* = 6478)	Total sample (*n* = 35 372)
Mean age (years)	54	51	50	49	52
Mean BMI (kg/m^2)	25.0	23.2	23.3	23.3	24.5
Educated to degree level (%)	23	32	37	37	27
Smokes daily (%)	9	6	7	7	8
Full-fat milk	13	6	10	10	12

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

(%)					
Supplements (%)	55	73	67	62	58

BMI, body mass index; UKWCS, UK Women's Cohort Study.

Overall, meat consumption (red meat and poultry) in the total cohort was found to be lower than in the general population, at a mean of 5 portions per week. The meat-eaters were slightly older, had a higher BMI, were less likely to have a degree and were more likely to smoke daily than the other dietary groups. Meat-eaters were also found to have more self-reported illness than the other groups, they were also more likely to use full-fat (whole) milk, but less likely to take dietary supplements than the other groups. All differences found were statistically significant, due to the large sample size (Cade *et al.* 2004).

Meat-eaters reported consuming the lowest number of servings of fruit or fruit dishes (10 per week) and vegetables or vegetable dishes (11 per week) compared with the other diet groups. With regard to nutrient intakes, the patterns found were similar to those observed in the EPIC-Oxford cohort (Davey *et al.* 2003). Although, in this case, the oily fish-eaters were found to have the highest total energy intakes, and the vegetarians the lowest, resulting in higher nutrient intakes in the oily fish group. Meat-eaters had the highest percentage of energy from protein and fat, as was the case for the EPIC-Oxford cohort, whereas the vegetarians had the highest percentage of energy from carbohydrate. Meat-eaters were also found to have the lowest intakes of carbohydrate, fibre, vitamin C, folate, calcium and iron, but the highest intakes of vitamin A and zinc, compared with all other groups.

One further study, which has examined differences in dietary patterns between high and low consumers of meat, is a Swedish Cohort Study of 11 648 subjects from the city of Malmo in Sweden (Elmstahl *et al.* 1999). Food and nutrient intake was assessed in this group using a combination of a 7-day menu book and an FFQ. Increasing red meat intake (adjusted for energy) was found to be associated with decreasing intake of fish, poultry, fruit, bread, cereals and cheese in both men and women. Meat intake was also found to be negatively correlated with vitamin C and fibre intake. The authors concluded that meat consumption was negatively associated with intake of food groups that are rich in antioxidants and fibre, and that the positive associations found between meat intake and certain types of cancer may be more related to this association than to components in meat *per se* (Elmstahl *et al.* 1999).

Summary

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

This small group of cohort studies (with large sample sizes) has highlighted some distinct differences in the diet and lifestyle characteristics of meat-eaters, compared with other dietary groups. In particular, meat-eaters seem to be generally older, have a higher BMI (which may be linked) and are more likely to smoke compared with non meat-eaters. As might be expected, nutrient intakes were found to differ between meat-eaters and other dietary groups. Meat-eaters were found to have higher protein, total fat and SFA intakes and lower carbohydrate and fibre intakes than non meat-eaters, and their consumption of fruit and vegetables was also lower. Marked differences in micronutrient intakes were also found.

However, it is noteworthy that these cohort studies do not differentiate between consumers of unprocessed and processed meat. A recent cross-sectional study of Irish adults indicates that it is important to distinguish between meat groups as there is a large variation in dietary quality between consumers of red meat, white meat and processed meat. For example, increasing processed meat intake has been found to be associated with a lower intake of wholemeal bread, fruit and vegetable and fish intake and poorer overall dietary quality (see Cosgrove *et al*. 2005b).

The variation in diet and lifestyle characteristics between meat-eaters and non meat-eaters may have important implications for associations between meat consumption and health and disease, which are discussed in the following section. However, vegetarians represent only a small minority of the population, for example in the UK it is estimated that around 3–7% of the population are vegetarian (Phillips 2005). Furthermore, the mortality rates of vegetarians are similar to those of comparable non-vegetarians, suggesting that much of this benefit may be attributed to non-dietary factors, such as lower prevalence of smoking or to other aspects of the diet other than the avoidance of meat and fish, *e.g.* higher intake of fruit and vegetables (Appleby *et al*. 2002).

Health aspects of red meat

Epidemiological studies

Nutritional epidemiology provides a means for identifying relationships between dietary factors and health in human populations consuming usual amounts of foods and nutrients. The main purpose is to identify potential causes of disease so that these can be modified in order to reduce the burden of disease. For a dietary factor to be the cause of ill-health, it must be demonstrated to occur before the onset of illness and its effect must not be due to chance, or some other confounding factor. Causality can only be tested in a carefully controlled experiment, however, where the exposure is changed and all other factors are held constant. A clinical trial or randomised controlled trial (RCT) attempts to do this. This type of approach is not always possible though and most studies are observational – where there is no intervention but researchers assess differences in the exposure and outcome of interest to see

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

whether a relationship exists. Although such studies cannot provide evidence of causality, they can be used to identify dietary factors that may be involved in the onset of disease.

There are four main types of observational study: ecological, cross-sectional, case-control and cohort (or prospective) studies. Each of these different types of studies has its limitations, but there is an accepted hierarchy of evidence (ecological < cross-sectional < case-control < cohort). Ecological studies compare patterns of disease in different populations with very different diets (such as different countries) but the problem with these types of studies is that there are many more potential determinants of disease other than the dietary factor under consideration, which may vary in prevalence in different parts of the world. In cross-sectional studies of individuals, both the dietary factor and disease, or risk of disease, are measured at the same time, which means it is not possible to assess whether any difference in diet occurred before, or as a consequence of, the disease. Case-control studies compare past dietary exposure to a particular dietary factor between groups of individuals with and without the disease outcome. The problem with these types of studies is that they are susceptible to recall and selection biases, in that people with the disease may recall their diet differently from healthy individuals. Cohort studies do not have this problem and therefore their findings are considered to be more reliable, however, these studies take a long time and are expensive to carry out. For this reason, cohort studies often rely on surrogate markers of a disease, *e.g.* cholesterol levels as a marker of heart disease risk.

Dietary data collection is another challenging aspect of nutritional epidemiology – accuracy in measurement of food and nutrient intake is particularly important in order to detect true associations. The more detailed method of using a 7-day food diary is considered to be more accurate than using an FFQ to assess dietary intake (see Bingham *et al.* 1994). Most prospective studies use FFQs to assess dietary intake as it would not be practical to use a more detailed method on a large cohorts of participants. However, it is recognised that all measures of diet have sources of bias (see Winkler 2005).

Cancer

Over the past 30 years, many studies have been published looking at associations between dietary factors and cancer incidence. The earlier studies mainly used a case-control design, while more recent studies have used a prospective cohort design. Very few intervention trials have been carried out looking at diet and cancer, mainly for methodological reasons, as people would have to be followed for very long periods of time and therefore compliance with any dietary intervention is likely to be low. It would also be unethical to ask participants to follow a diet that is hypothesised to lead to an increased risk of cancer. A small number of RCTs have looked at fibre, fruit and vegetable and fat intake in relation to the recurrence of colorectal adenomas (*e.g.* Schatzkin *et al.* 2000). This section has therefore focused on the evidence from recent prospective cohort studies.

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

Devising and implementing cohort studies to identify associations between diet and cancer is a complex and difficult process. Incidence rates for even common cancers are low, so cohort studies need very large sample sizes. Genetic factors also contribute to cancer risk and to identify the interaction between genetics and cancer, even larger cohorts are required (at least 100 000 people). At least 1000 cases of a particular cancer are needed to accurately identify the combined effect of relatively common risk factors, *e.g.* gene variant and lifestyle factors.

Most of the published literature on meat in relation to cancer development has focused on colorectal cancer (CRC). There have been some studies investigating possible associations between meat and other types of cancer, including gastric, breast, prostate and kidney cancers and cancer of the pancreas, however, the evidence in relation to these other types of cancer has been found to be weak or inconsistent (Department of Health, UK 1998; Key *et al.* 2004). This paper will therefore concentrate on the evidence in relation to meat and CRC risk.

CRC incidence

CRC is the third most common cancer in the world. In 2002, CRC was estimated to account for over a million new cancer cases worldwide (9.4% of all cancer cases) (Ferlay *et al.* 2004). Genetic changes associated with CRC are well established and inherited mutations in key genes are considered to be responsible for about 20% of cases. The remaining 80% of cases are sporadic (*i.e.* arise spontaneously) and appear to be influenced by environmental and lifestyle factors, such as diet and physical activity level. This is also supported by the findings of epidemiological studies (see below).

Incidence rates for CRC are approximately 10-fold higher in developed compared with developing countries (Ferlay *et al.* 2004). Changes in incidence rates have also been noted in populations over time. CRC was very rare in Japan in the 1960s; however, there has been an almost five-fold increase in CRC in Japanese men over the past 30 years, and incidence in Japanese men aged 55–60 is now twice that of men in the UK. This cannot all be attributable to genetic effects as there cannot have been such a rapid change in the Japanese gene pool over such a short period of time. Environmental factors such as diet therefore must play a part (Bingham & Riboli 2004).

Diet-related risk factors for CRC

It has been suggested that the contribution of diet to CRC incidence could be as much as 80% (Willett 1995). If this is the case, it means that the majority of CRC cases may be preventable. Few specific diet-related factors have been shown unequivocally to contribute to the pathogenesis of CRC. However, there is a general consensus that some aspects of the Western diet increase the risk of CRC, owing to the large variation in incidence between developed and developing countries (WHO 2003). Table 7 shows diet-related risk factors for which there is evidence of an association with CRC risk. Also, it has been shown that risk factors tend to

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

cluster, so individuals who are obese will often be physically inactive, smoke and consume low levels of fruit and vegetables and high levels of meat (Potter 1999).

Table 7. Diet-related risk factors for CRC (from Key *et al*. 2004)

Diet-related risk factor	Level of evidence
Overweight/obesity	Increases risk (best established diet-related risk factor)
Physical activity	Consistent association with reduced risk
Fruit and vegetables	*Probably* decreases risk
Red and processed meat	*Probably* increases risk
Alcohol	*Probably* contributes to a small increase in risk

CRC, colorectal cancer.

There is some evidence that several other diet-related factors may be associated with a reduced risk of CRC, including dietary fibre, folate, calcium and vitamin D. Evidence in relation to folate, calcium and vitamin D intake has not been firmly established, however, recent findings from the EPIC study have shown dietary fibre to be inversely related to CRC incidence (Bingham *et al*. 2003). Further detail on the EPIC study is given later in this section.

Meat and CRC

There has been a great deal of scientific interest in the association between red and processed meat intake and CRC over recent years, which has generated a number of reviews, including two large reports, one from the World Cancer Research Fund (WCRF) and another from the UK Committee on Medical Aspects of Food and Nutrition Policy (COMA). Both panels agreed that the results of epidemiological studies were not consistent. The conclusion of the WCRF (1997) report was that 'The evidence shows that red meat *probably* increases risk and processed meat *possibly* increases risk of colorectal cancer'. A new analysis from the WCRF is expected in 2006/7.

The COMA report (Department of Health, UK 1998) concluded that 'there is moderately consistent evidence from cohort studies of a positive association between the consumption of red or processed meat and risk of colorectal cancer'. This report recommended that the current, average level of red and processed meat intake in the UK should not increase. Those with high levels of intake (>140 g per day) were recommended to reduce their consumption.

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

In the UK, the incidence of CRC has increased substantially over the past 35 years, yet red meat intake has declined by around 25% over the same period. A similar pattern has been seen in other European countries, such as Norway, where the risk of CRC has increased by 50% over the same period (Hill 2002).

Table 8 shows the mean daily intake of different types of meat for 10 European countries from the EPIC study (women only shown) together with CRC incidence for each country. Meat consumption in the UK was found to be less than in many Mediterranean countries, such as Spain and Italy, and yet CRC incidence is higher in the UK than several southern European countries.

Table 8. Variation in meat consumption and CRC incidence across 10 EPIC countries

	Mean daily intake (g/day)					CRC incidence (ASR)
	All meat	Red meat	Poultry	Sausages	Other processed	
Denmark	88.3	44.1	16.8	15.6	9.8	19.2
Norway	88.6	28.5	10.6	22.9	23.6	16.8
Germany	84.3	28.6	13.0	29.4	11.5	15.7
the Netherlands	92.7	41.0	12.4	16.4	21.5	14.4
UK	72.3	24.6	24.0	9.3	13.0	12.4
France	106.0	44.4	21.8	12.2	17.8	11.8
Spain	99.2	37.8	24.4	13.1	16.5	11.3
Sweden	92.3	32.3	9.3	21.3	22.0	11.1
Italy	86.1	40.8	20.2	9.52	10.1	10.9
Greece	47.1	25.5	11.8	3.1	2.8	8.0

Data shown taken from EPIC calibration study (women only). Data adjusted for age, day of the week, season (Linseisen *et al*. 2002). CRC Incidence reported as age standardised (world) rate (ASR) per 100 000 (Ferlay *et al*. 2004).

CRC, colorectal cancer; EPIC, European Prospective Investigation into Cancer and Nutrition.

Table 9 is a summary of the main cohort studies carried out up to the year 2000 looking at meat consumption in relation to CRC risk (14 studies in total). These studies form the basis of two recent meta-analyses carried out by Sandhu *et al*. (2001) and Norat *et al*. (2002).

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

Table 9. Meat consumption and CRC: cohort studies (adapted from Sandhu *et al*. 2001 and Norat *et al*. 2002)

Author (year published)	Country/cohort details	No. in cohort	No. ofcases CRC	Type of meat	Partition	Relative risk (highest *vs.* lowest intake)	Signifi-cance
Phillips & Snowdon (1985)	Seventh-Day Adventist, USA	25 439	172	All meat	Freq./week	0.9	NS
Hirayama (1990)	Japan	265 113	725	All meat	Daily/occasional/ rare/none	Only reported by subsite	N/A
Willett *et al*. (1990)	Nurses' Health Study, USA	88 751	150	Red meat	Freq. per week/month	2.49	Sig.
				Processed meat	Freq. per week/month	1.21	NS
Thun *et al*. (1992)	Cancer Prevention Study, USA	5 746	1150	Red meat	g/day	Males 1.21	NS
						Females 1.05	NS

Note that the significance relates to the highest *vs.* the lowest level of intake. In some cases, there was a significant *P*-value for trend.

NS, not significant; Sig., significant; Freq., frequency; BMI, body mass index; CRC, colorectal cancer.

Sandhu *et al*. (2001) included in their review both published and unpublished prospective cohort studies that showed risk estimates for CRC associated with meat consumption. The definition of meat included lamb, beef and pork (some of the studies also included white meat, but where possible, inclusion of this was avoided) and processed meat included sausages, burgers, ham, bacon and other meat products. Studies that only classified people according to whether or not they ate meat were excluded, as the level of exposure in those who ate meat

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

could not be quantified. Thirteen studies were found to be eligible for inclusion in the meta-analysis.

Pooled results showed that a daily increase of 100 g in total or red meat intake (*i.e.* a substantial increase, see Table 8) was associated with a significant increase in CRC risk of 12–17%. In the case of processed meat, a daily increase of 25 g was associated with a significant 49% increased risk. Therefore, the review found an overall positive association between meat consumption and CRC risk, although there was marginally significant heterogeneity between studies (this could undermine the conclusions, if it were more significant). Evidence of publication bias among these studies was also found, *i.e.* there may be further studies that failed to find an effect that were not published. The review also suggests that processed meat intake may be a risk factor for CRC. However, the authors point out that only a few of the studies reviewed investigated the *independent* effect of meat intake and therefore the possibility that the overall association may be confounded or modified by other variables cannot be excluded (Sandhu *et al.* 2001).

The following year, Norat *et al.* (2002) published a meta-analysis of both case-control and cohort studies published between 1973 and 1999 which showed high intakes of both red and processed meat to be associated with an increased risk of CRC. Total meat intake (*i.e.* inclusive of poultry) was not found to be associated with increased risk. The review calculated a significant mean 35% increase in risk of CRC for the highest *vs.* the lowest level of red meat consumption. Additionally, the review calculated a significant mean 31% increase in risk for the highest *vs.* the lowest level of processed meat intake. A dose–response meta-analysis of relative risk was also carried out, which found the strongest association to be for processed meat. Consumption of 120 g per day of red meat (compared with no consumption) was estimated to be associated with a 24% increase in risk, while consumption of 30 g per day of processed meat (compared with no consumption) was estimated to lead to a 36% increase in risk of CRC (Norat *et al.* 2002).

Average intakes of red and processed meat in a number of European countries are shown in Table 1. For example, in Denmark men consume an average of 69.6 g of red meat and 51.9 g of processed meat per day (Linseisen *et al.* 2002). The authors are also careful to point out, however, that their estimates are based on a single dietary risk factor, and that individual dietary factors may not contribute independently to increased risk. Other factors, such as fruit, vegetable and fibre intake and physical activity are also important and risk of cancer may be more effectively reduced by tackling all diet-related and lifestyle risk factors together (Norat *et al.* 2002).

Some researchers have questioned the link between meat intake and CRC risk. Truswell (2002) reviewed 30 case-control studies from 16 different countries published up to 1999 and highlighted the fact that 20 of these showed no significant association between red meat intake and CRC risk, while four showed a clear association and six showed an association in only some

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

subgroups. Moreover, out of 15 cohort studies reviewed, significant associations between red meat intake and CRC risk were only found in three (Truswell 2002). The lack of significant associations may be attributable to small sample sizes, however, which can be overcome by carrying out a meta-analysis (*e.g.* Norat *et al.* 2002).

Truswell highlights the pooled analysis of cohort studies carried out by Key *et al.* (1998) showing that in five groups of vegetarians *vs.* socially matched controls (from three different countries), no difference in mortality rates from CRC was found between vegetarians and non-vegetarians (Truswell 2002). However, there were no quantitative estimates of the amounts of meat consumed by omnivores in these cohort studies (Bingham 1999).

Hill (2002) suggests the following explanation for these apparently contradictory findings. In the large prospective study carried out by Hirayama (1990), dietary data were collected at recruitment and regular follow-up stages, and therefore a large amount of dietary information was gathered. The data were stratified into four intake groups for meat (daily, often, sometimes and never consumed) and similarly analysed for vegetable intake. Daily meat consumers were found to have a much higher incidence of CRC than those who never consumed meat, with the other two groups intermediate. But simply looking at meat hides more complex relationships. For those individuals who never ate vegetables, meat intake was positively associated with CRC, however, for those who consumed green-yellow vegetables daily, there was an inverse association between meat intake and CRC risk. This suggests that meat intake may only be a risk factor in those who do not eat sufficient amounts of foods that are considered to be protective (Hill 2002).

Further support for this hypothesis comes from a prospective study carried out in America with a cohort of the Adventist Health Study (Singh & Fraser 1998). Singh and colleagues found that individuals with a high meat intake, a low intake of legumes (pulses) and a high body mass showed a three-fold increase in risk of colon cancer, relative to other patterns of these variables.

This may also help explain why many Mediterranean countries, which have a higher meat intake than the UK (see Table 8), have lower rates of CRC mortality, since vegetable and fibre intake tends to be much higher in Mediterranean countries than in northern European countries. Moreover, this could also help explain why meat intake only appears to be a risk factor in the highest intake groups (*i.e.* more than 140 g per day) as this level of intake could override the effect of protective factors provided by plant foods in the diet (Hill 2002).

Since the papers by Sandhu *et al.* (2001) and Norat *et al.* (2002), a number of other prospective studies have been published, the findings of which have been summarised in Table 10. A prospective study of American women from the Breast Cancer Detection Demonstration Project (BCDDP) cohort found no significant association between red or processed meat intake and CRC incidence (Flood *et al.* 2003). But in a combined analysis of

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

data from the Nurses' Health Study and the Health Professionals Follow-up Study, intake of beef, pork or lamb as a main dish, as well as processed meat intake were found to be significantly associated with colon cancer (but not rectal cancer) risk (Wei *et al.* 2003). A study published the following year in a cohort of Australians (The Melbourne Collaborative Cohort Study) found both fresh red meat and processed meat to be significantly associated with an increased risk of CRC; when data were analysed by cancer subsite, most of this association was found to be with rectal cancer (English *et al.* 2004). In a study of Swedish women (The Swedish Mammography Cohort), red meat was found to increase the risk of CRC, while no association was reported for processed meat intake. Again, data were analysed by cancer subsite, which showed red meat to be significantly associated with an increased risk of cancer of the distal colon (Larsson *et al.* 2004).

Table 10. Meat consumption and CRC: cohort studies published since 2002

Author (year published)	Country/cohort details	No. in cohort	No. of casesCRC	Type of meat	Partition	Relative risk (highest *vs.* lowest intake)	Signifi-cance	Adj
Flood *et al.* (2003)	Breast Cancer Detection Demonstration Project Follow-up Cohort, USA	45 496	487	All meat	g/1000 kcal	1.05	NS	Ene (ot var cor but to l cor
				Red meat	g/1000 kcal	1.1	NS	
				Processed meat	g/1000 kcal	1.0	NS	
Wei *et al.* (2003)	Nurses' Health Study and Health Professionals Follow-up Study, USA (combined analysis)	134 365	1478	Beef, pork or lamb as main dish	Servings/day	Only reported by subsite	N/A	Age BM sm phy act fan his alc fol cal
				Processed meat	Servings/day			

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

Note that the significance relates to the highest vs. the lowest level of intake. In some cases, there was a significant *P*-value for trend.

F&V, fruit and vegetable; NS, not significant; Sig., significant; Freq., frequency; CRC, colorectal cancer; EPIC, European Prospective Investigation into Cancer and Nutrition; SFA, saturated fatty acid.

More recently, Chao *et al.* (2005) published a paper looking at the effect of long-term meat consumption on the risk of incident colon and rectal cancers in a large cohort of adults living in America. Information on diet was collected using a questionnaire in 1982 and again using a validated FFQ in 1992/3. Red and processed meat consumption reported in 1992/3 was not shown to be significantly associated with CRC risk, in a model adjusting for age, lifestyle factors, energy intake and other dietary factors (see Table 10) (Chao *et al.* 2005).

The most recent paper to be published in this area is from the EPIC study (Norat *et al.* 2005). EPIC is the largest prospective study ever undertaken to investigate the relationship between diet and cancer, with 520 000 participants taking part from 10 European countries. This is sufficient size to investigate even the rarest cancers and provides enough statistical power to investigate any interactions, for example with genetic polymorphisms. This size of EPIC also enables between-person variability in food habits to be considered. Measurement error is further minimised by correcting results using more detailed dietary estimates from a subsample of the cohort (Bingham & Riboli 2004).

Sufficient cancer cases have now accumulated to look for links between diet and CRC. The recent EPIC paper looked at the relationship between red and processed meat, poultry and fish intake and CRC incidence. Processed meat was shown to be significantly associated with an increased risk of CRC, with higher intakes being associated with increasing incidence. The hazard ratio (HR) for the highest (>80 g per day) *vs.* the lowest (<10 g per day) level of processed meat intake was found to be 1.42 (95% CI: 1.09–1.86). Red meat intake was also found to be positively, but not significantly, associated with CRC incidence (see Table 10). When red and processed meat were analysed together, intake was shown to be significantly associated with CRC risk. The HR for the highest (>160 g per day) *vs.* the lowest (<20 g per day) level of red and processed meat intake was found to be 1.57 (95% CI: 1.13–2.17), although this association was no longer significant after adjustment for other covariates (HR = 1.35, 95% CI: 0.96–1.88). No association was found with intake of poultry, while fish was found to have a protective effect (Norat *et al.* 2005).

Previous EPIC data have found dietary fibre intake to be significantly and inversely associated with CRC and colon cancer incidence (but not rectal cancer). It has been demonstrated that, in populations with a low average intake of fibre, an approximate doubling of fibre intake (to 35 g per day) is associated with a 40% reduction in CRC risk (Bingham *et al.* 2003). These results

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

have subsequently been confirmed in a more recent analysis with a larger number of cases (Bingham 2005).

The recent EPIC paper also looked at whether the increased risk of CRC with high intakes of red and processed meat could be partially explained by low fibre intakes. Those participants with a high intake of red and processed meat who also had a high (>26 g per day in women and >28 g per day in men) intake of fibre were shown to have a lower risk of CRC (HR = 1.09, 95% CI: 0.83–1.42) than those with a high intake of red and processed meat and a medium or low (<17 g per day) intake of fibre (HR = 1.5, 95% CI: 1.15–1.97). In fact, the risk reduction associated with a high fibre intake was shown to be similar for all levels of red and processed meat intake (Norat *et al.* 2005). These results suggest that the increased risk which appears to be associated with high intakes of red and processed meat is attenuated in individuals who include plenty of dietary fibre from fruit, vegetables and wholegrain cereals in their diet.

Proposed mechanisms

A number of plausible mechanisms for the association between red and processed meat intake and CRC incidence have been suggested. However, so far, none of these potential mechanisms has been definitively established (Key *et al.* 2002).

The most plausible mechanisms identified so far to explain why red meat intake may be a risk factor for colorectal carcinogenesis, involve the meat-related mutagens heterocyclic amines, polycyclic aromatic hydrocarbons and N-nitroso compounds (Cross & Sinha 2004).

Heterocyclic amines (HCAs) are formed from amino acids, creatine, creatinine and sugars, when food is cooked at high temperatures, *e.g.* frying. Several studies have suggested a link between HCAs and CRC. However, at normal levels of human intake, it is unlikely that they could be the sole causative factor. Much higher levels of intake are required to induce cancer in animal models, although species differences must be taken into account. Furthermore, chicken is a major contributor to HCA intake and yet poultry consumption has not been found to be associated with CRC risk (Norat *et al.* 2005).

Polycyclic aromatic hydrocarbons (PAHs) are the by-product of the incomplete oxidation of organic materials. This occurs in food when meat is cooked over a flame and the juices or fat drip onto the heat source, which results in the flames containing PAHs that adhere to the surface of the food. However, the evidence for the role of PAHs in CRC is weaker than for HCAs (Cross & Sinha 2004).

Another possible mechanism is *via* the endogenous production of N-nitroso compounds (NOCs). NOCs are potent carcinogens which have been detected in small amounts in foods, *e.g.* processed meats containing nitrite. They can also be formed within the body from the nitrosation of amines, amides and urea, following high-protein diets. It was initially suggested that high red meat diets lead to an increase in the amount of partially digested meat residues

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

available for nitrosation in the large bowel (Bingham *et al.* 1996). However, recent human feeding studies have demonstrated a significant, dose-dependent increase in the endogenous formation of NOCs following high red meat diets (Bingham *et al.* 1996; Hughes *et al.* 2001) which is not observed when equivalent amounts of fish, white meat or vegetable protein are fed (Hughes *et al.* 2001; Cross *et al.* 2003). This suggests that the increase in NOCs measured in the faeces can be attributed to a specific effect of red meat. A subsequent study has specifically identified haem iron as the component in red meat that may be responsible for the increased levels of endogenous nitrosation (Cross *et al.* 2003). However, there are many thousands of NOCs and not all of them are carcinogenic. More research is needed to determine whether there is any risk associated with these NOCs present in faeces.

Gene–nutrient interactions

One question of interest is whether or not an individual's risk of cancer, resulting from his or her diet, is altered by inherited polymorphisms. For example, an individual who inherits a polymorphism that confers the fast-acting form of an enzyme that converts a carcinogen (*e.g.* in overcooked meat) into the active form, would be particularly susceptible to cancer, but *only* if he or she also consumes a diet high in red meat (Bingham & Riboli 2004). Some studies have suggested that individuals with the rapid variant of N-acetyltransferase 2, who also eat a lot of red meat, may be more susceptible to the development of CRC than those with the slow variant of this enzyme who eat a lot of red meat. Similarly, individuals inheriting the active forms of the cytochrome P450 enzyme involved in the activation of NOCs, are at a 40% increased risk of CRC (Le Marchand *et al.* 2002). Further data are required to clarify this association (Key *et al.* 2002).

It has been suggested that future studies investigating the link between diet and CRC, or other forms of cancer, should focus on the genetic polymorphisms that affect the risk of cancer development. This approach has its own intrinsic problems, however. Genes can have numerous polymorphisms and different groups of carcinogens can be found in a single food. Also, several genes may be involved in the regulation of particular enzymes. All of these factors could have different effects on the development of a particular type of cancer. Although cancer is a disease of genes, there is conclusive evidence that environmental and lifestyle factors are the predominant cause of somatic gene alterations that lead to most sporadic cancers. It is therefore unlikely that the identification of individual genomes can provide the best estimate of cancer risk, without detailed information on environmental exposure from large-scale epidemiological studies (Bingham & Riboli 2004).

Summary

The two meta-analyses carried out by Sandhu *et al.* (2001) and Norat *et al.* (2002) provide evidence from the combined results of cohort (and case-control) studies of an association between high red and processed meat intake and CRC risk. This is supported by the results of

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

some more recent large prospective studies, such as the EPIC cohort. EPIC results also indicate that processed meat intake may be a stronger risk factor for CRC than red meat intake (Norat *et al.* 2005). Although the results of prospective studies have not always been consistent, no studies so far have shown red or processed meat intake to be protective against CRC. Indeed, studies have consistently shown a positive association between high red and processed meat intake and CRC risk, even if this association is not always statistically significant.

The underlying mechanism for this association is still uncertain, although recent human intervention studies have suggested that the endogenous N-nitrosation arising from ingestion of haem iron (not inorganic iron or protein) could be the most likely mechanism (see Cross *et al.* 2003). This would also help explain why poultry, which contains much lower amounts of haem, is not associated with increased CRC risk. It has been suggested that processed meat intake is more strongly associated with CRC risk than unprocessed red meat, however, there is currently no straightforward mechanism that could explain this. Nitrites or nitrates added to meat during processing could increase exogenous exposure to nitrosamines and other NOCs, but not all processed meats contain added nitrites (Norat *et al.* 2005).

It is, however, important to put these findings in context. Red and processed meat intake is only one of a number of potential risk factors for CRC. Indeed, the most established diet-related risk factors for CRC are overweight and obesity and low physical activity level. Therefore reducing the risk of CRC involves modification of a number of dietary and lifestyle factors including maintaining a healthy bodyweight, increasing physical activity level and consuming more fruit and vegetables and dietary fibre (see Slattery 2000).

Moreover, whether the consumption of meat is an *independent* risk factor for CRC is still not certain. Colorectal carcinogenesis is a multi-step process involving many different factors; a number of biological pathways may be involved and an accumulation of alterations to DNA occur. It is therefore unlikely that factors determining CRC risk act in isolation. Furthermore, because not all studies have actually examined the independent effect of meat intake, it is not possible to exclude the possibility that the association may be confounded by other dietary, genetic or associated factors (Sandhu *et al.* 2001). Further studies with long-term follow-up, repeated measures of diet, more in-depth consideration of dietary patterns, more detailed measures of cooking methods, and genetic markers of susceptibility may therefore be required (Willett 2005).

Nevertheless, a picture seems to be emerging that patterns of food intake are important, *i.e.* any increased risk associated with a high intake of red and processed meat appears to be lessened if dietary fibre intakes are increased (see Norat *et al.* 2005). Thus lean red meat is unlikely to significantly increase the risk of CRC when consumed in moderation and as part of a healthy, balanced diet that includes plenty of fibre from fruit, vegetables, pulses and wholegrain cereals.

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

Cardiovascular disease

Cardiovascular disease (CVD), which includes coronary heart disease (CHD) and stroke, is the leading cause of death worldwide, accounting for 18 million deaths each year. It has a multifactorial aetiology. Although there are a number of unmodifiable risk factors, including a genetic predisposition, many of the major causes such as hypertension, obesity, diabetes, high blood cholesterol and high blood triglyceride concentrations are modifiable by diet, along with other lifestyle factors (*e.g.* physical activity and smoking cessation) (Stanner 2005).

Despite the presence of a number of potentially protective nutrients (*e.g.* selenium, *n*-3 fatty acids, B vitamins), meat has often been assumed to be a contributor to increased risk of heart disease because of its relatively high contribution to fat intakes and its perceived high content of SFAs. Prospective studies have demonstrated a positive association between meat and CVD risk, but any causal link remains to be established. For example, Fraser (1999) reported a significant association between beef consumption and fatal CHD in a male cohort of Seventh-Day Adventists, with those eating beef up to three times a week having a 1.9-fold increased risk, and those eating beef three or more times a week having a 2.3-fold increased risk, compared with vegetarians. However, the study found no association in women and no significant increase in risk of non-fatal myocardial infarction (MI). Subdivision of subjects in a pooled analysis of vegetarian studies into meat-eaters (those eating meat more than once a week), occasional meat-eaters (less than once a week), fish-eaters and vegetarians, demonstrated the death rate ratio to be significantly below 1 for occasional meat-eaters, fish-eaters and vegetarians, using the meat-eaters as the reference population (Key *et al.* 1998, 1999a). In the Nurses' Health Study, consumption of red meat was associated with an increased risk of CHD, after adjustment for age (relative risk for one additional serving per day was 1.43, 95% CI: 1.35–1.65) and this was attributed to its contribution to intake of SFAs (Hu *et al.* 1999a). However, the association was substantially attenuated and no longer significant in multivariate analyses. The ratio of red meat to poultry/fish consumption was more strongly associated with risk (multivariate relative risk was 1.32 comparing the top with the bottom quintile for this ratio, $P = 0.001$). In the Iowa Women's Health Study, higher consumption of red meat was significantly associated with CHD mortality (risk ratio 1.44, 95% CI: 1.06–1.94) (Kelemen *et al.* 2005). Among a cohort of men in the USA, intake of haem iron, particularly from red meat, was significantly related to increased risk of fatal CHD or non-fatal MI after adjustment for dietary cholesterol and fats (Ascherio *et al.* 1994). As meat-eaters vary from non or infrequent meat-eaters in a number of ways (*e.g.* they are often more likely to smoke and have a higher BMI; see section on *Dietary and lifestyle factors associated with red meat consumption*), it is very difficult for studies to isolate the effects of meat *per se,* even if they correct for a number of recognised potential confounders. Moreover, none of these investigations have attempted to distinguish between the effects of different types of meat, particularly lean *vs.* untrimmed meat or processed *vs.* unprocessed meat.

Dietary patterns and CVD

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

Studies that attempt to identify the effect of individual foods or nutrients on CVD risk are difficult to interpret because of strong correlations between foods or nutrients, particularly as dietary measures are often crude (*e.g.* rely on FFQs). This has led to increasing interest in examinations of dietary patterns in relation to risk.

Ecological studies, such as the Seven Countries Study, suggest that countries with a high intake of animal products in general, or meat in particular, experience high CHD death rates (Menotti *et al.* 1999). Mediterranean and Asian populations tend to have very low rates of CHD compared with northern European and other Western populations, and this has been attributed to their traditional diets providing higher intakes of vegetables, fruits, wholegrain products and fish, and lower amounts of red meat and high-fat dairy products (Trichopoulou *et al.* 2005). However, some regions of Mediterranean countries have a high meat intake (see Linseisen *et al.* 2002) and a major problem with these international comparisons is confounding by lifestyle variables such as physical activity and obesity and other aspects of diet, such as fibre intake.

Large prospective studies have shown mortality from CVD to be lower among vegetarians, and individuals eating a vegetarian diet tend to have lower levels of many established CVD risk factors including blood pressure, BMI and lipid levels (Phillips 2005). While reductions in risk among vegetarians compared with meat-eaters have not always reached significance, a collaborative analysis of five large vegetarian populations demonstrated an overall reduction of 24% in CHD mortality in vegetarians, which remained significant after adjustment for potential confounders (death rate ratio: 0.76, 95% CI: 0.62–0.94) (Key *et al.* 1998, 1999a). The risk reduction was greatest for deaths at younger ages, and risk of death was lowest among those who adhered most to the vegetarian diet. However, a further analysis of the pooled data found there was no reduction in mortality for those who had followed a vegetarian diet for 5 years or less. These data do not, however, justify advice to exclude meat from the diet as there are several aspects of a vegetarian diet (and lifestyle) apart from not eating meat that might reduce the risk. Indeed, many of the foods that would replace meat in a vegetarian dietary pattern (*i.e.* legumes, soy products, nuts and vegetables) could be causally protective against the disease.

There have been other attempts to examine the relation of different dietary patterns on CVD risk within prospective studies. Among nearly 45 000 men in the US Health Professionals Follow-up Study, Hu and colleagues categorised dietary patterns derived from an FFQ into a 'prudent' diet, similar to that found traditionally in Mediterranean countries (higher intakes of vegetables, fruit, legumes, wholegrains, fish and poultry) and a 'western' pattern characterised by higher intakes of red meat, processed meat, refined grains, sweets and desserts, French fries and high-fat dairy products (Hu *et al.* 2000). During 8 years of follow-up, those with a high score for the prudent pattern had a decreased risk of CHD after adjustment for potential confounders and possible beneficial nutrients such as folate and fibre. In contrast, those with a high score for the Western pattern had increased CHD risk after adjustment for potential

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

deleterious nutrients such as SFAs, *trans* fatty acids and cholesterol. Among women from the Nurses' Health Study, after 14 years of follow-up, the prudent pattern was associated with reduced stroke risk, while a high score for the Western pattern increased risk of total and ischaemic stroke (relative risk for ischaemic stroke was 1.56, 95% CI: 1.05–2.33, $P = 0.02$ for trend, when comparing the highest with lowest quintiles of the Western pattern) (Fung *et al.* 2004a). These studies suggest a protective effect of moderate meat, high plant food dietary patterns. However, in a similar approach, Osler *et al.* (2002) found no relationship between a 'prudent' diet (with plenty of wholemeal bread, fruit and vegetables) and a 'western' food pattern (containing more meat and white bread) and CHD risk, when controlling for potential confounders (BMI, smoking, exercise, education and alcohol drinking).

Studies have also investigated the effects of dietary patterns that include specifically lean meat on CVD risk factors. For example, in a small randomised trial, 26 men received two of three diets – a high-fat diet, a low-fat lacto-ovo-vegetarian diet and a low-fat diet containing lean meat (Kestin *et al.* 1989). Compared with the high-fat diet, both prudent diets significantly lowered total and LDL-cholesterol, as well as blood pressure (but significantly increased serum triglycerides – a recognised effect of low-fat diets, see Stanner 2005). The lacto-ovo-vegetarian diet had a significantly greater cholesterol-lowering effect (10%*vs.* 5% reduction in lean meat group) but blood pressure reductions were similar. The authors concluded that the partial substitution of lean meat for plant protein in a fat-modified diet did not negate the overall cardiovascular-risk lowering of the low-fat lacto-ovo-vegetarian diet. Thus dietary patterns that include lean meat in the context of a low fat intake may offer an alternative approach to a vegetarian diet for CVD prevention.

Fatty acids and associated risk of CVD

Dietary fats are regarded as having an important influence on CVD because of their effects on blood cholesterol levels. A high level of LDL-cholesterol, combined with a low level of HDL-cholesterol increases the risk of atherosclerosis, while a low level of LDL- and high level of HDL-cholesterol reduces the risk. Consuming a diet that is low in fat can reduce blood levels of total and LDL-cholesterol, while a high-fat diet is atherogenic. Laboratory studies have also shown that blood cholesterol can be influenced by the balance of different types of fatty acids in the diet; blood levels of LDL-cholesterol are lowered when some SFAs (*e.g.* myristic and palmitic acids) are replaced by MUFAs, PUFAs or carbohydrate. Fatty acids may also affect CVD risk *via* other mechanisms. For example, SFAs raise platelet activity, and thus increase the tendency of blood to clot, whereas PUFAs have the opposite effect. SFA intake may also be associated with reduced insulin sensitivity, a key factor in the development of the metabolic syndrome (Nugent 2004). Dietary recommendations to lower CVD risk have therefore emphasised the need for a reduction in total fat and, in particular the amount of SFAs in the diet.

Although the cholesterol-lowering effect of PUFAs is greater than that of MUFAs, when substituted for SFAs, MUFAs do not reduce the protective HDL-cholesterol to the same extent.

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

MUFAs compared to PUFAs are also less likely to be oxidised both in foods during cooking and processing, and in the body – oxidised lipids are implicated in protein and DNA damage, and in CVD risk. Moreover, low-fat, high-carbohydrate diets have been shown to increase plasma triglycerides and decrease beneficial HDL-cholesterol levels (Stanner 2005). Intervention studies using high MUFA diets have also shown potential beneficial effects on haemostasis, inflammation and coagulation (Kelly & Stanner 2003). This is likely to be a contributing factor in the ability of Mediterranean-style diets, which are rich in MUFAs, to protect against CVD. There is therefore considerable support within the scientific community for the idea that a moderate-fat diet that is high in unsaturated fatty acids may promote a better lipid profile than a low-fat, high-carbohydrate diet and offer a more effective approach for reducing CVD risk, particularly for people with type 2 diabetes, and such diets are likely to be easier to adhere to for those on weight-reducing diets (Stanner 2005).

The positive relationship between SFAs, cholesterol and CHD suggest that regular intake of foods high in SFAs and cholesterol may increase risk of CHD. Meat's contribution to SFA intake varies widely between countries (see section on the nutrient composition of meat). Overall, red meat contains similar proportions of MUFAs and SFAs, although the exact proportions of the fatty acids vary depending on its fat content. Lean meat is higher in PUFAs and lower in SFAs than untrimmed meat (lean meats contain as little as 2 g of SFAs per 100 g, while the visible fat of meat contains over 37 g/100 g). Furthermore, a substantial proportion of the SFAs in red meat is stearic acid, which has neutral effects on blood cholesterol levels (Kris-Etherton & Yu 1997), although it also contains smaller amounts of the cholesterol-raising fatty acids.

Dietary intervention studies have, however, suggested that while untrimmed meat is cholesterol-raising, this is not true of diets containing fat-trimmed lean meat. Most studies have demonstrated lean red meat to have similar effects on total, LDL- and HDL-cholesterol or triglyceride levels as white meat or soybean products (Li *et al.* 2005). In fact, a recent review of dietary intervention and cross-sectional studies concluded that diets low in SFAs and containing lean red meat are associated with a reduction in LDL-cholesterol levels in both healthy subjects and those with hypercholesterolaemia. Studies also suggest that lean meat does not have an adverse effect on blood lipids in patients with existing CVD (Watts *et al.* 1988). Lean red meat was also found to have no effect on thrombotic risk factors such as the production of thromboxane and prostacyclin, platelet function or haemostatic factors (Li *et al.* 2005).

Meat from ruminant animals contains some *trans* fatty acids as well as CLA (see section on the nutrient composition of meat). *Trans* fatty acids are recognised to have a more potent effect on blood cholesterol than SFAs, by raising levels of LDL-cholesterol and lipoprotein (a) and decreasing HDL-cholesterol levels, although this may not occur with the natural *trans* fats in meat and milk (BNF 1995a). Prospective studies, such as the Nurses' Health Study, have shown a high intake of *trans* fatty acids to increase risk of CHD (Hu *et al.* 1997; Oh *et al.* 2005) but

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

current European diets are generally sufficiently low in these fatty acids not to warrant concern (Hulshof *et al.* 1999). In Britain, current intake is low, at 1.2% of energy (Henderson *et al.* 2003a) (dietary reference value is 2% of energy), while reductions in intake are less apparent in North America. There have been reports of some interesting isomer-specific effects of CLA on the blood lipid profile in human subjects, however, findings have been inconsistent and this might be attributed to the variability of the dose level and/or the mix of CLA isomers used, particularly as results from animal studies show that specific isomers of CLA may be responsible for specific biological effects (Stanner 2005). Unfavourable effects of a high dose of one of the CLA isomers (*t*10*c*12 CLA, 3.4 g per day) has also been reported in relation to increased insulin resistance, oxidative stress and inflammatory biomarkers, and while such an intake is not obtained by diet alone, at present the effects of CLA on human health remain unclear (Tricon *et al.* 2005).

Meat, primarily lean meat, also contains medium (α-linolenic acid) and long-chain *n*-3 PUFAs (EPA, DPA and DHA). Despite being present at low levels, particularly when compared with oil-rich fish, intake of these fatty acids from red meat, mainly in the form of α-linolenic acid, is significant for the average consumer (see section on the nutrient composition of meat). RCTs with high doses of long-chain PUFAs from fish (Burr *et al.* 1989) or supplements (GISSI-Prevenzione Investigators 1999) in patients who had already had a heart attack, have demonstrated a reduction in cardiac events (*e.g.* death, non-fatal MI and non-fatal stroke). Prospective studies (*e.g.* the Nurses' Health Study) have also shown an inverse association between fish consumption and *n*-3 fatty acids and CHD deaths (Hu *et al.* 2002). The Physicians' Health Trial, a prospective study of over 20-years duration, reported a strong inverse dose relationship between blood phospholipid long-chain *n*-3 PUFA content at baseline and subsequent mortality from CHD (Albert *et al.* 2002).

Sodium and blood pressure

The relative risk of both CHD and stroke increases as blood pressure rises. In a recent review of the effect of sodium on blood pressure, the Scientific Advisory Committee on Nutrition (SACN) in the UK concluded that although studies that have prospectively collected 24-h urine (a good marker of sodium intake) suggest that a high salt intake has adverse effects on CVD mortality, there are insufficient reliable data on morbidity and premature mortality outcomes to reach clear conclusions (SACN 2003). However, SACN concluded that reducing the average salt intake in Britain would confer significant public health benefits by contributing to a reduction in CVD burden. Similar recommendations have been made elsewhere (American Heart Association 2000). Carcase meat contains very little sodium naturally, but salt is added to meat products for a variety of technical reasons and, as such, processed meat products make a substantial contribution to total salt intake. The meat industry has been working to reduce the salt content of meat products for some time (Matthews & Strong 2005).

Protein and CVD

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

Recent large epidemiological studies (*e.g.* INTERSALT, MRFIT and the National Diet and Nutrition Survey of British Adults) have found an inverse association between dietary protein intake and blood pressure (Elliott 2003; Hu 2005). Clinical trials have shown that increased plant protein (soy) intake can lower blood pressure but few feeding studies have investigated the effect of animal protein. However, in a parallel design study of 8-week duration, modest replacement of refined carbohydrate-rich foods (including bread, pasta, rice, potatoes and breakfast cereals) with protein in the form of lean red meat lowered blood pressure in 60 hypertensive individuals [24-h ambulatory systolic blood pressure fell by 4.0 mmHg (95% CI: 0.6–7.4)] (Hodgson *et al.* 2005). Emerging evidence from clinical trials also indicates that higher-protein diets may increase short-term weight loss (see *Obesity* section) and improve blood lipids (Parker *et al.* 2002; Layman *et al.* 2003), although claims for the latter have generally been made with interventions that resulted in a concomitant reduction in weight. A recent RCT of 20 moderately hypertriglyceridaemic subjects found no difference between high- and low-protein diets consumed for 6 weeks on fasting plasma or postprandial lipaemia, but the high-protein diet (with 24% of energy as protein derived from lean red meats) was found to attenuate the elevation in chylomicron concentration in response to ingestion of a high-fat mixed meal, compared with the low-protein diet (Mamo *et al.* 2005).

Hu *et al.* (1999b) specifically examined the relationship between protein intake and CHD risk in the Nurses' Health Study. They compared the highest quintile of protein intake (median 24% energy) with the lowest quintile (median 15% energy) and found a relative risk of CHD of 0.74 (95% CI: 0.59–0.94) after controlling for age, smoking, total energy intake, percentage of energy from specific types of fat and other coronary risk factors. Iso *et al.* (2001) found an inverse association between protein and stroke risk in the same cohort. The importance of the source of protein (*i.e.* animal- or plant-based) is currently not clear but different sources of protein appear to have different effects on CVD risk. In a recent review, Hu (2005) concluded that although optimal amounts and sources of protein cannot be determined at this time, evidence suggests a potential benefit of partially replacing refined carbohydrates with protein sources low in SFAs. Results of further large-scale epidemiological studies of protein, blood pressure and CVD are awaited.

Iron and associated risk of CVD

Two reviews in this area concluded that there was insufficient evidence to support an association between iron status and CHD (Sempos *et al.* 1996; Sempos & Looker 1999). This view was corroborated by a meta-analysis of prospective studies of iron status and CHD, which concluded that there was not enough good evidence to support an association (see Kelly 2002).

B vitamins and homocysteine status

While homocysteine is a normal constituent of the blood, there is now strong evidence from a variety of sources that elevated levels are linked with an increased risk of CVD, including stroke.

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

A meta-analysis of prospective studies showed that after adjustment for other cardiovascular risk factors, a 25% lowering in homocysteine was associated with about a 10% lower risk of CHD and 20% lower risk of stroke (Homocysteine Studies Collaboration 2002). Levels are usually very tightly controlled by three enzymes, two of which are folate-dependent. Therefore, a low folate status causes increased levels of homocysteine (Stanner 2005). While clinical trials have demonstrated that supplementation with folic acid lowers levels of homocysteine and that inclusion of vitamins B_6 and B_{12} (which are also involved in homocysteine metabolism) amplify this effect, the results of large-scale supplementation trials are awaited to provide further information on the role of these B vitamins and homocysteine in CVD. Red meat contains vitamins B_{12}, B_6 and folate and lower homocysteine levels have been reported in meat-eaters compared with vegetarians (Mann *et al.* 1999).

Selenium and associated risk of CVD

The role of selenium (present in moderate amounts in meat) in the activity of specific antioxidant enzymes, particularly glutathione peroxidase, has been well established (Holben & Smith 1999) and it is therefore considered to be an important antioxidant nutrient. Low levels of selenium have been associated with cardiomyopathy in China, but its importance in CVD remains controversial (Neve 1996). In case-control studies, patients with MI have low plasma selenium concentrations, but this could be a consequence of the disease (BNF 2001). Prospective studies investigating low selenium status and heart disease have produced mixed results (BNF 2001). The two studies that found an association (Salonen *et al.* 1982; Virtamo *et al.* 1985) were conducted in Finland, where selenium intake was very low, one showing a 3.6-fold increase in coronary deaths and a 2.7-fold increase in heart attacks among men with low serum selenium levels (<45 μg/L). In populations with higher selenium intakes, no associations were found (Miettinen *et al.* 1983; Ringstad & Fonnebo 1987; Salvini *et al.* 1995), suggesting that cardiovascular risk may only be increased by very low selenium status (BNF 2001).

Summary

Pooled analysis of prospective studies suggests that individuals adopting a vegetarian diet may be at a lower risk of CVD than those adopting other diets, but such data cannot be used to make specific associations about meat consumption. Some prospective studies have shown a modestly increased risk of CVD among meat-eaters compared with low meat-eaters, but controlling for potential confounders and other aspects of the diet remains difficult. While red meat contains SFAs, a high intake of which can have adverse effects on CVD risk factors such as blood cholesterol levels, it also contains other fatty acids (*n*-3 PUFAs, MUFAs) and nutrients (*e.g.* B vitamins and selenium) that offer potential cardioprotective benefits. Cohort studies have not been able to distinguish between the effects of different types of meat but feeding trials have not demonstrated lean meat to be hypercholesterolaemic or blood pressure raising or to have

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

any negative effect on thrombotic risk factors, which suggests that it can be promoted as part of a healthy diet for primary and secondary CVD prevention.

Obesity

Overweight and obesity is an increasing public health problem worldwide, affecting people of all ages and socio-economic groups. Globally, the prevalence of overweight and obesity is increasing in both adults and children and it is no longer restricted to affluent countries. Obesity is associated with an increased risk of chronic diseases including CVD, type 2 diabetes and some types of cancer (BNF 2004a).

Obesity is a complex disorder with a diverse range of causal factors. Most cases of obesity arise as the result of an adverse environment working on a susceptible genotype. Susceptibility may be mediated through a wide range of metabolic (*e.g.* control of fuel selection) and behavioural (*e.g.* binge eating) traits. One incontrovertible fact, however, is that for an individual to become obese, energy intake must be higher than energy expenditure, for an extended period of time. This wide range of aetiological factors makes obesity both a complex and challenging disorder (BNF 1999b).

Studies comparing meat-eaters to vegetarians have consistently found that vegetarians tend to have a lower BMI than comparable non-vegetarians, *e.g.* Key & Davey (1996). Average BMI varies substantially between cohorts, however, vegetarians have been shown to have a BMI that is, on average, slightly lower (1 kg/m^2) than non-vegetarians. This has been found in both men and women and in all age groups, resulting in a lower level of obesity in vegetarians compared with meat-eaters (Key *et al.* 1999b). The large EPIC-Oxford (UK) cohort comparing meat-eaters to fish-eaters, lacto-ovo-vegetarians and vegans has shown similar findings which were described earlier (Davey *et al.* 2003). It is difficult to establish exactly what determines these differences in BMI, the composition of the diet is likely to be of relevance, but other lifestyle factors, such as physical activity levels will also be important. In the EPIC-Oxford cohort, differences in macronutrient intakes were found to account for around 50% of the difference in mean BMI between meat-eaters and vegans (Spencer *et al.* 2003).

In terms of dietary factors related to the development of obesity, there is modest evidence that a high dietary fat to carbohydrate ratio in the diet is more likely to promote obesity development (BNF 1999b). Meat makes a relatively high contribution to dietary fat intakes; a recent UK dietary survey indicated that meat and meat products contribute around 23% of total fat intake (Henderson *et al.* 2003a). However, as discussed earlier, the fat content of carcase meat has recently been declining, while lean red meat can contain as little as 2% fat. As dietary fat comes from a variety of sources, reducing the fat content of the diet *as a whole* (target recommendation 35% of food energy) is a key feature of health policy activities.

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

There is also some evidence that it can be advantageous to include lean meat in weight loss diets, as high protein intakes have been found to lead to increased satiety (Stubbs 1995). Halton & Hu (2004) recently conducted a systematic review of studies investigating the effects of high-protein diets on thermogenesis, satiety, bodyweight and fat loss. Overall, they concluded that there is some evidence that diets higher in protein lead to increased weight loss and fat loss compared with diets lower in protein in the short-term (6-month period), but further longer-term studies are needed. Possible mechanisms to explain this effect include increased satiety from protein, decreased subsequent energy intake and the displacement of carbohydrate in higher-protein diets. It seems likely that several of these mechanisms work together and are interrelated (see Hu 2005).

Skov *et al.* (1999) showed that overweight and obese subjects randomised to either a high-carbohydrate (12% energy from protein) or a high-protein (25% energy from protein) *ad libitum* fat-reduced diet for a 6-month period, consumed less energy and lost more weight on the high-protein diet compared with the high-carbohydrate diet. These data are consistent with other *ad libitum* studies (*e.g.* Weigle *et al.* 2005) and suggest that, at least in the short-term, high-protein diets induce greater satiety and lead to lower subsequent energy intake compared with lower-protein diets (Hu 2005).

There is also evidence that foods higher in protein induce increased energy expenditure (diet-induced thermogenesis) compared with foods with a higher carbohydrate content. This may be another plausible mechanism through which higher-protein diets may help with weight loss (Halton & Hu 2004). There is also some suggestion that a diet rich in animal protein may induce greater energy expenditure than a diet with the same energy and protein content, but in which the protein was from a vegetable source (Mikkelsen *et al.* 2000). Further investigation is needed, however, to assess the long-term effects of high-protein diets before any public health recommendations can be made.

Summary

Studies comparing meat-eaters with vegetarians have consistently found that vegetarians tend to have a lower BMI than comparable non-vegetarians, but this association cannot be attributed to meat intake *per se*. Obesity is a complex disorder with a diverse range of causal factors and therefore to identify one dietary factor as the cause would be a gross oversimplification of a complex process.

There is growing evidence (from short-term studies) to suggest that higher-protein diets lead to increased satiety and reduced subsequent energy intake and may therefore help enhance weight loss, compared with lower-protein diets, in the short term. However, further studies are required to assess the long-term effects of high-protein diets.

Type 2 diabetes

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

The prevalence of type 2 diabetes is increasing rapidly worldwide and this is thought to be linked to the increasing prevalence of obesity. Currently, an estimated 120 million people worldwide are affected by type 2 diabetes and the incidence has been predicted to double to 215 million by 2010. There are a number of risk factors for type 2 diabetes, the most important being genetic predisposition, obesity and physical activity level (BNF 2004b).

Several prospective studies have suggested that a 'western' dietary pattern, characterised by higher consumption of red and processed meats, French fries, refined grains and sweets and desserts, is associated with an increased risk of type 2 diabetes, whereas a more prudent dietary pattern, characterised by higher intakes of fruit and vegetables, fish, poultry and wholegrains has been associated with a reduced risk (van Dam *et al.* 2002a; Fung *et al.* 2004b). Many factors can confound such associations but recently it has been speculated that processed meat intake may be independently associated with the risk of type 2 diabetes (see Table 11).

Table 11. Meat consumption and type 2 diabetes: prospective cohort studies

Author (year published)	Country/cohort details	No. in cohort	No. of cases of diabetes	Type of meat	Partition	Relative risk (highest *vs.* lowest intake)	Significance	A
Fung *et al.* (2004b)	American women	69 554	2699	Red meat	Servings	1.26*	Sig.	
				Processed meat	Servings	1.38*	Sig.	
Song *et al.* (2004)	Women's Health Study, USA	37 309	1558	Red meat	Freq./week	1.28	Sig.	
				Processed meat	Freq./week	1.23	Sig.	
Schulze *et al.* (2003)	Nurses' Health Study II, USA	91 246	741	Red meat	Freq./week	1.44	NS	
				Processed meat	Freq./week	1.82	Sig.	

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

* Relative risk (RR) for diabetes for every 1-serving increase in intake.

NS, not significant; Sig., significant; Freq., frequency; BMI, body mass index.

The hypothesis that high meat intake may be linked to an increased risk of type 2 diabetes first arose from ecological and migrant studies (Kawate *et al.* 1979; Pratley 1998). Also, the Seventh-Day Adventists study comparing vegetarians with non-vegetarians found meat intake to be associated with a higher risk of diagnosed type 2 diabetes, after correcting for bodyweight, physical activity and other dietary factors (Snowdon & Phillips 1985). Since then, a few prospective studies, primarily from one group in the USA, have investigated the possible link between red and processed meat intake and type 2 diabetes. The results of these studies are summarised in Table 11; the limitations of ecological and prospective studies have been discussed earlier in this section.

In the large cohort of male participants of the Health Professionals Follow-up Study, frequent consumption of processed meat (five servings or more per week) was associated with a higher risk of type 2 diabetes, however, there was no significant association with red meat intake (van Dam *et al.* 2002b). A similar finding was demonstrated in a large cohort of women in the Nurses' Health Study. Participants consuming processed meat five times or more per week were shown to have a 82% (95% CI: 1.34–2.46) increased risk of developing type 2 diabetes compared with those consuming processed meat less than once per week, after adjusting for confounders, including other dietary factors. Red meat was not found to be significantly associated with type 2 diabetes after correction for the other dietary factors (Schulze *et al.* 2003).

More recently, in the Women's Health Study, both red and processed meat intakes were found to be significantly associated with an increased risk of type 2 diabetes, the strongest association being with frequent consumption of processed meat (particularly bacon and hot dogs). These results remained significant after further adjustment for total fat intake, dietary fibre, magnesium and glycaemic load. Intakes of total cholesterol, animal protein and haem iron were also shown to be significantly associated with increased risk (Song *et al.* 2004). Finally, in another cohort of American women, both red and processed meat intake were found to be associated with type 2 diabetes; again the association was found to be strongest for processed meat intake (Fung *et al.* 2004b).

The results of these large cohort studies suggest that frequent consumption of processed meat may increase the risk of developing type 2 diabetes, but there is less evidence for an association with red meat intake. However, the underlying mechanism by which red or processed meat may influence diabetes risk is unclear. Processed meats are a major source of nitrites in the diet and N-nitrosamines, formed in foods or the stomach by the interaction of nitrites with amines, have also been detected in processed meats such as bacon and sausages.

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

Some N-nitrosamines have been found to be toxic to pancreatic α-cells (LeDoux *et al.* 1986) and consumption of foods high in nitrites and N-nitrosamines has been shown to be positively associated with the risk of type 1 diabetes in a number of studies (*e.g.* Virtanen *et al.* 1994). However, it is less clear whether *N*-nitrosamines are involved in the development of type 2 diabetes (van Dam *et al.* 2002b). Advanced glycation end products (AGEs) and lipoxidation end products produced during the cooking or processing of meat have also been suggested as an explanation (Song *et al.* 2004).

Summary

Evidence from a small number of recent cohort studies suggests that a high intake of processed meat may be associated with an increased risk of type 2 diabetes. However, to date there have been no intervention studies (the means of demonstrating cause and effect) and purported mechanisms are merely speculative. The evidence that exists points to any effect being linked with processed rather than lean red meat, and processed meat products can be high in fat. Hence the findings are in accord with current recommendations to reduce fat intake and to include lean meat in the diet, in moderation.

People with type 2 diabetes, like the general population, are recommended to follow a healthy balanced diet, low in fat (especially SFAs) and rich in fruit, vegetables and wholegrain cereals, to maintain a healthy bodyweight and to stay physically active (BNF 2004b). There is no evidence to suggest that lean red meat cannot be recommended, in moderation, as part of a healthy balanced diet for people with type 2 diabetes. In fact, single-meal intervention studies suggest that an energy restricted, high-protein, low-fat diet (that includes lean red meat) may actually help improve overall glucose control in type 2 diabetes (*e.g.* Gannon *et al.* 2003).

Discussion

Data from national dietary surveys indicate that intakes of meat vary widely between countries, and between subgroups of the population within countries. However, some similarities have been found; for example, women tend to consume less meat than men. As different methodologies are adopted to collect information on food intake, it is difficult to make direct comparisons between data from different countries, although the recent EPIC study has provided comparable food intake information for a number of European countries.

Another challenging aspect of investigating patterns of red meat intake is that the composition of different types of meat varies widely. For example, lean red meat is low in total fat, SFAs and salt, whereas untrimmed meat is higher in both total fat and SFAs; processed meat is generally higher in salt and fat and often contains other components, such as nitrites, which are added during processing. Moreover, the composition of different processed meats and the types of processed meat consumed vary widely between countries. It is therefore important to distinguish between different types of meat when looking at patterns of intake, as well as

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

considering the health effects of red meat. This is not always straightforward, however, as definitions vary and not all studies distinguish between different kinds of meat consumed.

Red meat provides many important nutrients, particularly protein, long-chain *n*-3 fatty acids, iron, zinc, selenium, vitamin D and vitamin B_{12}. Red meat is a well-recognised source of bioavailable iron, contributing around 20% of iron intake, on average, in developed countries. In the light of current low levels of iron intake in many developed countries, particularly among women, meat has the potential to make an important contribution to intakes. In some countries, for example the UK, there are also concerns over low intakes of selenium. Meat is one of the main providers of selenium in the diet, particularly in parts of the world where selenium levels in the cereals consumed are low. Red meat is also classified as a *source* of zinc, in EU food labelling terms (BNF 2002). Red meat consumption provides 30–40% of the recommended zinc intake in some countries. As with iron, the zinc in meat is in a highly bioavailable form, making meat a more 'efficient' provider of this mineral. Concern exists, *e.g.* in the UK, about the zinc intakes of some population groups.

Red meat is also a good source of vitamin D, which may be more easily utilised than the vitamin D present in other foodstuffs. Meat can therefore provide a useful dietary source for those individuals who do not get enough vitamin D through sunlight exposure (the main route for most people). Red meat also contains a range of B vitamins, although the levels vary between different types of meat, in particular it is classified as a *rich source* of vitamin B_{12}, in EU food labelling terms. As this vitamin is only found naturally in foods of animal origin, subgroups of the population who do not consume meat or animal products, may have inadequate intakes.

Although red meat is seen as a contributor to SFAs intake, lean red meat actually contains a higher proportion of unsaturated fatty acids. Meat, primarily lean meat, also contains the long-chain *n*-3 fatty acids, EPA, DPA and DHA. Although levels of these are low, there are few other food sources (the richest source being oily fish), and therefore lean red meat can make a contribution to intake of these fatty acids. Furthermore, much work is currently underway to identify methods through which to alter the fatty acid profile of foods, such as meat, in order to reflect a positive fatty acid profile in terms of heart health.

Various research studies have been conducted to try to determine whether there is a link between red and processed meat intake and a number of chronic diseases, including CRC, CVD, obesity and type 2 diabetes. Evidence from prospective cohort studies indicates that high intakes of red and processed meat (*e.g.* >160 g per day) contribute to an increased risk of CRC (*e.g.* Norat *et al.* 2005). Obtaining definitive evidence to confirm the effects of specific dietary factors on cancer risk is a challenging process (the shortcomings of epidemiological studies have been discussed earlier). Prospective cohort studies provide the best available evidence regarding associations between diet and cancer, but they have their limitations, in particular, the complex nature of the diet makes it very difficult to measure precise levels of food and nutrient intake. Furthermore, it is very difficult to tease out the independent effects of

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

individual dietary factors, as dietary patterns tend to cluster, *e.g.* individuals with high intakes of processed meat have also been shown to have low intakes of fruit and vegetables (see Cosgrove *et al.* 2005b). The recent EPIC study of a large pan-European cohort helps to overcome measurement error, as statistical power is increased by using large numbers of subjects with greater heterogeneity in dietary habits. Therefore, the EPIC study currently provides the strongest available evidence regarding associations between dietary factors and the risk of cancer.

With regard to CVD, some cohort studies have shown an association between high meat intake and increased CVD risk, but most of these have combined meat intake from all sources and have not been able to distinguish between the effects of different types of meat (*e.g.* lean, processed or untrimmed meat). However, intervention studies have not indicated that lean red meat has any adverse effect on blood cholesterol levels, blood pressure or other CVD risk factors.

Meat-eaters have been shown to have a higher BMI than comparable vegetarians (Key *et al.* 1999b); however, evidence of an association between meat consumption and obesity does not automatically indicate there is a causal link. Furthermore, there are problems with interpreting studies comparing meat-eaters with vegetarians, as vegetarians tend to be more health-orientated generally, *e.g.* consuming more fruit and vegetables and having higher levels of physical activity. Obesity is a complex disorder with a diverse range of causal factors and to identify one dietary factor as causal would be a gross oversimplification. Finally, with regard to type 2 diabetes, there is evidence from a few large cohort studies in the USA that frequent consumption of processed meat (*e.g.* 5 times or more per week) may increase the risk of type 2 diabetes development. However, further studies are needed to confirm these findings.

With respect to CRC risk, the currently available evidence suggests that the UK guidelines set by COMA are still appropriate, that is that individuals' consumption of red and processed meat should not rise and that higher consumers (>140 g per day or 12–14 portions per week) should consider a reduction in intake (Department of Health, UK 1998). It is worth noting that recommendations in different countries vary somewhat; for example, the Australian Cancer Council of New South Wales (NSW) recommends that people consume moderate amounts of red meat (65–100 g of cooked red meat, 3–4 times a week) and limit consumption of processed meats (The Cancer Council NSW 2003). The American Cancer Society recommends limiting consumption of red meats, especially those high in fat and processed meat (American Cancer Society 2001).

Average daily intakes of red and processed meat in most countries are still below the level thought to increase the risk of CRC. For example, average intakes of red meat in Europe (in men) range from 40 g per day in the UK to 74 g per day in Spain, while average intakes of processed meat (in men) range from 10 g per day in Greece to 83.2 g per day in Germany

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

(see Table 1) and therefore it is only the small proportion of high consumers of meat and meat products that may need to consider a reduction in consumption.

The majority of the population in most developed countries consume meat and meat products, and meat makes a significant contribution to nutrient intake for most individuals. In addition, meat can be a versatile food that adds variety to eating occasions and is enjoyed by many. Some people choose not to eat meat, for a variety of reasons, but as there is no evidence that a moderate intake of lean red meat has any negative effects on health, there is currently no real scientific justification for excluding it from the diet. Therefore, as recommended in healthy eating advice around the world, lean red meat, consumed in moderation, can be promoted as part of a healthy balanced diet.

Acknowledgement

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References

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U.S. Patent No. 11,224,241

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Attachment 9b

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007    cr-mnu|||||||
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035    ##$a(CONSER)---00242328-
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United Kingdom
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260    ##$a[Oxford, England] : $bBlackwell Science
310    ##$aQuarterly
336    ##$atext $btxt $2rdacontent
337    ##$acomputer $bc $2rdamedia
338    ##$aonline resource $bcr $2rdacarrier
362    1#$aPrint began with vol. 4, published in 1977.
500    ##$aRefereed/Peer-reviewed
500    ##$aRefereed/Peer-reviewed
520    ##$aNutrition bulletin's focus is the interface between the
needs of nutritionists in research, health promotion and teaching,
and those working in the food industry. Topics addressed in reviews
cover the many and diverse areas that together comprise nutrition
science, including the biochemical and physiological basis for the
role of various nutrients in the body, discussion of observational
(epidemiological) associations between diet/nutrients and aspects
of health.
538    ##$aMode of access: World Wide Web.
588    0#$aVol. 25, issue 1 (Mar. 2000); title from table of
contents page (viewed November 1, 2000).
588    1#$aVol. 29, issue 1 (Mar. 2004) (viewed Apr 6, 2004).
650    #0$aNutrition $vPeriodicals.
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650    #2$aNutrition Disorders
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780    00$tBNF bulletin $w(CKB)991042744462606 $w(DLC)sn80008224
710    2#$aBritish Nutrition Foundation.
906    ##$aJOURNAL
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Attachment 9c

LIBRARY OF CONGRESS AUTHORITIES

LC control no.: sh2008108541
LCCN Permalink: https://lccn.loc.gov/sh2008108541
HEADING: Nutrition Periodicals

000 00448cz a2200169n 450
001 7460925
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906 __ |**t** 8888 |**u** te00 |**v** 0
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Attachment 10a

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655 Avenue of the Americas, New York, NY 10010
040    ##$aDLC $cDLC $dOCL $dNSD $dOCL $dNST $dSER $dRCS $dOCL $dNST
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510    2#$aIndex medicus $x0019-3879
510    2#$aLife sciences collection
525    ##$aV.173 includes a separate supplement.
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555    0#$aAuth., 1-3(1976-78) bd w/3; auth., 1-30(1976-84) bd w/30;
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auth., 1-100(1976-91) bd w/100.
580    ##$aVols. for 1996- includes separately paged section called:
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electronically in Gene-combis on the World-Wide Web.
650    #0$aGenetic engineering $vPeriodicals
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901    ##$cSer
936    ##$a15 July 1992
948    ##$aLTI 07/21/2009
948    ##$aLTI 09/04/2009
948    ##$aLTI 02/03/2010
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Attachment 10b

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LCCN Permalink: https://lccn.loc.gov/sh2008117864
HEADING: Genetic engineering Periodicals

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667 __ |**a** Record generated for validation purposes.
670 __ |**a** Work cat.: Methods in gene technology, c1991-c1994
906 __ |**t** 8888 |**u** te00 |**v** 0
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Case No.: IPR2023-00207
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LC control no.: sh 85086580
LCCN Permalink: https://lccn.loc.gov/sh85086580
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906 __ |**t** 8727 |**u** fk03 |**v** 1
953 __ |**a** xx00 |**b** ff03

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Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

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LC control no.: sh 85111851
LCCN Permalink: https://lccn.loc.gov/sh85111851
HEADING: Recombinant DNA

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550 __ |**a** Genetic recombination
550 __ |**a** Genetic vectors
670 __ |**a** Catalogue of bacteria, phages, and rDNA vectors, 1985.
906 __ |**t** 8930 |**u** fk04 |**v** 0
953 __ |**a** xx00 |**b** ff01

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BLvl	s	**Form**		**Conf**	0	**Freq**	e	**MRec**		**Ctry**	ne
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022 0 0378-1119 $l 0378-1119 $2 j
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032 008729 $b USPS
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245 00 Gene.
246 3 Gene-combis
260 Amsterdam : $b Elsevier/North-Holland, $c 1976-
300 volumes $b illustrations $c 24 cm
310 Biweekly, $b <15 July 1992-
321 Bimonthly, $b 1976-
321 Monthly, $b <Jan. 1979-
336 text $b txt $2 rdacontent
337 unmediated $b n $2 rdamedia
338 volume $b nc $2 rdacarrier
362 0 v. 1-

500 "An international journal focusing on gene cloning and gene structure and function."
510 2 Chemical abstracts $x 0009-2258
530 Also issued via World Wide Web by subscription through ScienceDirect (Online service).
580 Vols. for 1996- includes separately paged section called: Gene-combis which is printed version of articles published electronically in Gene-combis on the World-Wide Web.
580 Online version has open access mirror journal: Gene X (Online), published 2019-2020.
588 1 Vol. 295, no. 1 (July 24, 2002).
650 0 Genetic engineering $v Periodicals.
650 0 Molecular cloning $v Periodicals.
650 0 Recombinant DNA $v Periodicals.
650 0 Genetic recombination.
650 2 Molecular Biology $0 (DNLM)D008967
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655 4 Internet Resources.
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856 41 $u http://www.sciencedirect.com/science/journal/03781119

Attachment 11a

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082    00$a664 $223
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245    00$aVolatile sulfur compounds in food / $cMichael C. Qian,
Xuetong Fan,  Kanjana Mahattanatawee, editor[s] ; sponsored by the
ACS Division of Agricultural and Food Chemistry, Inc.
260    ##$aWashington, DC : $bAmerican Chemical Society, $cc2011.
300    ##$ax, 355 p. : $bill. ; $c24 cm.
490    1#$aACS symposium series ; $v1068
504    ##$aIncludes bibliographical references and index.
650    #0$aFood $xSulfur content.
650    #0$aSulfur compounds $vCongresses.
650    #0$aFood $xAnalysis $vCongresses.
700    1#$aQian, Michael.
700    1#$aFan, Xuetong.
700    1#$aMahattanatawee, Kanjana.
710    2#$aAmerican Chemical Society. $bDivision of Agricultural and
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830    #0$aACS symposium series ; $v1068.
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Attachment 11b

LIBRARY OF CONGRESS AUTHORITIES

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670 __ |**a** Work cat: 2011029690: Volatile sulfur compounds in food, c2011.
910 __ |**a** Proposal saved by tc03 on 09/08/2011 at 10:43:19
906 __ |**t** 1125 |**v** 1

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Attachment 11c

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

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LCCN Permalink: https://lccn.loc.gov/sh85130373
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LC control no.: sh 85031115

LCCN Permalink: https://lccn.loc.gov/sh85031115

HEADING: Congresses and conventions

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360 __ |**i** subdivision |**a** Congresses |**i** under subjects; and names of individual congresses

450 __ |**a** Colloquia

450 __ |**a** Colloquiums

450 __ |**a** Conferences

450 __ |**a** Conventions (Congresses)

450 __ |**a** International conferences, congresses and conventions

450 __ |**a** Symposia

450 __ |**a** Symposiums

550 __ |**w** g |**a** Intellectual cooperation

550 __ |**a** Convention facilities

670 __ |**a** Random House Kernerman Webster's college dictionary, ©2010, via TheFreeDictionary.com, Aug. 29, 2013 |**b** (colloquium n., pl. -quiums, -quia a conference at which scholars or other experts present papers on and discuss a specific topic)

670 __ |**a** The American heritage dictionary of the English language, ©2000, via TheFreeDictionary.com, Aug. 29, 2013 |**b** (colloquium n. pl. colloquiums or colloquia 1. An informal meeting for the exchange of views. 2. An academic seminar on a broad field of study, usually led by a different lecturer at each meeting.)

670 __ |**a** Merriam-Webster online, Aug. 29, 2013 |**b** (colloqium plural colloquiums or -quia: a usually academic meeting at which specialists deliver addresses on a topic or on related topics and then answer questions relating to them)

906 __ |**t** 9921 |**u** te04 |**v** 0

910 __ |**a** Record saved by ec04 on 01/17/2014 at 16:02:37

910 __ |**a** Record saved by ec11 on 09/03/2013 at 08:55:33

953 __ |**a** xx00 |**b** sg08

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Washington, DC 20540

Library of Congress Authorities
URL: http://authorities.loc.gov/
Library of Congress Online Catalog
URL: https://catalog.loc.gov/

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Attachment 11e

LIBRARY OF CONGRESS AUTHORITIES

LC control no.: sh2008103843
LCCN Permalink: https://lccn.loc.gov/sh2008103843
HEADING: Food Analysis Congresses

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040 __ |**a** DLC |**b** eng |**c** DLC
150 __ |**a** Food |**x** Analysis |**v** Congresses
667 __ |**a** Record generated for validation purposes.
670 __ |**a** Work cat.: Headspace analysis of foods and flavors, 2001
906 __ |**t** 8888 |**u** te00 |**v** 0
953 __ |**a** te00

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Mailing Address:
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Washington, DC 20540

Library of Congress Authorities
URL: http://authorities.loc.gov/
Library of Congress Online Catalog
URL: https://catalog.loc.gov/

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Attachment 11f

Copyright United States Copyright Office

Try the Copyright Public Records System (CPRS) pilot with enhanced search features and filters.

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Public Catalog

Copyright Catalog (1978 to present)
Search Request: Left Anchored Title = Volatile Sulfur Compounds in Food
Search Results: Displaying 1 of 1 entries

previous next

Labeled View

Volatile Sulfur Compounds in Food, SS1068.

Type of Work: Text
Registration Number / Date: TX0007498381 / 2012-03-08
Application Title: Volatile Sulfur Compounds in Food, SS1068.
Title: Volatile Sulfur Compounds in Food, SS1068.
Description: Book, 355 p.
Copyright Claimant: American Chemical Society (ACS), Transfer: By written agreement. Address: 1155 Sixteenth St., N.W., Washington, DC, 20036, United States.
Date of Creation: 2011
Date of Publication: 2011-08-24
Nation of First Publication: United States
Authorship on Application: Michael C Qian; Domicile: United States; Citizenship: United States. Authorship: editing.
Xuetong Fan; Domicile: United States; Citizenship: United States. Authorship: editing.
Kanjana Mahattanatawee; Domicile: Thailand; Citizenship: Thailand. Authorship: editing.
Rights and Permissions: Robert W Hauserman, 1155 Sixteenth St., N.W., Washington, DC, 20036, United States, (202) 452-2120, (703) 887-

8496, b_hauserman@acs.org

Copyright Note: C.O. correspondence.

ISBN: 9780841226166

Names: Qian, Michael C
Fan, Xuetong
Mahattanatawee, Kanjana
American Chemical Society (ACS)

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Attachment 12a

MARC Bibliographic Record

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Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

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210 0_ $aJ. agric. food chem.

222 _0 $aJournal of agricultural and food chemistry

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246 17 $aAgricultural and food chemistry

260 $aEaston, Pa. :$bAmerican Chemical Society, Books and Journals Division.

300 $avolumes :$billustrations ;$c31 cm

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

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321        $aBimonthly,$b<, Jan./Feb. 1976-?>
321        $aBiweekly,$bApr. 1, 1953-<Dec. 22, 1954>
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362    1_  $aBegan with Apr. 1, 1953 issue.$zCf. OCLC.
500        $aDescription based on: Vol. 25, no. 1 (Jan.-Feb. 1977); title from cover.
500        $a"An American Chemical Society publication."
500        $aPlace of publication varies.
530        $aAlso available on the Internet.
530        $aAlso available on microfiche and microfim.
510    2_  $aBiological abstracts$x0006-3169
510    2_  $aBibliography of agriculture$x0006-1530
510    2_  $aChemical abstracts$x0009-2258
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Case No.: IPR2023-00207
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510 2_ $aCoal abstracts$x0309-4979

510 2_ $aEnergy information abstracts$x0147-6521

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510 2_ $aEnvironment abstracts$x0093-3287

510 2_ $aExcerpta medica

510 0_ $aIndex medicus$x0019-3879

510 2_ $aInternational aerospace abstracts$x0020-5842

510 0_ $aNuclear science abstracts$x0029-5612

510 2_ $aPESTDOC

510 2_ $aRINGDOC

510 2_ $aSelected water resources abstracts$x0037-136X

510 2_ $aVETDOC

550 $aVols. for 1953-<54> published by the American Chemical Society; <Jan./Feb. 1976-> by the Books and Journals Division, American Chemical Society.

650 _0 $aAgricultural chemistry$vPeriodicals.

650 _0 $aFood$xAnalysis$vPeriodicals.

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

650 _0 $aPlants$xNutrition$vPeriodicals.

650 _2 $aFood$xchemistry$vperiodicals.

650 _2 $aChemistry, Agricultural$vperiodicals.

710 2_ $aAmerican Chemical Society.

710 2_ $aAmerican Chemical Society.$bBooks and Journals Division.

776 1_ $tJournal of agriculture and food chemistry$x0021-8561$w(OCoLC)07385304

997 $aMARCIVE

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260 $aWashington, DC :$bAmerican Chemical Society,$c1953-

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310 $aWeekly,$b2012-

321 $aBiweekly,$b2002-2011

321 $aFrequency varies,$b1953-2001

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337 $acomputer$bc$2rdamedia

338 $aonline resource$bcr$2rdacarrier

362 1_ $aPrint began with vol. 1, no. 1 (Apr. 1953).

500 $aRefereed/Peer-reviewed

525 $aSome issues accompanied by supplementary materials or supporting information.

588 $aDescription based on print version record, (ACS Publications, viewed Aug. 29, 2006); title from contents page.

588 $aLatest issue consulted: Volume 69, issue 43 (November 3, 2021), (ACS Publications, viewed Nov. 9, 2021).

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906          $aJOURNAL
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Attachment 12b

LIBRARY OF CONGRESS AUTHORITIES

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HEADING: Agricultural chemistry Periodicals

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906 __ |**t** 8888 |**u** tc00 |**v** 0
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Attachment 12c

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

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906 __ |**t** 0220 |**u** te03 |**v** 1
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URL: http://authorities.loc.gov/
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OCLC 1800249 **No holdings in XXX - 596 other holdings**

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245 00 Journal of agricultural and food chemistry.
246 17 Agricultural and food chemistry
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310 Weekly, $b 2012-
321 Biweekly, $b 2002-2011
321 Monthly, $b <Dec. 1990-2001
321 Bimonthly, $b Jan./Feb. 1960-Nov./Dec. 1989
321 Monthly, $b 1955-1959
321 Biweekly, $b Apr. 1, 1953-<Dec. 22, 1954
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338 volume $b nc $2 rdacarrier
362 0 v. 1- Apr. 1, 1953-

510 2 Chemical abstracts $x 0009-2258
525 Some issues <1978-Jan. 1995 accompanied by supplementary material on microfiche; Feb. 1995-<Feb. 1998, by supporting information on microfiche; current (1995-) supporting information also available online.
530 Also issued on microfiche, microfilm, and online.
550 Volumes for 1953-<54 published by the American Chemical Society; <Jan./Feb. 1976- by the Books and Journals Division, American Chemical Society.
650 0 Agricultural chemistry $v Periodicals.
650 0 Food $x Analysis $v Periodicals.
650 0 Plants $x Nutrition $v Periodicals.
650 2 Chemistry, Agricultural. $0 (DNLM)D002622
650 2 Food-Processing Industry. $0 (DNLM)D005525
650 6 Chimie agricole $v Périodiques.
650 6 Aliments $x Analyse $v Périodiques.
650 6 Plantes $x Nutrition $v Périodiques.
650 7 58.34 food technology. $0 (NL-LeOCL)07760671X $2 bcl
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710 2 American Chemical Society. $b Books and Journals Division.
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Attachment 13a

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050 00 $aTP372.5$b.H43 2006

082 00 $a664/.5$222

100 1_ $aReineccius, Gary.

245 10 $aFlavor chemistry and technology /$bGary Reineccius.

250 $aSecond edition.

264 _1 $aBoca Raton :$bTaylor & Francis,$c2006.

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500 $aRev. ed. of: Flavor chemistry and technology / by Henry B. Heath and Gary Reineccius. c1986.

504 $aIncludes bibliographical references and index.

650 _0 $aFlavor.

650 _0 $aFlavoring essences.

700 1_ $aHeath, Henry B.$tFlavor chemistry and technology.

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Attachment 13b

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U.S. Patent No. 11,224,241

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Attachment 13c

LIBRARY OF CONGRESS AUTHORITIES

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LCCN Permalink: https://lccn.loc.gov/sh85049035
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150 __ |**a** Flavoring essences
550 __ |**w** g |**a** Essences and essential oils
550 __ |**w** g |**a** Food additives
906 __ |**t** 9430 |**u** te04 |**v** 0
953 __ |**a** xx00 |**b** ta25

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Copyright United States Copyright Office

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Public Catalog

Copyright Catalog (1978 to present)
Search Request: Left Anchored Title = Flavor Chemistry and Technology
Search Results: Displaying 3 of 4 entries

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Edition: 2nd ed.
Imprint: Boca Raton, FL : Taylor & Francis, 2006.
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Date of Creation: 2005
Date of Publication: 2005-07-11
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Names: Reineccius, Gary
Taylor & Francis

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Case No.: IPR2023-00207
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JOURNAL ARTICLE

Sustainability of meat-based and plant-based diets and the environment

David Pimentel ✉, Marcia Pimentel

The American Journal of Clinical Nutrition, Volume 78, Issue 3, September 2003, Pages 660S–663S,
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ABSTRACT

Worldwide, an estimated 2 billion people live primarily on a meat-based diet, while an estimated 4 billion live primarily on a plant-based diet. The US food production system uses about 50% of the total US land area, 80% of the fresh water, and 17% of the fossil energy used in the country. The heavy dependence on fossil energy suggests that the US food system, whether meat-based or plant-based, is not sustainable. The use of land and energy resources devoted to an average meat-based diet compared with a lactoovovegetarian (plant-based) diet is analyzed in this report. In both diets, the daily quantity of calories consumed are kept constant at about 3533 kcal per person. The meat-based food system requires more energy, land, and water resources than the lactoovovegetarian diet. In this limited sense, the lactoovovegetarian diet is more sustainable than the average American meat-based diet.

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Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

Keywords: Meat-based diet, plant-based diet, environment, natural resources, fossil, energy, fuel

Topic: calories, diet, food, fossils, meat, plants, plant-based diet

Issue Section: Environmental impact of vegetarian diets

INTRODUCTION

Worldwide, an estimated 2 billion people live primarily on a meat-based diet, while an estimated 4 billion live primarily on a plant-based diet. The shortages of cropland, fresh water, and energy resources require most of the 4 billion people to live on a plant-based diet. The World Health Organization recently reported that more than 3 billion people are malnourished (1, 2). This is the largest number and proportion of malnourished people ever recorded in history. In large measure, the food shortage and malnourishment problem is primarily related to rapid population growth in the world plus the declining per capita availability of land, water, and energy resources (3).

Like the world population, the US population continues to grow rapidly. The US population doubled in the past 60 y and is projected to double again in the next 70 y (4) (**Figure 1**). The US food production system uses about 50% of the total US land area, approximately 80% of the fresh water, and 17% of the fossil energy used in the country (3). The heavy dependence on fossil energy suggests that the US food system, whether meat-based or plant-based, is not sustainable. The use of land and energy resources devoted to an average meat-based diet compared with a lactoovovegetarian (plant-based) diet is analyzed in this report. In both diets, the daily quantity of calories consumed was kept constant at about 3533 kcal per person.

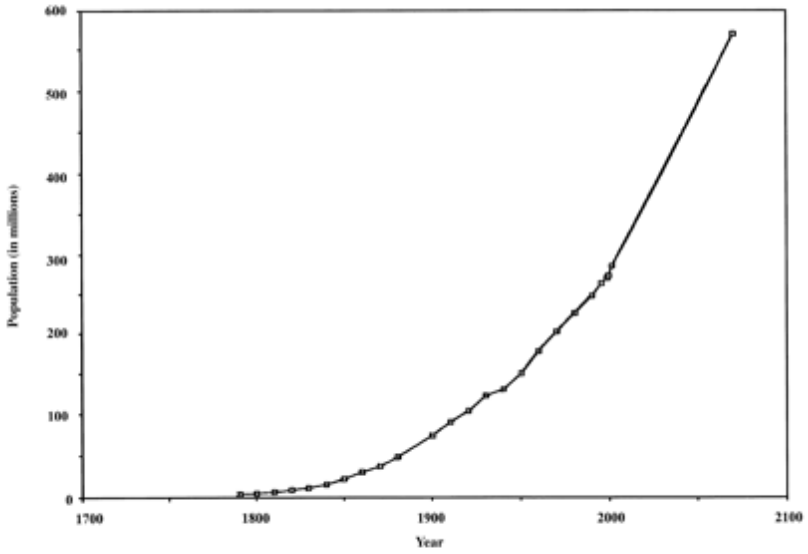
FIGURE 1.

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Case No.: IPR2023-00207
U.S. Patent No. 11,224,241



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Projection of US population growth in the next 70 y (4).

LACTOOVOVEGETARIAN DIET

The lactoovovegetarian diet was selected for this analysis because most vegetarians are on this or some modified version of this diet. In addition, the American Heart Association reported that the lactoovovegetarian diet enables individuals to meet basic nutrient needs (5).

A comparison of the calorie and food consumption of a lactoovovegetarian diet and a meat-based diet is provided in **Table 1**. In the lactoovovegetarian diet, the meat and fish calories were replaced by proportionately increasing most other foods consumed in Table 1 in the vegetarian diet except sugar and sweeteners, fats, and vegetable oils. The total weight of food consumed was slightly higher (1002 kg per year) in the lactoovovegetarian diet than in the meat-based diet (995 kg per year). The most food calories consumed in both diets were associated with food grains, and the second largest amount of calories consumed was from sugar and sweeteners.

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TABLE 1 Per capita food consumption, energy, and protein of foods of a meat-based compared with a lactoovovegetarian diet in the United States

Skip to Main Content

Food	Meat-based diet[1]	Energy	Protein	Lactoovovegetarian diet[2]	Energy	Protein

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

	kg	*kcal*	*g*	*kg*	*kcal*	*g*
Food grain	114	849	24.9	152	1132	33.2
Pulses (legumes)	4.3	40	2.0	7.5	70	4.5
Vegetables	239	147	6.6	286	155	8.8
Oil crops	6	71	3.0	8	95	4.0
Fruit	109	122	1.4	112	122	1.9
Meat	124	452	41.1	0	0	0
Fish	20.3	28	4.7	0	0	0
Dairy products	256	385	22.5	307.1	473	30.0
Eggs	14.5	55	4.2	19.2	73	5.6
Vegetable oils	24	548	0.2	25	570	0.2
Animal fats	6.7	127	0.1	6.7	127	0.1
Sugar and sweeteners	74	686	0.2	74	686	0.2
Nuts	3.1	23	0.6	4.0	30	0.8
Total	994.9	3533	111.5	1001.5	3533	89.3
Feed grains[3]	816.0	—	—	450.0	—	—

1 Data from FAOSTAT (6).

2 Estimated.

3 Feed grains are cereal grains fed to livestock.

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The amount of feed grains used to produce the animal products (milk and eggs) consumed in the lactoovovegetarian diet was about half (450 kg) the amount of feed grains fed to the livestock (816 kg) to produce the animal products consumed in the meat-based diet (Table 1). This is expected because of the relatively large amount of animal products consumed in the meat-based diet (7). Less than 0.4 ha of cropland was used to produce the food for the

Skip to Main Content
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Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

vegetarian-based diet, whereas about 0.5 ha of cropland was used in the meat-based diet (8). This reflects the larger amount of land needed to produce the meat-based diet (Table 1).

The major fossil energy inputs for grain, vegetable, and forage production include fertilizers, agricultural machinery, fuel, irrigation, and pesticides (8, 9). The energy inputs vary according to the crops being grown (10). When these inputs are balanced against their energy and protein content, grains and some legumes, such as soybeans, are produced more efficiently in terms of energy inputs than vegetables, fruits, and animal products (8). In the United States, the average protein yield from a grain crop such as corn is 720 kg/ha (10). To produce 1 kcal of plant protein requires an input of about 2.2 kcal of fossil energy (10).

MEAT-BASED DIET

The meat-based diet differs from the vegetarian diet in that 124 kg of meat and 20.3 kg of fish are consumed per year (Table 1). Note that the number of calories is the same for both diets because the vegetarian foods consumed were proportionately increased to make sure that both diets contained the same number of calories. The total calories in the meat and fish consumed per day was 480 kcal. The foods in the meat-based diet providing the most calories were food grains and sugar and sweeteners—similar to the lactoovovegetarian diet.

In the United States, more than 9 billion livestock are maintained to supply the animal protein consumed each year (11). This livestock population on average outweighs the US human population by about 5 times. Some livestock, such as poultry and hogs, consume only grains, whereas dairy cattle, beef cattle, and lambs consume both grains and forage. At present, the US livestock population consumes more than 7 times as much grain as is consumed directly by the entire American population (11). The amount of grains fed to US livestock is sufficient to feed about 840 million people who follow a plant-based diet (7). From the US livestock population, a total of about 8 million tons (metric) of animal protein is produced annually. With an average distribution assumed, this protein is sufficient to supply about 77 g of animal protein daily per American. With the addition of about 35 g of available plant protein consumed per person, a total of 112 g of protein is available per capita in the United States per day (11). Note that the recommended daily allowance (RDA) for adults day is 56 g of protein from a mixed diet. Therefore, based on these data, each American consumes about twice the RDA for protein. Americans on average are eating too much and are consuming about 1000 kcal in excess per day per capita (12, 13). The protein consumed per day on the lactoovovegetarian diet is 89 g per day. This is significantly lower than the 112 g for the meat-based diet but still much higher than the RDA of 56 g per day.

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Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

About 124 kg of meat is eaten per American per year (6). Of the meat eaten, beef amounts to 44 kg, pork 31 kg, poultry 48 kg, and other meats 1 kg. Additional animal protein is obtained from the consumption of milk, eggs, and fish. For every 1 kg of high-quality animal protein produced, livestock are fed about 6 kg of plant protein. In the conversion of plant protein to animal protein, there are 2 principal inputs or costs: 1) the direct costs of production of the harvest animal, including its feed; and 2) the indirect costs for maintaining the breeding herds.

Fossil energy is expended in livestock production systems (**Table 2**). For example, broiler chicken production is the most efficient, with an input of 4 kcal of fossil energy for each 1 kcal of broiler protein produced. The broiler system is primarily dependent on grain. Turkey, also a grain-fed system, is next in efficiency, with a ratio of 10:1. Milk production, based on a mixture of two-thirds grain and one-third forage, is relatively efficient, with a ratio of 14:1. Both pork and egg production also depend on grain. Pork production has a ratio of 14:1, whereas egg production has a 39:1 ratio.

TABLE 2 Animal production in the United States and the fossil energy required to produce 1 kcal of animal protein

Livestock and animal products	Production volume[1]	Ratio of energy input to protein output[2]
	$\times 10^6$	*kcal*
Lamb	7	57:1
Beef cattle	74	40:1
Eggs	77000	39:1
Swine	60	14:1
Dairy (milk)	13	14:1
Turkeys	273	10:1
Broilers	8000	4:1

1 Data from US Department of Agriculture (11).

2 Data from Pimentel (9).

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Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

The 2 livestock systems depending most heavily on forage but also using significant amounts of grain are the beef and lamb production systems (**Table 3**). The beef system has a ratio of 40:1, while the lamb has the highest, with a ratio of 57:1 (Table 2). If these animals were fed on only good-quality pasture, the energy inputs could be reduced by about half.

TABLE 3 Grain and forage inputs per kilogram of animal product produced

Livestock	Grain[1]	Forage[2]
	kg	*kg*
Lamb	21	30
Beef cattle	13	30
Eggs	11	—
Swine	5.9	—
Turkeys	3.8	—
Broilers	2.3	—
Dairy (milk)	0.7	1

1 Data from US Department of Agriculture (11).

2 Data from Morrison (14) and Heitschmidt et al (15).

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The average fossil energy input for all the animal protein production systems studied is 25 kcal fossil energy input per 1 kcal of protein produced (Table 2). This energy input is more than 11 times greater than that for grain protein production, which is about 2.2 kcal of fossil energy input per 1 kcal of plant protein produced (**Table 4**). This is for corn and assumes 9% protein in the corn. Animal protein is a complete protein based on its amino acid profile and has about 1.4 times the biological value of grain protein (8).

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TABLE 4 Energy inputs and costs of corn production per hectare in the United States

Inputs	Quantity	Energy	Cost
		kcal×1000	*$*

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

Labor (h)[1]	11.4 (16)[2]	462	114.00[3]
Machinery (kg)	55 (8)	1018 (17)	103.21 (18)
Diesel (L)	42.2 (19, 20)	481 (17)	8.87 (21)
Gasoline (L)	32.4 (19, 20)	328 (17)	9.40 (21)
Nitrogen (kg)	144.6 (22)	2688 (23)	89.65 (21)
Phosphorus (kg)	62.8 (22)	260 (23)	34.54 (21)
Potassium (kg)	54.9 (22)	179 (23)	17.02 (21)
Lime (kg)	699 (22)	220 (17)	139.80 (16)
Seeds (kg)	21 (8)	520 (17)	74.81 (24)
Irrigation (cm)	33.7 (25)	320 (17)	123.00
Herbicides (kg)	3.2 (22)	320 (17)	64.00[4]
Insecticides (kg)	0.92 (22)	92 (17)	18.40[4]
Electricity (kWh)	13.2 (19, 20)	34 (17)	2.38[5]
Transportation (kg)[6]	151	125 (17)	45.30[7]
Total (kg yield)	7965 (27)	7047[8]	844.38

1 It is assumed that a person works 2000 h/y and uses an average of 8100 L oil equivalents/y.

2 Reference.

3 It is assumed that farm labor is paid $10/h.

4 It is assumed that herbicide and insecticide prices are $20/kg.

5 The price of electricity is $0.07/kWh (26).

6 Goods transported include machinery, fuels, and seeds that were shipped an estimated 1000 km.

7 Transport was estimated to cost $0.30/kg.

8 Ratio of kcal input to output = 1:4.07.

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Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

LAND RESOURCES

More than 99.2% of US food is produced on land, while < 0.8% comes from oceans and other aquatic ecosystems. The continued use and productivity of the land is a growing concern because of the rapid rate of soil erosion and degradation throughout the United States and the world. Each year about 90% of US cropland loses soil at a rate 13 times above the sustainable rate of 1 ton/ha/y (28). Also, US pastures and rangelands are losing soil at an average of 6 tons/ha/y. About 60% of United States pastureland is being overgrazed and is subject to accelerated erosion.

The concern about high rates of soil erosion in the United States and the world is evident when it is understood that it takes approximately 500 y to replace 25 mm (1 in) of lost soil (28). Clearly, a farmer cannot wait for the replacement of 25 mm of soil. Commercial fertilizers can replace some nutrient loss resulting from soil erosion, but this requires large inputs of fossil energy.

WATER RESOURCES

Agricultural production, including livestock production, consumes more fresh water than any other activity in the United States. Western agricultural irrigation accounts for 85% of the fresh water consumed (29). The water required to produce various foods and forage crops ranges from 500 to 2000 L of water per kilogram of crop produced. For instance, a hectare of US corn transpires more than 5 million L of water during the 3-mo growing season. If irrigation is required, more than 10 million L of water must be applied. Even with 800–1000 mm of annual rainfall in the US Corn Belt, corn usually suffers from lack of water in late July, when the corn is growing the most.

Producing 1 kg of animal protein requires about 100 times more water than producing 1 kg of grain protein (8). Livestock directly uses only 1.3% of the total water used in agriculture. However, when the water required for forage and grain production is included, the water requirements for livestock production dramatically increase. For example, producing 1 k fresh beef may require about 13 kg of grain and 30 kg of hay (17). This much forage and g requires about 100 000 L of water to produce the 100 kg of hay, and 5400 L for the 4 kg of grain. On rangeland for forage production, more than 200 000 L of water are needed to produce 1 kg of beef (30). Animals vary in the amounts of water required for their production. In contrast to beef, 1 kg of broiler can be produced with about 2.3 kg of grain requiring approximately 3500 L of water.

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Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

CONCLUSION

Both the meat-based average American diet and the lactoovovegetarian diet require significant quantities of nonrenewable fossil energy to produce. Thus, both food systems are not sustainable in the long term based on heavy fossil energy requirements. However, the meat-based diet requires more energy, land, and water resources than the lactoovovegetarian diet. In this limited sense, the lactoovovegetarian diet is more sustainable than the average American meat-based diet.

The major threat to future survival and to US natural resources is rapid population growth. The US population of 285 million is projected to double to 570 million in the next 70 y, which will place greater stress on the already-limited supply of energy, land, and water resources. These vital resources will have to be divided among ever greater numbers of people.

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FOOTNOTES

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Case No.: IPR2023-00207
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Published: 18 December 2009

Meat and Morality: Alternatives to Factory Farming

Evelyn B. Pluhar

Journal of Agricultural and Environmental Ethics **23**, 455–468 (2010) | Cite this article

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> factory farming (FAK-tuh-ree FAHR-ming) noun: an industrialized system of producing meat, eggs, and milk in large-scale facilities where the animal is treated as a machine
> (Wordsmith 2008)

Abstract

Scientists have shown that the practice of factory farming is an increasingly urgent danger to human health, the environment, and nonhuman animal welfare. For all these reasons, moral agents must consider alternatives. Vegetarian food production, humane food animal farming, and in-vitro meat production are all explored from a variety of ethical perspectives, especially utilitarian and rights-based viewpoints, all in the light of current U.S. and European initiatives in the public and private sectors. It is concluded that vegetarianism and potentially in-vitro meat production are the best-justified options.

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Notes

1. No statistics are available for the number of aquatic animals slaughtered; however, it is thought that this number is likely equal to the land animals killed (HSUS 2008).

2. For an example of the latter sort of view see Frey (1989) and Frey and Paton (1989). Frey rejects speciesism but embraces experimentation and meat consumption. He consistently concludes that mentally disadvantaged humans could justifiably be vivisected. For his current thoughts on the justifiability of factory farming, see Frey (2004).

3. For Tom Regan's most recent reply to the predation argument, see Regan (2001, p. 19). See also Pluhar (1995, p. 277).

4. The one case where supplementation or fortification is required for vegans concerns vitamin B-12. Found in dairy products, eggs, and meat, B-12 does not occur naturally in plants. Fortified sources of B-12, such as cereals, are readily available.

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362    0#$aVol. 4, no. 1 (1991)-
500    ##$aTitle from cover
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Attachment 16c

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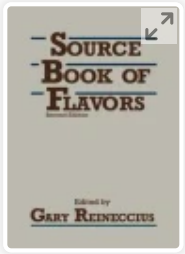
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650 0 Agriculture $x Moral and ethical aspects $v Periodicals.
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Attachment 17a

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U.S. Patent No. 11,224,241



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Process Flavors

Charles Manley

Chapter

724 Accesses | **4** Citations

Abstract

Flavors may be produced by a large variety of methods, from the purely synthetic approach, to the extraction of volatile components from materials of botanical origin. Science and technology have led us to more sophisticated ways of creating flavors. An area of knowledge that has developed into a major commercial venture for the flavor industry is the production of process flavors. The term “process flavor” has been used to define a group of flavors or flavoring ingredients that are produced from precursor materials via some type of processing technique(s). The major processing techniques in use today are thermal processing and enzyme modification. Both techniques have been in commercial use for approximately 25 years and currently represent a significant part of the flavors industry’s commercial and research activities. A summary of the general types of process flavors is given in Table 5-1.

Keywords

American Chemical Society **Maillard Reaction** **Glyceryl Ether**

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

Thermal Generation **Cheddar Cheese**

These keywords were added by machine and not by the authors. This process is experimental and the keywords may be updated as the learning algorithm improves.

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BOOK

Flavor, fragrance, and odor analysis

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Case No.: IPR2023-00207
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Analysis of α-dicarbonyl compounds and volatiles formed in Maillard reaction model systems

Jiyoon Cha, Trishna Debnath & Kwang-Geun Lee

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Abstract

In this study, production of three α-dicarbonyl compounds (α-DCs) including glyoxal (GO), methylglyoxal (MGO), and diacetyl (DA) as well as volatile flavor compounds was analyzed using Maillard reaction (MR) model systems. A total of 16 model systems were assembled using four amino acids and four reducing sugars, and reactions were performed at 160 °C and pH 9. Determination of α-DCs was conducted using a gas chromatography/nitrogen phosphorous detector (GC-NPD) after derivatization and liquid-liquid extraction. α-DC levels in MR model systems were 5.92 to 39.10 μg/mL of GO, 3.66 to 151.88 μg/ml of MGO, and 1.10 to 6.12 μg/mL of DA. The highest concentration of total α-DCs was found in the fructose-threonine model system and the lowest concentration in the lactose-cysteine model system. Volatile flavor compounds were analyzed using solid-phase micro-extraction (SPME) followed by GC-mass spectrometry (GC-MS). Different volatile flavor compound profiles were identified in the different MR model systems. Higher concentrations of α-DCs and volatile flavor

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

compounds were observed in monosaccharide-amino acid MR model systems compared with disaccharide-amino acid model systems.

Introduction

The Maillard reaction is the most basic chemical reaction occurring in many foods, in the form of a non-enzymatic browning reaction between a reducing sugar and an amino acid. Maillard reaction products (MRPs) generally have a positive impact on taste, color, and flavor of food, but some of them can have a negative toxic impact as well.

α-Dicarbonyl compounds (α-DCs) are yellow colored, low molecular weight organic compounds containing two carbonyl groups on the α-carbon[1]. They are formed from sugar fragmentation during non-enzymatic browning and are intermediates in caramelization and the Maillard reaction[2], they are also formed during oxidative degradation. In addition, α-DCs exist in fermented foods and beverages. Approximately 18 kinds of α-DCs have been identified in various foods, among which glyoxal (GO), methylglyoxal (MGO), and diacetyl (DA) are the most representative. Recent studies have found that α-DCs can pose a safety risk[3]. Amoroso *et al.*[3] reported the cytotoxic effect of α-DCs *in vitro*[3]. According to their results, both undigested and digested α-DCs induce cytotoxicity in human cells and inhibit human DNA repair enzymes. Further, Morgan *et al.*[4] reported respiratory toxicity of diacetyl in C57Bl/6 mice reproducing characteristics of human obliterative bronchiolitis. In addition, α-DCs were reported as precursors of toxic substances such as 4(5)-methylimidazole, heterocyclic flavor compounds with respiratory toxicity[5]. α-DCs react with free amino group of proteins to form the Advanced glycation endproducts (AGEs) which associated with diabetes and kidney disease in the human body[6]. It has been reported that inhalation of glyoxal causes local irritation of the eyes and respiratory organs. Oral exposure to glyoxal may also cause congestion of gastrointestinal tract, lung, and kidney[7]. In this study, α-DCs were analyzed as potential undesirable products of the Maillard reaction.

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

Previous studies have reported α-DC analysis through derivatization to quinoxalines by o-phenylenediamine (OPD) to terminate the Maillard reaction[8,9]. This reaction easily occurs at room temperature in a slightly basic solution[10], and allows detection using various analytical methods including liquid chromatography, gas chromatography-mass spectrometry, and gas chromatography-nitrogen phosphorous detection[11].

Aroma compounds in Maillard reactions are formed based on types of sugars and amino acids present, temperature, reaction time, and pH[12]. Over 100 types of such aroma compounds have been found in conventional flavor studies. Among them, roasted and sweet flavors are accepted as having positive effects in foods such as coffee, caramel sauce, and bread. Pyrazines, representative compounds of the roasted flavor, are formed by reaction of amines and α-dicarbonyls through Strecker degradation[13,14]. Formation of furaneols, representative compounds of the sweet flavor, occurs through the 2, 3-enolization pathway leading to 1-deoxyosones as intermediates[15].

MRPs that consist mainly of low-molecular volatile compounds are difficult to sample and analyze, and individual compound analysis has been conducted in such cases. Studies of MRPs with sensory characteristics have also been performed. However, studies comparing desirable aroma compounds and undesirable toxic compounds are not common. In this study, we compared α-dicarbonyl compounds and aroma compounds produced in reducing sugar–amino acid model systems. This work puts down the bases for studies on desirable preference factor like flavors and undesirable toxic substances.

Materials and Methods

Chemicals

D-Glucose, D-fructose, D-maltose, D-lactose, L-lysine, L-serine, L-threonine, L-cysteine, L-norvaline, glyoxal, methylglyoxal, diacetyl, quinoxaline, 2-methylquinoxaline, 2,3-dimethylquinoxaline, o-phenylenediamine dihydrochloride, 1-methylpyrazole, C8-C20 alkane standard, methyl cinnamate, and sodium chloride were purchased from Sigma-Aldrich (St. Louis, MO). Water and ethyl acetate were obtained from J.T. Baker

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

(Philipsburg, NJ, USA). All solutions were stored at 4 °C until analysis. Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS, 50 µm film thickness) solid phase microextraction (SPME) fiber, polypropylene hold caps, and polytetrafluoroethylene (PTFE)/silicone septa were purchased from Supelco Inc. (Belfonte, PA, USA).

Preparation of Maillard reaction model system solutions

Equimolar solutions (0.1 M) of four reducing sugars (glucose, fructose, lactose, and maltose) and four amino acids (lysine, serine, threonine, and cysteine) were prepared in distilled water and pH was set to 9. Solutions were placed in swing-top bottles and reactions were performed at 160 °C for 2 h in an oven (OF-22, Jeiotech Co., Seoul, Korea) to instigate the Maillard reaction. All solutions were cooled under running cold water to room temperature immediately in order to stop the reaction progress. All samples were stored at 4 °C until they were analyzed.

Analysis of α-Dicarbonyl compounds

Quantification and analysis of α-DCs produced in the Maillard reaction model systems was performed using o-phenylenediamine dihydrochloride derivatization reported in a previous study[1]. A 3 mL volume of each sample and 2 mL of o-phenylenediamine dihydrochloride were placed in 20 mL vials. The mixtures were set to pH 12 and stirred for 2 h at 600 rpm for derivatization of α-DCs to quinoxalines. Then, 5 mL of ethyl acetate was added to the mixtures and shaken for extraction. Extracted solutions were analyzed using GC-NPD. The concentrations used to perform standard calibration curve were 0.5, 1, 5, 10, 50, 100 µg/ml.

An Agilent 6890 N gas chromatograph with a nitrogen phosphorous detector was used for analysis of α-DCs. A DB-WAX column (30 m × 250 × 0.25 µm; J&W Scientific, Folsom, CA) was used for separation. Helium was used as carrier gas at constant flow of 1.5 mL/min. Injection was set to the splitless mode at 260 °C. Oven temperature was held at

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

40 °C for 2 min and then increased to 170 °C at 20 °C/min and held for 15 min. Detector temperature was set at 300 °C. Nitrogen was used as make-up gas at a flow rate of 5 mL/min.

Analysis of aroma compounds

Aroma compounds were analyzed using SPME (Solid phase micro-extration). A 5-mL volume of sample was added to a 20 mL headspace vial containing 0.75 g of sodium chloride. 5 µL of methyl cinnamate (internal standard, 100 µg/mL), 10 µL of alkane standard (10 µg/mL), and a magnetic stirring bar were added and the vial sealed with a PTFE cap. Subsequently, samples were stirred for 30 min at 70 °C to reach equilibrium. Volatile flavor compound extraction was carried out by injecting a fiber into the vial for adsorption at 70 °C for 10 min. Then, the fiber was inserted into the GC injector port and held for 10 min to desorb the volatile flavor compounds.

An Agilent 7820 A gas chromatograph and a 5977E mass spectrometer were used for volatile flavor compound detection. Separation of volatile aroma compounds was performed using a DB-WAX column (60 m × 250 × 0.25 µm). The oven was held at 40 °C for 5 min, then raised to 185 °C at 5 °C/min and held for 20 min, and then raised to 200 °C at 10 °C/min and held for 5 min. Helium was used as carrier gas at a constant flow rate of 0.8 mL/min. Injection mode was set to splitless at 230 °C. The detector was set to the scan mode and the scan range was from 20 to 550 m/z. Volatile flavor compounds were identified based on Kovats index on DB-WAX, co-injection, and mass spectrum from the NIST library. The quantification of each flavor compound was displayed as peak area ratio (PAR, peak area of each compound/that of internal standard).

Statistical analysis

All samples were analyzed in triplicate and the analysis results are presented as mean ± standard deviation.

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

Results and Discussion

Analysis of α-DCs in Maillard reaction model systems

Reaction model solutions were prepared with glucose and lysine at four temperatures (100, 120, 140 and 160 °C) and four pH levels (3,5,7, and 9) to simulate Maillard reaction model system conditions. Level of different α-DCs produced in the reactions, as shown in Table 1 was 0.36 to 16.78 µg/mL of GO, trace to 50.81 µg/mL of MGO, and N.D. to 2.27 µg/mL of DA. Higher pH and temperature resulted in greater production of total α-DCs including GO, MGO, and DA. Conditions yielding the maximum concentration of total α-DCs were 160 °C and pH 9. These conditions were then used to study the reducing sugar-amino acid model systems. As shown in Table 2, a total of 16 model systems were used with combinations of four amino acids (lysine, serine, threonine, and cysteine) and four reducing sugars (glucose, fructose, maltose, and lactose). Under the above conditions, basic amino acids containing hydroxyl groups have higher reactivity with α-DCs than acidic and nonpolar amino acids[16]. Levels of α-DCs in MR model systems were 5.92 to 39.10 µg/mL of GO, 3.66 to 151.88 µg/mL of MGO, and 1.10 to 6.12 µg/mL of DA. The highest concentration of α-DCs was detected in the fructose-threonine model system and the lowest concentration in the lactose-cysteine model system. In addition, DA concentration was 1.66 to 6.12 µg/mL in lactose model systems, compared with other reducing sugar model systems. These results indicated that α-DCs were produced mainly from monosaccharides rather than from disaccharides. Hollnagel and Kroh reported that monosaccharides forms more α-DCs than disaccharides[2]. Different from our results among monosaccharides glucose formed more α-DCs than fructose[2]. In the report they suggested that fructose forms more cyclic compounds rather than fragmentation products such as α-DCs. However, Maillard reaction conditions were different and it could give rise to the conflicting results.

Table 1 Concentrations (µg/mL) of glyoxal (GO), methylglyoxal (MGO), and diacetyl (DA) produced in the glucose–lysine Maillard reaction model system.

Full size table >

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

Table 2 Concentrations (µg/mL) of glyoxal (GO), methylglyoxal (MGO), and diacetyl (DA) produced in reducing sugar and amino acid Maillard reaction model systems at 160 °C and pH 9.

Full size table >

Aroma compounds formed in Maillard reaction model systems

Volatile flavor compounds formed in the reaction model systems were identified. Results of analysis showed that volatile flavor compounds were primarily composed of eight compounds representing roasted flavor (2,3-dimethyl pyrazine, 2,5-dimethyl pyrazine, 2,6- dimethyl pyrazine, 2,3,5-trimethyl pyrazine, 2-ethyl-3,6-dimethyl pyrazine, 2-ethyl-3,5- dimethyl pyrazine, 2,3-dimethyl-5-methylpyrazine, and 2-Acetylthiazole) and five representing sweet flavor (benzaldehyde, 5-methylfurfural, furaneol, homofyraneol, and norfuraneol).

Table 3 shows results from glucose (Glu)–amino acid (lysine, serine, threonine and cysteine) model systems. Total peak area ratio (PAR) in Glu–Lys, Glu–Ser, Glu–Thr and Glu–Cys model systems was 1.3606, 0.7369, 1.5351, and 0.2322, respectively. The compound 2,5-dimethyl pyrazine representing odor of chocolate and roasted nuts was detected significantly in Glu–-Lys, Glu–Ser, Glu–Thr, and Glu–Cys model systems with PAR of 1.0248, 0.4728, 0.6512, and 0.0692, respectively[17]. The PARs of 2,5-dimethyl pyrazine were 26–75% in the model systems compared to total PAR. In the previous study 2, 5-dimethyl pyrazine was reported as a main flavor in Maillard reaction model systems[13].

Table 3 Aroma compounds produced in glucose (Glu)–amino acid (lysine (Lys), serine (Ser), threonine (Thr), and cysteine (Cys)) Maillard reaction model systems.

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

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In the Glu–Thr model system, 2-ethyl-3,5-dimethylpyrazine was the highest detected with peak area ratio 0.7304. In addition, 2-acetylthiazole representing popcorn-like[18] flavor had a peak area ratio of 0.0223 in the Glu–Cys sulfur model system. Benzaldehyde, representing sweet burnt sugar and roasted almond flavors[19] had a peak area ratio of 0.0140, 0.0064, 0.0059, and 0.0124, respectively in Glu–Lys, Glu–Ser, Glu–Thr, and Glu–Cys model systems. 2,3-Dimethyl-5-methylpyrazine, representing roasted flavor and 5-Methylfurfural, Furaneol, Homofuraneol, and Norfuraneol representing sweet flavor were not detected.

Table 4 shows results from fructose (Fru)–amino acid (Lys, Ser, Thr, and Cys) model systems. Total peak area ratio in Fru–Lys, Fru–Ser, Fru–Thr, and Fru–Cys model systems was 1.1592, 0.7983, 2.6741, and 0.1341, respectively. The compound 2,5-Dimethyl pyrazine was detected with peak area ratio of 0.9241, 0.4980, 1.0404, and 0.0396, respectively in Fru–Lys, Fru–Ser, Fru–Thr, and Fru–Cys model systems. In the Fru–Cys model system, 2, 6-dimethyl pyrazine was the highest detected with peak area ratio 0.0567. In addition, 2-acetylthiazole showed a peak area ratio of 0.0140 in the Fru–Cys model system. The compounds 2,3-Dimethyl-5-methylpyrazine, 5-Methylfurfural, Furaneol, Homofuraneol, and Norfuraneol were not detected.

Table 4 Aroma compounds produced in fructose (Fru)–amino acid (lysine (Lys), serine (Ser), threonine (Thr), and cysteine (Cys)) Maillard reaction model systems.

Full size table >

Table 5 shows results from maltose (Mal)–amino acid (Lys, Ser, Thr, and Cys) model systems. Total peak area ratio in Mal–Lys, Mal–Ser, Mal–Thr and Mal–Cys model systems

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

was 0.0066, 0.0161, 0.0217, and 0.0110, respectively. Benzaldehyde was detected with peak area ratio of 0.0041, 0.0051, 0.0053, and 0.0110, respectively in Mal–Lys, Mal–Ser, Mal–Thr, and Mal–Cys model systems. The compounds 2,3-dimethyl pyrazine, 2,6-dimethyl pyrazine, 2-ethyl-3,5-dimethylpyrazine, 2,3-Dimethyl-5-methylpyrazine, 2-acethylthiazole, 5-methylfurfural, Furaneol, Homofuraneol, and Norfuraneol were not detected.

Table 5 Aroma compounds produced in maltose (Mal)–amino acid (lysine (Lys), serine (Ser), threonine (Thr), and cysteine (Cys)) Maillard reaction model systems.

Full size table >

Table 6 shows results from lactose (Lac)–amino acid (Lys, Ser, Thr, and Cys) model systems. Total peak area ratio in Lac–Lys, Lac–Ser, Lac–Thr, and Lac–Cys model systems was 0.0053, 0.0063, 0.5224, and 0.0043, respectively. Benzaldehyde was detected with peak area ratio of 0.0053, 0.0063, 0.0043, and 0.0043, respectively in Lac– Lys, Lac–Ser, Lac–Thr, and Lac–Cys model systems. In the Lac–Thr model system, 2,5-dimethyl pyrazine, 2,3,5-trimetyl pyrazine, 2-ethyl-3,6-dimethylpyrazine, and 2-ethyl-3,5-dimethylpyrazine were present with peak area of 0.0070, 0.04961, 0.0086, and 0.0064, respectively. The compounds 2, 3-dimethyl pyrazine, 2,6-dimethyl pyrazine, 2,3-Dimethyl-5-methylpyrazine, 2-acethylthiazole, 5-Methylfurfural, Furaneol, Homofuraneol, and Norfuraneol were not detected. Overall, in the reducing sugar and amino acid Maillard reaction model systems, roasted flavors were produced at higher levels while sweet flavor was produced at lesser levels.

Table 6 Aroma compounds produced in lactose (Lac)–amino acid (lysine (Lys), serine (Ser), threonine (Thr), and cysteine (Cys)) Maillard reaction model systems.

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

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Conclusions

In this study, desirable volatile flavor compounds and undesirable α-dicarbonyl compounds formed during the Maillard reaction were analyzed using reducing sugar–amino acid model systems. The levels of α-DCs produced in MR model systems were 1.10 to 151.88 μg/mL. The highest concentration of total α-DCs was found in the fructose–threonine model system and the lowest concentration of total α-DCs was found in the lactose–cysteine model system. Different volatile flavor compound profiles were identified from different MR model systems. Higher concentrations of α-DCs and volatile flavor compounds were observed in monosaccharide–amino acid Maillard model systems than in disaccharide–amino acid model systems. As a future work, the balanced study between desirable flavor compounds and toxic compounds such as α-DCs are supposed to be carried out in our laboratory.

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Acknowledgements

This study was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT, & Future

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Jiyoon Cha, Trishna Debnath & Kwang-Geun Lee

Contributions

J. Cha and K. Lee wrote the main manuscript text and T. Debnath prepared Tables 4 and 5.

Corresponding author

Correspondence to Kwang-Geun Lee.

Ethics declarations

Competing Interests

This work of the authors has been funded by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT, & Future Planning (NRF-2015R1A2A2A01005772, NRF-2018R1A2B6002634) and the Agriculture, Food and Rural Affairs Research Centre Support Program, Ministry of Agriculture, Food and Rural Affairs, Republic of Korea). We, the authors declare no potential conflict of interest.

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588 Description based on [v.] 1, article no. 1 (14 June 2011); title from PDF running title (viewed Aug. 16, 2011).
588 Latest issue consulted: V. 10, Article number: 21152 (03 December 2020) (Nature website, viewed December 3, 2020).
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670 __ |**a** Random House |**b** (environmental science)

670 __ |**a** Barnhart dict. of new English |**b** (environmental science, group of sciences dealing with the environment)

670 __ |**a** Biol. & agr. index: |**b** v. 44, p. 668 (Environmental sciences)

670 __ |**a** LC data base, 3/24/92 |**b** (environmental sciences, environmental science)

670 __ |**a** Hennepin |**b** (Environmental sciences)

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020 $z 0841210306
050 00 TP372.55.M35 $b M34 1983
060 00 TP 372.55.M35 $b M219 1982
070 QD1.A45 $b no.215
082 00 664/.028 $2 19
084 58.34 $2 bcl
090 $b
245 04 The Maillard reaction in foods and nutrition / $c George R. Waller, editor, Milton S. Feather, editor.
260 Washington, D.C. : $b American Chemical Society, $c 1983.
300 xii, 585 pages : $b illustrations ; $c 24 cm
336 text $b txt $2 rdacontent
337 unmediated $b n $2 rdamedia
338 volume $b nc $2 rdacarrier
490 1 ACS symposium series, $x 0097-6157 ; $v 215
500 "Based on a symposium jointly sponsored by the Divisions of Agricultural and Food Chemistry, and Carbohydrate Chemistry at the 183rd meeting of the American Chemical Society, Las Vegas, Nevada, March 28-April 2, 1982."
504 Includes bibliographical references and index.
505 0 Seventy years of the Maillard reaction / Sin'itiro Kawamura -- A new mechanism of the Maillard reaction involving sugar fragmentation and free radical formation / Mitsuo Namiki and Tateki Hayashi -- Analytical use of fluorescence-producing reactions of lipid- and carbohydrate-derived carbonyl groups with amine end groups of polyamide powder / W.L. Porter, E.D. Black, A.M. Drolet, and J.G. Kapsalis -- Strecker degradation products from (1-^{13}C)-D-glucose and glycine / Tomas Nyhammar, Kjell Olsson, and Per-Åke Pernemalm -- Nitrite interactions in model Maillard browning systems / Gerald F. Russell --

Studies on the color development in stored plantation white sugars / Hung-Tsai Cheng, Wai-Fuk Lin, and Chung-Ren Wang -- Colored compounds formed by the interaction of glycine and xylose / H.E. Nursten and Rosemary O'Reilly -- Conditions for the synthesis of antioxidative arginine-xylose Maillard reaction products / G.R. Waller, R.W. Beckel, and B.O. Adeleye -- The variety of odors produced in Maillard model systems and how they are influenced by reaction conditions / M.J. Lane and H.E. Nursten -- Characteristics of some new flavoring materials produced by the Maillard reaction / E. Dworschák, V. Tarján, and S. Turos -- The Maillard reaction and meat flavor / Milton E. Bailey -- Sensory properties of volatile Maillard reaction products and related compounds : a literature review / Susan Fors -- Mechanism responsible for warmed-over flavor in cooked meat / A.M. Pearson and J.I. Gray -- Maillard technology : manufacturing applications in food products / James P. Danehy and Bernard Wolnak -- Maillard reaction products as indicator compounds for optimizing drying and storage conditions / K. Eichner and W. Wolf -- Characterization of antioxidative Maillard reaction products from histidine and glucose / H. Lingnert, C.E. Eriksson, and G.R. Waller -- The effect of browned and unbrowned corn products on absorption of zinc, iron, and copper in humans / P.E. Johnson, G. Lykken, J. Mahalko, D. Milne, L. Inman, H.H. Sandstead, W.J. Garcia, and G.E. Inglett -- Nutritional value of foods and feeds of plant origin : relationship to composition and processing / J.E. Knipfel, J.G. McLeod, and T.N. McCaig -- Effect of the Maillard browning reaction on the nutritive value of breads and pizza crusts / C.C. Tsen, P.R.K. Reddy, S.K. El-Samahy, and C.W. Gehrke -- Loss of available lysine in protein in a model Maillard reaction system / Barbee W. Tucker, Victor Riddle, and John Liston -- Effect of a glucose-lysine reaction mixture on protein and carbohydrate digestion and absorption / Rickard Öste, Inger Björck, Arne Dahlqvist, Margaretha Jägerstad, Per Sjödin, and Hans Sjöstrom -- Determination of available lysine by various procedures in Maillard-type products / H.F. Erbersdobler and T.R. Anderson -- Nonenzymatic glycosylation and browning of proteins in vivo / V.M. Monnier and A. Cerami -- Maillard reactions of therapeutic interest / L. Mester, L. Szabados, and M. Mester -- Nutritional and toxicological effects of Maillard browned protein ingestion in the rat / Stephen J. Pintauro, Tung-Ching Lee, and Clinton O. Chichester -- Mutagens in cooked foods : possible consequences of the Maillard reaction / William Barnes, Neil E. Spingarn, Claire Garvie-Gould, Loretto L. Vuolo, Y.Y. Wang, and John H. Weisburger -- Creatinine and Maillard reaction products as precursors of mutagenic compounds formed in fried beef / M. Jägerstad, A. Laser Reuterswärd, R. Öste, A. Dahlqvist, S. Grivas, K. Olsson, and T. Nyhammar -- Mutagens produced by heating foods / M. Nagao, S. Sato, and T. Sugimura -- Formation of mutagens by the Maillard reaction / Hirohisa Omura, Nazma Jahan, Kazuki Shinohara, and Hiroki Murakami.
540 Current Copyright Fee: GBP36.92 $c 0. $5 Uk
520 Abstract: Various aspects of the Maillard reaction (a non-enzymatic reaction that gives food its flavor and color during frying, roasting, and baking) are discussed for food scientists and nutritionists in 29 technical papers. The papers are organized into 7 sections, covering: historical development; chemical aspects (6 papers); flavors, tastes, and odors of cooked foods (6 papers); food technology aspects (3 papers); nutritional aspects, with emphasis on lysine losses (6 papers); in vivo Maillard reactions (2 papers); and toxicological aspects, with emphasis on mutagenproduction (5 papers). A literature review of the sensory properties of almost 450 Maillard reaction products is included. (wz).
650 0 Maillard reaction $v Congresses.
650 0 Food industry and trade $v Congresses.
650 0 Food handling.
650 0 Nutrition.
650 2 Food Handling $0 (DNLM)D005511
650 2 Food Technology $0 (DNLM)D005524
650 2 Nutritional Physiological Phenomena $0 (DNLM)D009747
650 6 Réaction de Maillard $0 (CaQQLa)201-0120706 $v Congrès. $0 (CaQQLa)201-0378219
650 6 Aliments $x Manipulation. $0 (CaQQLa)201-0052849

650 6 Nutrition. $0 (CaQQLa)201-0064996
650 7 Food industry and trade. $2 fast $0 (OCoLC)fst00930843
650 7 Maillard reaction. $2 fast $0 (OCoLC)fst01006114
650 17 Maillardreacties. $2 gtt
653 0 Food industry and trade
653 0 Maillard reaction
653 00 aminozuren
653 00 amino acids
653 00 chemische samenstelling
653 00 chemical composition
653 00 maillard-reactie
653 00 evenwicht
653 00 equilibrium
653 00 voedselsamenstelling
653 00 food composition
653 00 voedingsmiddelen
653 00 foods
653 00 monosaccharіden
653 00 monosaccharides
653 00 voedingswaarde
653 00 nutritive value
653 00 reactiemechanisme
653 00 reaction mechanism
653 10 Chemistry of Food Components
653 10 Chemie van voedselcomponenten
655 2 Congress $0 (DNLM)D016423
655 7 Conference papers and proceedings. $2 fast $0 (OCoLC)fst01423772
655 7 Conference papers and proceedings. $2 lcgft
655 7 Actes de congrès. $2 rvmgf $0 (CaQQLa)RVMGF-000001049
700 1 Waller, George R.
700 1 Feather, Milton S., $d 1936-
710 2 American Chemical Society. $b Division of Agricultural and Food Chemistry.
710 2 American Chemical Society. $b Division of Carbohydrate Chemistry.
776 08 $i Online version: $t Maillard reaction in foods and nutrition. $d Washington, D.C. : American Chemical Society, 1983 $w (OCoLC)706868785
830 0 ACS symposium series ; $v 215. $x 0097-6157
856 41 $u http://pubs.acs.org/doi/book/10.1021/bk-1983-0215 $z Rutgers restricted $z Full text available from ACS Symposium Series (1974-2012)

Attachment 20c

LC control no.: sh 85079891
LCCN Permalink: https://lccn.loc.gov/sh85079891
HEADING: Maillard reaction

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005 20120326102513.0
008 860211i| anannbabn |a ana
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053 _0 |**a** RB171 |**c** Pathology
053 _0 |**a** TP372.55.M35 |**c** Food processing
150 __ |**a** Maillard reaction
450 __ |**a** Browning, Nonenzymatic
450 __ |**a** Browning reaction
450 __ |**a** Nonenzymatic browning
550 __ |**w** g |**a** Chemical reactions
670 __ |**a** Food for health, 1986 |**b** (Maillard reaction: Nonenzymatic browning that takes place upon heating or prolonged storage of food)
670 __ |**a** McGraw Hill dict. sci. tech. |**b** (Maillard reaction; Browning reaction)
670 __ |**a** MESH |**b** (Maillard reaction x Browning reaction)
906 __ |**t** 9638 |**u** te04 |**v** 0
953 __ |**a** xx00 |**b** ym11

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MARC Display | Labelled Display

LC control no.: sh 85050282

LCCN Permalink: https://lccn.loc.gov/sh85050282

HEADING: Food industry and trade

000 02292cz a2200397n 450

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035 __ |**a** (DLC)sh 85050282

035 __ |**a** (DLC)48631

040 __ |**a** DLC |**c** DLC |**d** DLC

053 _0 |**a** HD9000 |**b** HD9495 |**c** Economics

053 _0 |**a** TP368 |**b** TP456 |**c** Processing and manufacture

150 __ |**a** Food industry and trade

360 __ |**i** subdivision |**a** Postharvest technology |**i** under individual food crops, e.g. |**a** Corn--Postharvest technology; |**i** and subdivision |**a** Processing |**i** under individual food animals and crops, e.g. |**a** Cattle--Processing; Corn--Processing

450 __ |**a** Food |**x** Processing

450 __ |**a** Food preparation industry

450 __ |**a** Food processing

450 __ |**a** Food processing industry

450 __ |**a** Food technology

450 __ |**a** Food trade

550 __ |**w** g |**a** Agricultural processing industries

550 __ |**a** Processed foods

670 __ |**a** Institute of Food Technologists web site, viewed Oct. 23, 2018 |**b** (Food science is the study of the physical, biological, and chemical makeup of food; and the concepts underlying food processing. Food technology is the application of food science to the selection, preservation, processing, packaging, distribution, and use of safe food)

680 __ |**i** Here are entered works on the processing of food in general and on the marketing of processed food products. Works on the processing of specific kinds of food products are entered under the heading for the specific product with the subdivision Processing or

under the heading for the industry. General works on the processing of agricultural products are entered under |**a** Agricultural processing. |**i** Works on a specific aspect or method of processing are entered under the heading for the specific process.

681 __ |**i** Note under |**a** Agricultural processing
906 __ |**t** 9946 |**u** te04 |**v** 0
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952 __ |**a** LC-BRF
953 __ |**a** xx00 |**b** ta27

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Attachment 20e

Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

LC control no.: sh 85050276
LCCN Permalink: https://lccn.loc.gov/sh85050276
HEADING: Food handling

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953 __ |**a** xx00 |**b** ta27

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245 0  0  ‡a Archives of biochemistry and biophysics.
260       ‡a New York, ‡b Academic Press.
300       ‡a v. ; ‡b ill. ‡c 23 cm.
310       ‡a Semimonthly
321       ‡a 14 nos. a year, ‡b 1977-
321       ‡a 2 issues per month
362 0     ‡a v. 31- Mar. 1951-
510 0     ‡a Biological abstracts ‡x 0006-3169
510 0     ‡a Chemical abstracts ‡x 0009-2258
510 0     ‡a Nuclear science abstracts ‡x 0029-5612
510 0     ‡a International aerospace abstracts ‡x 0020-5842
510 0     ‡a Bibliography of agriculture ‡x 0006-1530
555       ‡a Vols. 1-75, 1942-58. 1 v. (Includes index to the journal under its earlier title)
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780 0  0  ‡t Archives of biochemistry ‡x 0096-9621
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863 4  0  ‡8 1 ‡a 31-373 ‡i 1951-1999
865 4  1  ‡8 1 ‡a 316/324 ‡p B
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999 f f    ‡i 18378c95-92f5-4018-8408-cef54c6491d1 ‡s 17b886b3-5aef-4515-a96a-ba98212cb5e3
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Case No.: IPR2023-00207
U.S. Patent No. 11,224,241

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HEADING: Biophysics Periodicals

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150 __ |**a** Biophysics |**v** Periodicals
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670 __ |**a** Work cat.: Recent research developments in biophysics & biochemistry, 2001-
906 __ |**t** 8888 |**u** te00 |**v** 0
953 __ |**a** te00

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