JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Isolation and Composition of Chromoplasts from Tomatoes

LINN U. HANSEN* AND MEI-CHEN M. CHIU

Western Regional Research Center, U.S. Department of Agriculture, 800 Buchanan Street, Berkeley, California 94710-1105

The fruit of the tomato plant is composed of elongated tomato cells filled with organelles called chromoplasts (plastids). These plastids scattered throughout the cell are rich in nutrients, particularly protein (33%) and lipids (20%). They can be released from the cells by rupture of their cell membranes and then isolated. Plastids and their cell contents can be utilized by the food-processing industry for the preparation of special food products. This study was designed to examine the macronutrient content of isolated tomato plastids and, therefore, determine its potential nutritional value. Use of tomato plastids in pasta sauces and rice dishes, salsa, and extrusion products would increase the nutritional value of the product. Because glucose has been removed in the process of plastid isolation, tomato plastids are useful in the diets of diabetics and cardiovascular patients, as well as for patients in need of weight reduction. Composition comparison of tomato plastid is made with tomato paste, from which glucose has not been removed. Many people require low-sugar products for medical reasons (diabetics and those with cardiovascular disease) and others for weight loss. Therefore, tomato chromoplasts having high protein and lipid contents and low sugar content may be useful in meeting these particular human needs.

KEYWORDS: Tomato chromoplasts; wheat flour; rice flour; nutrition; macronutrients

INTRODUCTION

The fruit of the tomato plant (*Lycopersicon esculentum*) is largely composed of elongated tomato cells (pericarp) filled with organelles called chromoplasts (carotenoid-containing plastids) as depicted in **Figure 1**. The carotenoid lycopene, a micronutrient, is responsible for the deep red color of the plastids and is believed to have anticancer and anticholesterol properties because of its antioxidant properties (*1*). The chromoplast (plastid) is a biologically encapsulated membrane structure containing concentrated nutrients. The numerous plastids scattered throughout the cell are rich in macronutrients, particularly in protein and lipids. The plastids can be released from the tomato cells by rupture of their membranes and then isolated. Plant cells have plastids, but animal cells do not. It is a distinguishing anatomical feature between them. The tomato chromoplasts are the site of the biosynthesis of protein, lipid, carotenoid (lycopene), starch, and sugar (*2*, *3*).

In the history of studying diets for humans, there have been numerous epidemiological studies based on populations and their diets, which have indicated that certain diets are more beneficial for human health and longevity than others. The Mediterranean countries of Italy, Crete, France, and Spain have a diet consisting of large amounts of fruits and vegetables (tomatoes), bread, pasta, rice, olive oil as the principal fat, garlic, lean red meat in limited amounts, and moderate amounts of fish. From population studies (epidemiology), researchers have found that cardiovascular disease is lower and the life span extended on this type of diet (*4*−*6*). Many people require low-sugar products for medical reasons (diabetics and those with cardiovascular disease) and others for weight loss. Therefore, tomato chromoplasts having high protein and lipid contents and low sugar content may be useful in meeting these human needs.

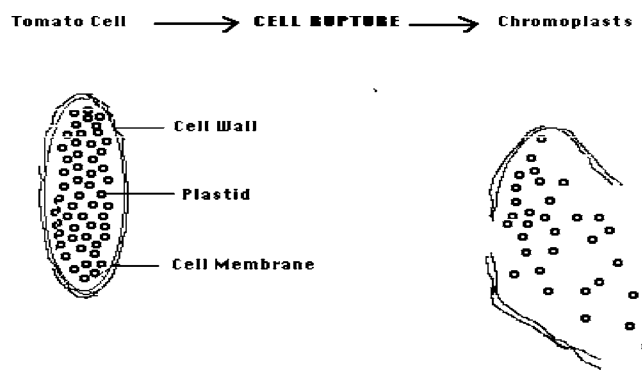

Figure 1. Chromoplasts released from the tomato cell (schematic).

This study was designed to examine the macronutrient content of the isolated plastids and, therefore, their potential nutritional value by determining their protein and essential amino acid contents, total fat, essential fatty acids, and dietary fiber, carbohydrate, and ash contents. Tomato paste, another tomato product, is compared to the tomato plastids. Using these data, we were able to evaluate some food categories that may be supplemented nutritionally by the addition of tomato plastids to them. The nutrients found in the plastids are believed to play

* Author to whom correspondence should be addressed (e-mail luh@pw.usda.gov).

10.1021/jf058013p
Published on Web 07/28/2005

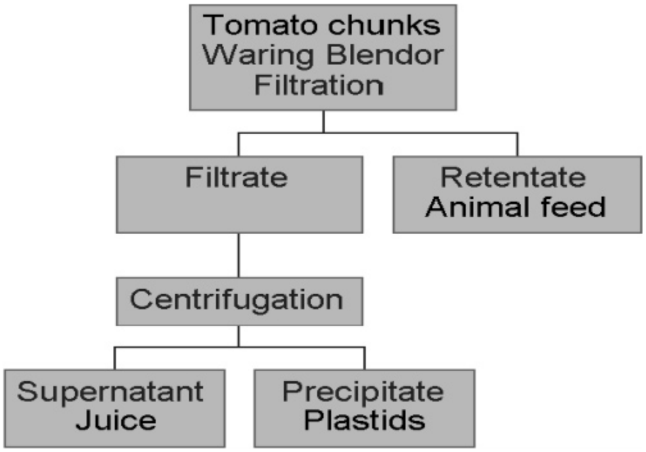

Figure 2. Flow sheet for the preparation of tomato plastids.

an important role in the prevention of cardiovascular disease (*7*), digestive diseases (constipation and diverticulitis), and some digestive tract cancers (*8*, *9*).

The plastids and their cell contents can be utilized by the food-processing industry for the preparation of special food products having enriched nutritional and health value. Plastids from tomatoes have been studied in basic plant research but not for food purposes as in our study. Tomato plastids could be used to increase the nutritional value of sauces, soups, tomato bouillon (concentrated stock), and wheat- and rice-based extruded products, when added to them. The paper is based on epidemiology findings, biology, chemistry, and nutritional research studies and biochemical methodology.

MATERIALS AND METHODS

Biological Isolation of Tomato Plastids (Figure 2). Chilled (10 °C) whole tomato chunks (*L. esculentum* var. Shady Lady from Lewis Farms, CA) were cut in ca. 1 × 3 cm pieces. The cut pieces (100 g) were put into a Waring blender containing 100 mL of 0.1 M citrate buffer (pH 6.5 at 10 °C). The blender was run at full speed for 5 s to release the plastids from the pericarp cells. Blending time is critical for the isolation of the plastids. An increase in blending time, determined experimentally, resulted in the tearing of the chromoplast membranes. Seeds, epidermis (skins), membranes, and cell wall materials (pomace) were removed by filtration through cheesecloth and glass wool. The filtrate, containing the plastids, was then centrifuged for 30 min in a Sorvall centrifuge at 5000 rpm and 10 °C. The deep red intact chromoplasts (∼80 mg/100 g of tomato) were centrifuged and isolated from the amber supernatant liquid (129 mL). They were pooled to eliminate individual tomato variations.

Proximate Analysis of Tomato Plastids. The pooled plastids were dried in a vacuum oven and then analyzed in duplicate for protein, lipid, ash, and dietary fiber. Protein concentration was determined using a Leco apparatus, analyzed for lipids with a Dionex ASE 200 accelerated solvent extractor at 125 °C with a 428 nitrogen determinator (*10*, *11*). Plastid samples (1 g) were petroleum ether extracted. Ash analysis was determined using a vacuum oven set at 550 °C for 16 h. Duplicate samples were run.

Amino Acid Analysis. Amino acids of tomato plastids were determined according to an AOAC International official method (*12*). Cystine and methionine residues were performic acid oxidized to cysteic acid and methionine sulfone, respetively. Sodium metabisulfite was added to decompose the performic acid. The protein was then hydrolyzed with 6 N HCl for 24 h at 110 °C. The released amino acids were separated and identified on a column of sulfonated polystyrene resin with ninhydrin postcolumn derivatization (A_{570nm}) according to a method developed by Spackman et al. (*13*).

Total Dietary Fiber. Soluble and insoluble dietary fiber (plant materials that are nondigestible by humans) contents were determined according to an enzymatic−gravimetric method developed by Prosky et al. (*14*). Vacuum-dried tomato plastids were defatted with petroleum ether, gelatinized, and digested with heat-stable α-amylase (Termamyl) from Novo, protease, and amyloglucosidase from Sigma Chemical Co. to remove protein and starch components. The enzyme digest was filtered. The residue (insoluble dietary fiber) was washed with 95% ethanol and then acetone, dried, weighed, and then corrected for protein and ash contents. The filtrate containing the soluble dietary fiber was precipitated with 4 volumes of 95% ethanol. The precipitate was filtered, then washed with 78% ethanol, 95% ethanol, and then acetone, dried, and weighed. Total dietary fiber was also determined (*15*).

Fatty Acid Analysis. Fatty acid analysis of tomato plastids was measured by gas chromatography. Fatty acid methyl esters (FAME) of tomato plastids (pooled preparation) were prepared according to the boron trifluoride method and then measured by gas chromatography according to an AOAC International method (*16*).

Tomato plastid−FAME compounds and standard FAME compounds (2 μL) were run on a Supelco column (SP-2380; 30 m × 0.25 mm) with the oven set at 80 °C for 5 min and then ramped at 4 °C/min to 280 °C, using a flame ionization detector.

Table 1. Composition and Nutritional Value of Tomato Plastids, Tomato Paste, and Cereals (White Wheat Flour and White Rice Flour)

nutrient[a]	tomato plastids (%)	tomato paste[b] (%)	wheat flour[b] (%)	rice flour[b] (%)
protein[c]	33.1	13.0	11.7	6.8
lipid	19.6	5.0	0.1	1.6
carbohydrates	18.0	83.0	86.6	91.0
total dietary fiber	21.1		3.1	1.0
insoluble	17.7		1.6	0.8
soluble	3.4		1.2	0.2
ash	8.2		0.5	0.7

[a] Dry basis. [b] (*33*) Compositions of Foods: Vegetables and Vegetable Products. (*34*) Compositions of Foods: Cereal Grains; rice flour (white): wheat flour (white). [c] Protein factors (tomato plastids and commercial tomato paste, 6.25; white wheat flour 5.95; rice flour, 5.95) used in calculations.

RESULTS AND DISCUSSION

Isolation of Tomato Plastids. The three major fractions obtained from the preparation of tomato plastids are animal feed called pomace containing the seeds, epidermis, and membranes after blending and filtration and the filtrate. The filtrate is further centrifuged, and the liquid supernatant (juice) and the tomato plastids are obtained (**Figure 2**). Pomace has been traditionally used as animal feed, particularly for swine, which, after suitable growth, are processed into pork products. The clear amber supernatant liquid is high in ascorbic acid, sugars, and other water-soluble compounds. A large amount of the total carbohydrate consists of soluble sugars in tomato paste (**Table 1**), whereas the total carbohydrate in tomato plastids is reduced due to this process. This fraction could be used as a juice itself or incorporated into mixed fruit drinks. The precipitated tomato plastids could be used to increase the nutritional and health value of sauces, salsa, soups, stews, tomato bouillon (concentrated stock), rice, and wheat products, and extruded wheat and rice flour food formulas.

Tomato Plastid Protein. Tomato plastids have a much higher protein concentration (33.1%) compared to wheat flour (11.7%) and rice (6.8%) shown in **Table 1**. Tomatoes are often utilized in food dishes with pasta and rice. Because rice and wheat flours have low protein concentrations, tomato plastid protein at 33% would serve to increase the protein concentration of meals prepared with pasta or rice for undernourished people. Spinach chloroplasts, another plastid, have been reported to have high protein (70%) and lipid concentrations (20%), similar to other plastids having high protein and lipid concentrations (*17*).

Table 2. Amino Acid Content of Tomato Plastids

amino acid	%	μmol/mg	mg/g
glutamic	0.478	0.0325	161.5
aspartic	0.312	0.0234	105.4
phenylalanine	0.276	0.0167	93.2
leucine	0.253	0.0192	85.5
lysine	0.212	0.0450	71.6
arginine	0.188	0.0108	63.5
alanine	0.173	0.0194	58.4
serine	0.168	0.0160	56.8
isoleucine	0.155	0.0188	52.4
threonine	0.147	0.0124	49.7
glycine	0.142	0.0189	48.0
valine	0.141	0.0121	47.6
proline	0.138	0.0128	46.6
tyrosine	0.121	0.0067	40.9
tryptophan	0.084	0.0041	28.4
histidine	0.093	0.0060	31.4
methionine	0.053	0.0036	17.9
cystine/cysteine	0.048	0.0040	6.2

Hansen et al. have increased the protein level of rice flour from 6 to 25% by using an enzymatic process (*18*). The high-protein rice flour was designed to meet the needs of normal and malnourished young children. The addition of tomato plastids to pasta dishes and extruded products could also prevent protein calorie malnutrition in impoverished adults and because it should be inexpensive to produce. There are two new products, the plastids and the juice, from the process. The amino acid concentration of tomato plastids (**Table 2**) showed that glutamic acid, aspartic acid, and phenylalanine were the most abundant. Glutamic acid and aspartic acid are nonessential amino acids. They are nonessential because other compounds in humans can make them. They do, however, have important functions. Aspartic acid produces some amino acids in the citric acid cycle and removes toxic ammonia from the blood vessels. Glutamic acid combines with ammonia and forms glutamine, which is then transported to the liver, where it is detoxified. The salt of this compound, monosodium glutamate, is used as a flavor additive.

People who have the disease phenylketonuria (PKU) have a defective gene for phenylalanine hydroxylase, causing phenylalanine, an essential amino acid, to accumulate. This disease leads to severe mental retardation in infants and adults if not treated (*19*). The disease is an inherited one involving both parents with defective genes. However, it is a rare disease, 1 in 10000. People with this condition should avoid high-protein foods such as meat, milk, eggs, and tomato plastids (**Table 2**) to prevent mental retardation. The sweetener aspartame, use in some diet sodas, should also be avoided for people having this disease.

The essential amino acids (EAA) are the amino acids that the human body is unable to synthesize and, therefore, is dependent on dietary sources for them. They are listed in **Table 3**. Tomato plastids have a good balance of EAA. A comparison of amino acids of tomato plastids with rice or wheat flour shows that the essential amino acid lysine of tomato plastids, needed for growth, could supplement that of white rice flour and white wheat flour, leading to a more complete protein in these foods. Friedman (*20*) provides a detailed discussion of the nutritional value of proteins from different food sources.

In contrast, meats (beef, lamb, and pork) have a complete EAA profile so that it is unnecessary to add any specific amino acids or proteins to them to improve their nutritional value; the addition of amino acids or proteins may be harmful in some instances. For example, methionine, an essential amino acid, can lose a methyl group, forming homocysteine by the action of methyl transferase (**Figure 3**). Homocysteine buildup is considered to be a cardiovascular risk factor. Folic acid and vitamins B_6 and B_{12} can reverse this reaction (*21*). It has not been established whether homocysteine is an indicator of cardiovascular disease or a causative factor. Beef, veal, and pork have a high content of methionine compared to white rice and wheat flours (**Figure 4**). Tomato plastids have a low content of methionine (**Table 2**) compared to other amino acids (AA). For meat-eaters a diet rich in fruits and vegetables (green leafy vegetables and beans) would help to reduce the homocysteine content by providing folic acid and vitamin B_6 to reverse the reaction of the methyl transferase.

Table 3. EAA[a] of Tomato Plastids, Rice Flour (White), and Wheat Flour (White)

food source	amino acid	AA (mg/g of protein)	EAA[a] (mg/g of protein)	ratio[b]
tomato plastid	leucine	85.5	66	1.30
	lysine	71.6	58	1.23
	isoleucine	52.4	28	1.87
	threonine	49.7	34	1.46
	tryptophan	28.4	11	2.58
	histidine	31.4	19	1.65
	valine	47.6	35	1.36
	Met + Cys	24.1	25	0.96
	Phe + Tyr	134.1	63	2.13
rice flour[c]	leucine	81.9	66	1.24
	lysine	34.8	58	0.60
	isoleucine	41.0	28	1.46
	threonine	35.5	34	1.04
	tryptophan	12.1	11	1.10
	histidine	25.0	19	1.32
	valine	58.5	35	1.67
	Met + Cys	42.2	25	1.69
	Phe + Tyr	106.0	63	1.68
wheat flour[c]	leucine	68.6	66	1.04
	lysine	22.0	58	0.38
	isoleucine	34.0	28	1.21
	threonine	27.2	34	0.80
	tryptophan	12.3	11	1.12
	histidine	22.2	19	1.17
	valine	40.3	35	1.15
	Met + Cys	39.0	25	1.56
	Phe + Tyr	80.5	63	1.28

[a] (*36*) essential amino acids. [b] Ratio = AA/EAA. [c] (*34*) USDA Human Nutrition Service Information. Composition of Foods: Cereal Grains.

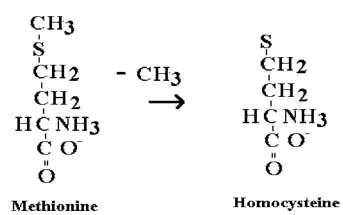

Figure 3. Reaction of methionine; loss of a methyl group forming homocysteine.

Dietary Fiber. Total dietary fiber percentage for tomato plastids is 21.1%, much larger than that of rice flour (1.0%) and wheat flour (3.1%). Soluble dietary fiber of tomato-plastids (3.4%) is much higher than that of white wheat flour (1.2%) and white rice flour (0.2%) (**Table 1**). Insoluble dietary fiber was higher in tomato plastids (17.7%) than in rice flour (0.8%) and wheat flour (1.6%). Therefore, the addition of tomato plastids to an extruded-product formula, made from white wheat flour or rice flour dishes, would enrich them with dietary fiber.

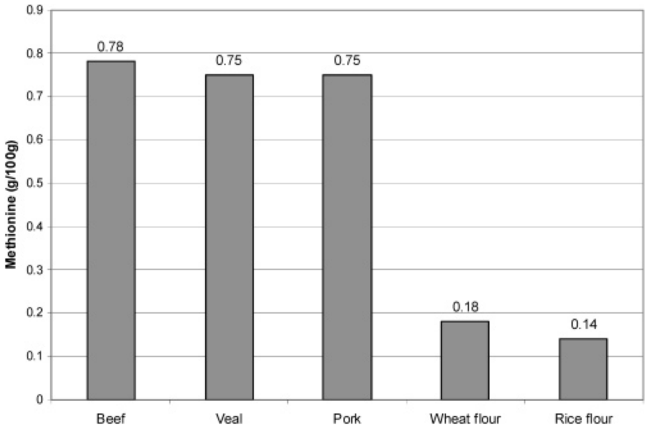

Figure 4. Methionine content of specified foods: tomato plastid water 89%, methionine 0.053 (g/100 g); (*34*) wheat flour (white) water 11.9%, AA; rice flour (white) water 11.9%, AA; (*35*) beef (top sirloin, choice, cooked, broiled) water 61%, AA; veal (shoulder, lean, cooked, braised) water 56.4%, AA; pork (loin, lean, cooked, broiled) water 60.7%, AA.

The soluble dietary fiber of fruits is composed largely of pectin (polymers of galacturonic acids bound by α-glycosidic linkages). Human digestive enzymes are unable to hydrolyze these polymer linkages. However, endogenous bacteria in the colon are able to break these linkages, forming butyric acid. Butyric acid and similar acids can induce cancerous cells to die, by a process called apoptosis (*22*, *23*). These acids are believed to be specifically toxic to colon cancer cells and not to normal colonic cells.

Some of the bile salts and bile acids are also trapped by the gel-like pectin in the intestine and then excreted. Keys et al. (*24*) have studied the effects of fiber and pectin in the diet and serum cholesterol concentration levels in man. Rats fed pectin−cholesterol diets had lower serum and liver cholesterol values than animals fed cellulose−cholesterol diets (*25*, *26*). Sixty-two healthy human subjects were fed different fiber diets (citrus−pectin, fruits, and vegetables, wheat bran, and a low-fiber diet) (*27*). Serum cholesterol decreased in the subjects fed pectin or the fruit and vegetable diets but not in those fed wheat bran or low-fiber diets. However, the effect of pectin in reducing serum cholesterol has been reported to be less than that obtained by the reduction of total fats and saturated fats in the diet (*28*).

The high concentration values of soluble dietary fiber for tomato plastids (**Table 1**) compared to other vegetables indicate that it probably could reduce serum and liver low-density lipoprotein (LDL) cholesterol levels and thus be beneficial in the prevention of arteriosclerosis. Pectin increases bile acid excretion, resulting in reduced serum and liver cholesterol concentrations in rats (*29*).

The insoluble fiber diet containing cellulose is believed to accelerate intestinal transit time of fecal matter and increase fecal amount due to water absorption. Thus, it is believed that insoluble dietary fiber may prevent constipation, diverticulitis, and digestive tract cancers by removal of toxic metabolites and by stimulating contractions and movement of food material in the intestinal tract.

Fatty Acids. An intake of 30% total fat or less of total energy has been recommended by many nutritionists in the United States, with only 10% coming from saturated fatty acids and the rest coming from monounsaturated (10%) and polyunsaturated fatty acids (10%), to decrease cardiovascular disease prevalent in the country.

Total fat concentrations of tomato plastids, wheat flour (white), and rice flour (white) are listed in **Table 1**. The dietary fatty acid profile of tomato plastids is shown in **Table 4**. The plasma membranes are composed mainly of phopholipids and glycolipids containing fatty acids forming a lipid bilayer. The polyunsaturated fatty acids, linoleic and linolenic, are the essential fatty acids. They are not synthesized by the human body and, therefore, must be obtained from the dietary intake of foods containing them. Tomato plastids are an excellent source of the essential fatty acid linoleic acid, a polyunsaturated acid (C18:2) that is a cholesterol-lowering fatty acid.

Table 4. Profile of Fatty Acid Composition of Tomato Plastids

fatty acid	carbon no.: bond (=)[a]	%
saturated		
palmitic acid	C16: 0	37.02
stearic acid	C18: 0	3.03
arachidic acid	C20: 0	8.51
monosaturated		
oleic	C18: 1	1.01
polyunsaturated		
linoleic acid[b]	C18: 2	47.02
linolenic acid[b]	C18: 3	2.18
arachiadonic acid[b]	C20: 4	0.37

[a] Number of carbons and double bonds in the compound. [b] Essential fatty acids.

Linoleic acid aids in the prevention of platelet aggregation leading to blood clotting in the blood vessels and is described by Renaud (*30*). The oxidation of LDL has been reviewed by Abbey and others (*31*). Linolenic acid is able to reduce the incidence of cardiac arrest in rats induced by artery occlusion resulting in an increased blood flow to the heart.

Palmitic acid, the saturated fatty acid found in large quantities in tomato plastids, has also been found in human milk and some milk products. Palmitic acid (C16:0) and oleic acid (C18:1) have a neutral effect on serum cholesterol, when the dietary intake of cholesterol is low as shown in Cebus monkeys (*32*).

Conclusions. Tomato plastids have the potential to increase the nutritional and health value of the human diet because of their high protein content and excellent essential amino acid content, particularly lysine. They could also contribute to the prevention and amelioration of protein calorie malnutrition due to their high protein concentration and essential amino acids if added to wheat flour (white) and rice flour (white). Because of their high content of linoleic acid, an essential fatty acid that reduces serum cholesterol, they may prevent the development of cardiovascular disease. They could also play a role in the prevention of atherosclerosis by decreasing the deposit of fatty substances, LDL-cholesterol, in the arteries. The insoluble dietary fiber of tomato plastids prevents constipation and diverticulitis. Soluble dietary fiber (pectin) increases bile acid excretion, resulting in reduced serum and liver cholesterol levels in rats. The use of tomato plastids in pasta dishes, sauces, and extruded products of rice and wheat would meet some of the special dietary needs of some people. Some people require low-sugar products for medical reasons (diabetics, those having cardiovascular disease, and others for weight loss for health reasons). Therefore, tomato plastids having high protein and lipid contents and low sugar content can be useful in meeting these human needs. The isolation process provides two new nutritional products, tomato plastids and an amber juice, to the consumer.

ACKNOWLEDGMENT

We appreciate the fine technical work performed by Jose Gomez of our laboratory (WRRC) and the technical assistance of Latasha Hilliard and Njeri Pratt, ACS Seed Students.

LITERATURE CITED

(1) Agarwal, S.; Rao, A. V. Tomato lycopene and low-density lipoprotein oxidation: a human dietary intervention study. *Lipids* **1998**, *33*, 981−984.

(2) Galili, G. Regulation of lysine and threonine biosynthesis. *Plant Cell* **1995**, *7*, 899−906.

(3) Ohirogge, J.; Brouse, J. Lipid biosynthesis. *Plant Cell* **1995**, *7*, 957−970.

(4) Ferro-Luzzi, A.; Bianca, F. Mediterranean diet, Italian style: prototype of a healthy diet. *Am. J. Clin. Nutr.* **1995**, *61S*, 1338−1345.

(5) Seira-Majem, L.; Ribas, L.; Tresserras, R.; Ngo, J.; Salleras, L. How could changes in the diet explain changes in coronary heart disease mortality in Spain? The Spanish paradox. *Am. J. Clin. Nutr.* **1995**, *61S*, 1351−1359.

(6) Trichopoulou, A.; Kouris-Blazos, A.; Vassilakou, T.; Gnardellis, C.; Polychronopoulos, E.; Venizelos, M.; Lagiou, P.; Wahlqrist, M. L.; Trichopoulou, T. Diet and survival of elderly Greeks: a link to the past. *Am. J. Clin. Nutr.* **1995**, *61S*, 1346−1350.

(7) Spiller, G. A. Health effects of Mediterranean diets and monounsaturated fats. *Cereal Foods World* **1961**, *36* (9), 787−854.

(8) Jenkins, D. J. A. Dietary fiber and other anti-nutrients: metabolic effects and therapeutic implications. In *Nutritional Pharmacology—Current Topics in Nutrition and Disease*; Spiller, G. H., Ed.; Wiley: New York, 1981; Vol. 4; pp 117−145.

(9) Franceschi, S.; Bidoli, E.; La Vecchia, C.; Talamini, R.; D'Avanzo, B.; Negri, E. Tomatoes and the risk of digestive tract cancers. *Int. J. Cancer* **1994**, *59*, 181−184.

(10) AOCS. Ba 4e. Protein nitrogen by combustion. *Official Methods of Analysis*; American Oil Chemists' Society: Champaign, IL, 1995.

(11) Bicsak, R. C. Comparison of Kjeldahl method for the determination of crude protein in cereal grains and oil with a generic combustion method. Collaborative study. *AOAC Int.* **1993**, *76* (4), 780.

(12) AAOC International. Amino acid anlaysis. *Official Methods*, 17th ed.; 2000; Method 994.12 Chapter 4, pp 6−14.

(13) Spackman, D. H.; Stein, W. H.; Moore, S. Automatic recording apparatus for use in the chromatography of amino acids. *Anal. Chem.* **1958**, *30*, 1190−1206.

(14) Prosky, L.; Asp, N.; Furda, I.; DeVries, J. W.; Schweizer, T. F.; Harland, B. F. Determination of dietary fiber in foods and food products. *J. Assoc. Off. Anal. Chem.* **1985**, *68* (4), 677−679.

(15) Schweizer, T. F.; Wursch, P. Analysis of dietary fibre. *J. Sci. Food Agric*. **1979**, *30*, 613−619.

(16) AOAC International. Methyl esters of fatty acids in oils and fats. Gas chromatographic method. *Official Methods*, 16th ed.; AOAC International: Washington, DC, 1998; Method 963.22, Chapter 41, p 18D.

(17) Gunning, B. Cell plastids. In *McGraw-Hill Encyclopedia of Science and Technology*, 9th ed.; 2002; Vol. 3, pp 652−654.

(18) Hansen, L. P.; Hosek, R.; Callan, M.; Jones, F. The development of high-protein rice flour for early childhood feeding. *Food Technol.* **1981**, *35*, 38−42.

(19) Phenylketonuria. Medline Plus and The National Institutes of Health; http://www.nlm.nih.gov/medlineplus/phenylketonuria.html.

(20) Friedman, M. Nutritional value of proteins from different food sources. A review. *J. Agric. Food Chem.* **1996**, *44*, 6−29.

(21) Boushey, C. J.; Beresford, S. A.; Omenn, G. S.; Motulsky, A. G. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. *JAMA—J. Am. Med. Assoc.* **1995**, *274*, 1049−1057.

(22) Hague, A.; Manning, A. M.; Hanlon, K. A.; Huschtscha, L. I.; Hart, D.; Paraskeva, C. Sodium butyrate induces apoptosis in human colonic cell lines in a p53 independent pathway. Implications for a possible role of dietary fiber in the prevention of large-bowel cancer. *Int. J. Cancer* **1993**, *55*, 498.

(23) Smith, J. G.; Yokoyama, W. H.; German, J. B. Butyric acid from the diet: actions at the level of gene expression. *Crit. Rev. Food Sci.* **1998**, *38* (4), 259−297.

(24) Keys, A.; Anderson, J. T.; Grande, R. Serum cholesterol response to changes in the diet. *Metabolism* **1965**, *14*, 747−758.

(25) Anderson, J. W.; Jones, A. E.; Riddell-Mason, S. Ten different dietary fibers have significantly different effects on serum and liver lipids of cholesterol-fed diets. *J. Nutr.* **1994**, *124*, 78−83.

(26) Kay, R. M.; Judd, P. A.; Truswell, A. S. The effect of pectin on serum cholesterol. *Am. J. Clin. Nutr.* **1978**, *31*, 562−563.

(27) Stasse-Wolthuis, M.; Albers, H. F. F.; van Jeveren, J. G. C.; de Jong, J. W.; Hautvast, J. G. A.; Hermus, R. J. J.; Katan, M. B.; Brydon, W. G.; Eastood, M. A. Influence of dietary fiber from vegetables and fruits, bran or citrus pectin on serum lipids, fecal lipids, and colonic function. *Am. J. Clin. Nutr.* **1980**, *33* (8), 1745−1756.

(28) Hegsted, D. M.; McGrandy, R. B.; Meyers, M.; Stare, F. J. Quantitative effects of dietary fat on serum cholesterol in men. *Am. J. Clin. Nutr.* **1965**, *17*, 281−295.

(29) Garca-Diez, F.; Garcia-Mediavilla, V.; Bayon, J. E.; Gonzalez-Gallego, J. Pectin feeding influences fecal bile acid excretion, hepatic bile acid and cholesterol synthesis and serum cholesterol in rats. *J. Nutr.* **1996**, *126* (7), 1766−1771.

(30) Renaud, S. Linoleic acid, platelet aggregation and myocardial infarction. *Atherosclerosis* **1990**, *80*, 255−266.

(31) Abbey, M.; Billing, B. G.; Noakes, M.; Hirata, F.; Nestel, P. J. Oxidation of low-density lipoproteins: intra-individual variability and the effect of dietary supplementation. *Am. J. Clin. Nutr.* **1993**, *57*, 391−399.

(32) Khosla, P.; Hayes, K. C. Dietary palmitic acid raises plasma LDL cholesterol relative to oleic acid only at a high intake of cholesterol. *Biochim. Biophys. Acta* **1993**, *1210*, 13−22.

(33) USDA. *Composition of Foods: Vegetables and Vegetable Products*; Haytowitz, D. B., Matthews, R. H., Eds.; Human Nutrition Service Information 8-11; U.S. GPO: Washington, DC, 1989; tomato paste, p 460.

(34) USDA. *Composition of Foods: Cereal Grains and Pasta*; Drake, D. L., Gebhardt, S. E., Matthews, R. H., Eds.; Human Nutrition Service Information 8-20; U.S. GPO: Washington, DC, 1989; rice flour (white) and wheat flour (white), pp 76, 96.

(35) USDA. *Composition of Foods*; Human Nutrition Service Information; U.S. GPO: Washington, DC; 8-13 (beef) 1989, p 358; 8-17 (veal) 1989, p 149; 8-10 (pork) p 54.

(36) FAO/WHO/UNU. *EAA (Essential Amino Acids)*; Rome, Italy, 1971.

Received for review January 4, 2005. Accepted May 31, 2005.

JF058013P