Comprehensive REVIEWS in Food Science and Food Safety

Health Benefits of Punicic Acid: A Review

P. Aruna, D. Venkataramanamma, Alok Kumar Singh, and R.P. Singh

Abstract: Punicic acid (PA) is a polyunsaturated fatty acid (18:3 n-5), which is classified as a conjugated linolenic acid. PA is also referred as a "super CLnA" whose effect is even more potent than that of an ordinary CLnA. It is found mainly in the seeds of pomegranate fruit (*Punica granatum)* and *Trichoxanthes kirilowii* and some other minor sources. It possesses a wide array of biological properties including antidiabetic, antiobesity, antiproliferative, and anticarcinogenic activity against various forms of cancer. In spite of this, PA has not been explored as a nutraceutical or as an ingredient of food products which can be aimed at specific consumer target groups. This review details the various health-beneficial properties of PA and explores the possibilities of its utilization as an active ingredient in various food products.

Keywords: conjugated linolenic acid, eleostearic acid, pomegranate seed oil, punicic acid

Practical Application: Punicic acid, with its wide array of health-beneficial properties, needs to be utilized as a compound or as a main ingredient of pomegranate seed oil in various food formulations. This would not only add value to the waste from the pomegranate industry, but also would contribute to waste management solutions.

Introduction

Pomegranate *(Punica granatum L.)* seeds are considered as waste by fruit processing industries, but are also a treasure of pharmaceutical and nutraceutical compounds (Ali and others 2014). Pomegranate seed oil (PSO) consists of a complex array of fatty acids approximately 80% of which are 18-carbon molecules with 3 alternating double bonds (also known as trienoic acids). Research has shown that trienoic fatty acids, such as those that occur in PSO, possess more potent physiological activity than dienoic fatty acids (with two alternating double bonds, also referred to as conjugated linoleic acid; CLA). The specific trienoic fatty acid found in PSO is referred to as punicic acid (PA), which is a polyunsaturated fatty acid (18:3 n-5), also called trichosanic acid, *cis* 9, *trans* 11, *cis* 13 acid with IUPAC name 9Z,11E,13Z-octadeca-9,11,13-trienoic acid (Franzke and others 1982). PA has also been known as a "super CLA," whose effect is more potent than ordinary CLnA (Melo and others 2014; www.morretec.com, http://extract-herb.com).

This review encompasses various aspects of PA, including its occurrence, metabolism, biochemical, and health-beneficial properties. The possibilities of its application in food products has also been discussed.

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Occurrence/Sources

PSO is one of the 6 plant components known that contain conjugated fatty acids and with an exceptional amount of PA. It is also found in bitter gourd (*Momordica balsamina L.*; Lotti and others 1973), *M. charantia* (Mukherjee and Bhattacharya 2006), *Trichosanthes bracteata* (Kittur and others 1993), *T. kirilowii* (Yong and others 1995), and snake gourd seed oil (*T. anguina;* Lansky and Newman 2007). The other sources of conjugated fatty acids (Figure 1) are the seeds of pot marigold (α- and β-calendic acid), Calendula (α-calendic acid), Catalpa (catalpic acid), and Jacaranda (jacaric acid; Tanaka and others 2011). Conjugated fatty acids have been shown to inhibit eicosanoid metabolism in the synthesis of prostaglandins from arachidonic acid and this provides them with natural anti-inflammatory properties (www.morretec.com).

Different varieties of pomegranates grown all over the globe have been shown to possess as low as 6% and up to 24% (w/w) oil in the seeds, with PA as one of the major components (70% to 85%) of the oil which is synthesized *in situ* from linoleic acid (Hopkins and Chisholm 1968; Hornung and others 2002; Lansky and Newman 2007).

Iran is one of the major producers of pomegranates in the world. The oil from the seeds of 5 different varieties of pomegranate were analyzed and shown to possess >70% PA in the fatty acid fraction of the seed oil (Habibnia and others 2012). Some 25 pomegranate varieties from 2 different regions of Iran were studied for the composition of the seed oils by Fadavi and others (2006). The oil content ranged from 6.6% to 19.3% with linolenic acid (LnA) (C18:3) as the major fatty acid (31.8% to 86.6%), followed by linoleic (0.7% to 24.4%) and oleic acid (0.4% to 17.4%). The inter-varietal differences in fatty acid compositions were shown and they could be useful to establish chemotaxonomic differences. Dadashi and others (2013) showed that the pomegranate seeds of 4 Iranian

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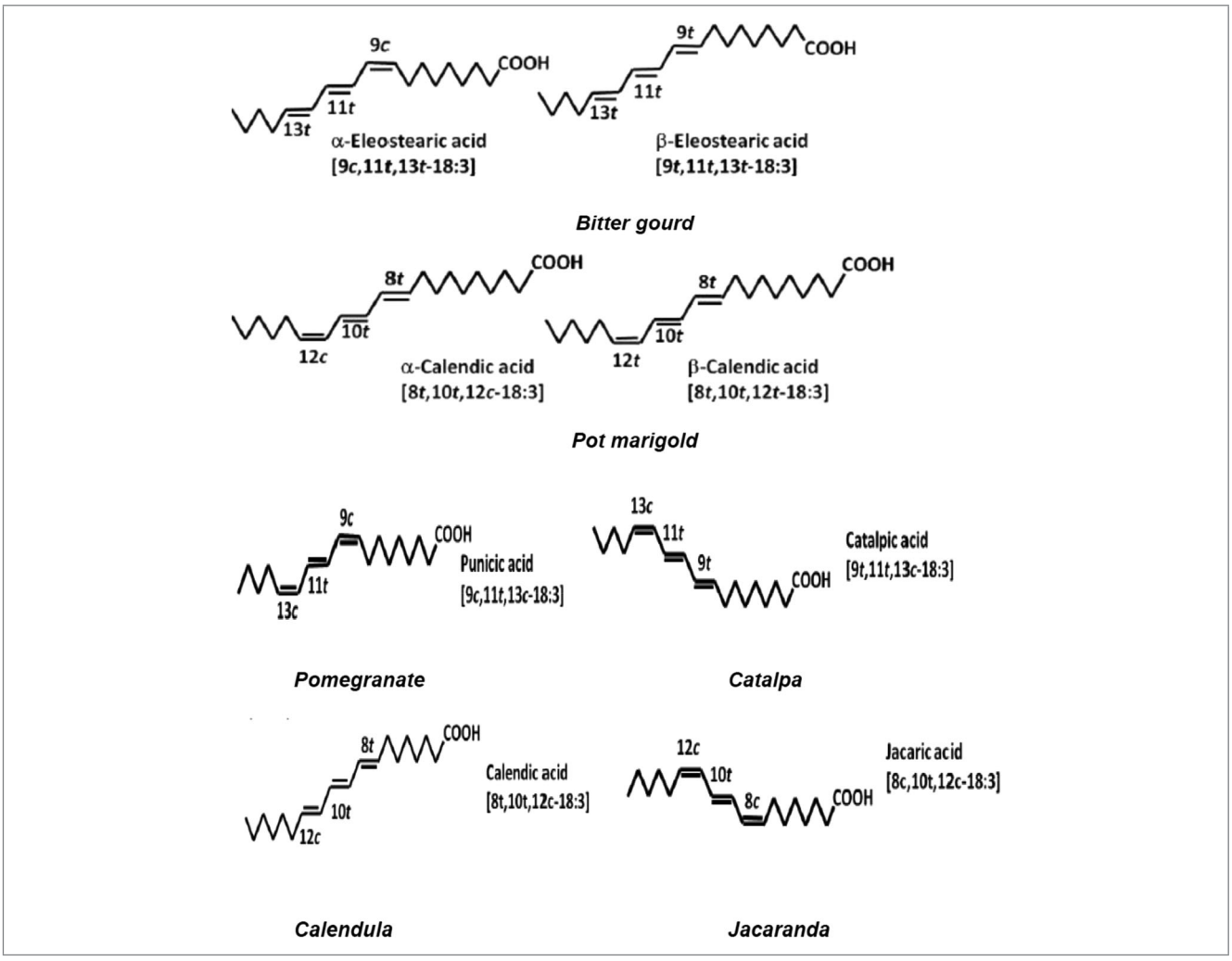

Figure 1–Various sources of conjugated linolenic acid (Tanaka and others 2011).

varieties (Abanmahi, Malas, Pust Sefid, and Shahvar) possessed oil contents in the range of 13.5% to 16.9% with the highest content in the Pust Sefid variety. The content of PA in these varieties was in the range of 72.07% (Shahvar) to 73.31% (Malas).

Kyralan and others (2009) studied the seed oil content and fatty acid composition of 15 pomegranate cultivars of Turkey. The oil content was in the range between 13.95% and 24.13% (db) with PA content in the range of 70.42% to 76.17%. Pande and Akoh (2009) investigated 6 pomegranate cultivars from Georgia and reported that the seeds had an average lipid content of 19.2% with PA as the predominant fatty acid (83.4% in one of the varieties). Soetjipto and others (2010) investigated the content and composition of fatty acids in seed oil of red and purple pomegranate varieties from Indonesia. The total oil content of red and purple pomegranate were 12.8% and 10.3% (dw), respectively. The PA content of total lipid of purple PSO (0% to 25%) was higher than that of red pomegranate (9% to 16%), while the neutral lipids of red pomegranate showed higher PA contents (54% to 75%) than the purple pomegranates (14% to 55%). Glycolipids of red pomegranates contained PA (0% to 42%). The PA content of the phospholipid fraction of red pomegranate was higher (0% to 22%) than purple pomegranate (0% to 2%). In conclusion, the seed oil of the purple variety was better than the red one. In general, pomegranate seeds consist of 12% to 20% oil (PSO) on a weight basis. Elbandy and Ashoush (2012) also reported tocopherol-rich PSO from Egypt with 88.4% polyunsaturated fatty acid.

Verardo and others (2014) studied the lipid composition of pomegranate seeds from Israel, Spain, Turkey, Iran, and Tunisia. The total lipid content of the pomegranate seeds varied between approximately 8% and 16%. The PSO contained large amounts of PA in the range of 74% to 85% of the total fatty acids. The presence of 2 tocotrienols was identified and reported for the first time in PSO.

Apart from pomegranate, CLnA are also found in tung oil, catalpa seed oil, balsam pear seed oil, and cherry seed oil (Takagi and Itabashi 1981). α-Eleostearic acid (EA) and PA are the 2 typical conjugated trienoic fatty acid isomers of conjugated LnA (CLnA) found in seed oils. Bitter gourd (*M. charantia*) and snake gourd (*Trichosanthes anguina*) are 2 common vegetables of Asia and widely consumed by the Indian and other Asian populations. The oil from *M. charantia* seeds is rich in α-EA acid, while *T. anguina* oil is rich in PA. The differences in the effectiveness of these oils are assigned to their antagonistic cis-transmolecular arrangement of the CLnA. α-EA is more effective than PA due to its high trans content (Saha and others 2012a). Jing and others (2012) studied the composition of fatty acids form the oil of the seeds of *T. kirilowii* Maxim from 4 different geographical locations and concluded that the *T. kirilowii* seeds were especially rich in PA,

and their contents were not influenced by geographical location; the minute variations in some proximate compositions may be caused by ecological, temperature, climate, technical, and cultural conditions.

Yuan and others (2011) studied the distribution pattern of lipids and CLnA contents in *T. kirilowii*, pomegranate, and bitter melon seed oils. The oil from 3 pomegranate (Sanbaiyu, Qingpiruanzi, and Tianluzi) and 6 bitter melon cultivars (Changbai, Manyouqing, Bingchengyihao, Kaihua-1, Kaihua-2, and Qingfeng) from China were shown to be rich in CLnA with PA as the main CLnA in *T. kirilowii* and pomegranate, while α-EA was predominant in bitter melon. Natural resources of CLnA, especially edible *T. kirilowii* seed, could be a potential dietary source of CLA, following CLnA metabolism.

Biosynthesis of CLnA

CLA is an 18-carbon fatty acid with 2 alternating double bonds. PSO consists of a variety of fatty acids; about 80% of these are 18 carbon molecules with 3 alternating double bonds. These are called *trienoic* fatty acids which, in general, possess a more potent physiological activity than the *dienoic* fatty acids (Pomegranate . . . The Fabled Fruit™; www.morretec.com/img/Pomegranate_Brochure.pdf). The specific trienoic fatty acid in PSO is referred to as PA. In lab rats, it was found that PA was converted to the CLA rumenic acid (9Z11E-CLA, Tsuzuki and others 2006).

PA is derived from linoleic acid (18:2Δcis9, cis12) by a fatty acid conjugase which converts a cis-Δ12 double bond into a conjugated trans-cis-double-bond system (http://www.metacyc.org/META/new-image?type = PATHWAY&object = PWY-5374).

Biosynthesis of PA was studied by Hornung and others (2002) who speculated the involvement of (11, 14)-linoleoyl desaturase activity for the conversion of a cis-double-bond at position δ12 into a cis-trans double-bond system. The 2 cDNAs from pomegranate seeds encoding for these enzymes were cloned, sequenced, and expressed in *Saccharomyces cerevisiae*. The analysis of the fatty acids produced by recombinant yeast points out that one of the cDNA codes for δ12-acyl-lipid-desaturase, while the other codes for (1,4)-acyl-lipid-desaturase that converts the cis-double-bond at the δ12-position of linoleic acid or γ-LnA, but not α-LnA, into a conjugated cis-trans double-bond system.

Iwabuchi and others (2003) described the biosynthesis of CLnA with the help of conjugases, which modify the existing double bond to produce the conjugated double bonds with or without introducing a new double bond (Figure 2). The cDNAs from *T. kirilowii* and *P. granatum*, designated as TkFac and PgFac, respectively, were isolated, which encoded a class of conjugases to form trans- δ11, cis- δ13 double bonds. TkFac and PgFac thus showed to have both Δ12-oleate desaturase and conjugase activities, and support the concept that fatty acid-modifying enzymes might have originated from fatty acid desaturases. Expression of TkFac and PgFac in *Arabidopsis* seeds resulted in accumulation of PA up to 10% (w/w) of the total seed oil. Even in yeast cells grown without exogenous fatty acids, TkFac and PgFac expression resulted in PA accumulation accompanied by 16:2 δ9cis,12cis, and 18:2 δ9cis,12cis production. Thus, the products from TkFac and PgFac are bifunctional enzymes with both conjugase and δ 12-oleate desaturase activities. Also, 16:2 δ9cis, 12cis, and 18:3 δ9cis, 12cis, 15cis, as well as 18:2 δ9cis, 12cis, may be potential substrates for the conjugases to form trans- δ11 and cis- δ13 double bonds.

Effect of Extraction Procedure

The extraction of PSO was carried out using hexane (Elbandy and Ashoush 2012; Dadashi and others 2013), supercritical CO_2 (SC-CO_2) extraction (Guangmin and others 2009; May 2014), and cold press (Habibnia and others 2012; Ali and others 2014). The PSO extracted by cold-pressing possessed superior physicochemical properties and quality as compared to that extracted with organic solvents (Ali and others 2014). The method results in extraction of a highly nutritious oil without environmental impacts. In SC-CO_2 extraction, the extraction pressure was shown to be the dominant factor to affect the PSO yield (Guangmin and others 2012), and the PSO possessed high contents of PA and γ-tocopherol which were slightly increased by increasing extraction pressure and temperature. Sargolzaei and Moghaddam (2013) developed an effective intelligent system for predicting the effects of temperature and pressure on PSO yield by SC-CO_2 process and reported that properly developed back-propagation neural network and radial basis function neural network could be used as successful predicting tools for SC-CO_2 oil extraction. Ahangari and Sargolzaei (2012) reported the superiority of PSO extracted with hexane over SC-CO_2 and subcritical propane extracted PSO. SC-CO_2 and subcritical propane could extract only 59% and 77% of the total PSO (extracted with Soxhlet extraction using hexane), respectively.

Yuan and others (2013) optimized ultrasound-assisted extraction of PSO and obtained the best yield with a solid-liquid ratio of 1:12 g/mL and an ultrasonic extraction temperature of 51°C at 70 W for 40 min. The PSO yield was 18.75% and the major fatty acids were PA (64.73%), linoleic acid (9.68%), and oleic acid (8.91%). Tian and others (2013) studied the ultrasonic-assisted extraction of PSO with various solvents. The yield of PSO was high with petroleum ether, followed by n-hexane, ethyl acetate, diethyl ether, acetone, and isopropanol. With petroleum ether, other variables like solid:solvent ratio, ultrasonic power, temperature, and time used for extraction were also optimized by response surface methodology. The yield of ultrasonic-assisted extraction of PSO was higher than by Soxhlet or supercritical fluid extraction, and the composition of PSO obtained by these methods was significantly different.

Abbasi and others (2008) studied the effect of different extraction methods on the total phenolic contents of the oil extracted from pomegranate seeds of the Malas variety from Iran and reported that combination of SC-CO_2 and different operational conditions resulted in extraction of varying amounts of phenolic compounds in PSO. Increases in pressure, temperature, and volume of modifier (water, ethanol, or hexane) resulted in decreased total phenolic contents of the extracted oils.

Biological Properties

Recent studies indicate that the potent therapeutic and preventive properties of PA may help the human body to fight against cancers, obesity, diabetes, and heart disease. The following sections describe various aspects of health-beneficial properties of PA.

Antidiabetic and antiobesity properties

Arao and others (2004a) reported on the hypolipemic activity of PA using human hepatoma HepG2 cells. PA significantly decreased apolipo protein B100 secretion, which is a constituent of low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) and acts as signals for their binding and internalization, when compared with α-LnA. The uptake of ^{14}C-oleate into newly synthesized triacylglycerol (TAG) was also better decreased by PA

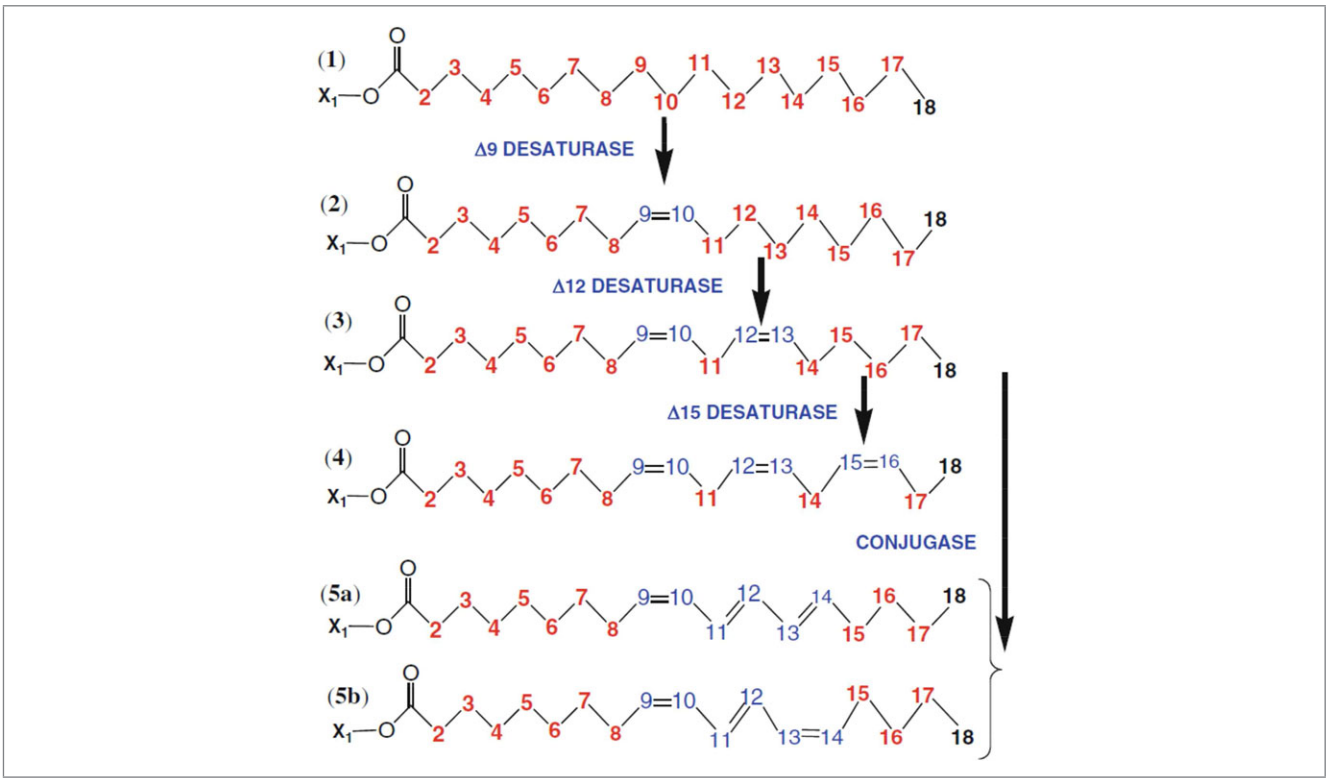

Figure 2–Schematic representation of the reaction catalyzed and products formed by conjugases and desaturases, (1) stearic acid, (2) (9Z) oleic acid, (3) (9Z, 12Z) linoleic acid, (4) (9Z, 12Z, 15Z) α-linolenic acid, (5a) (9Z, 11E, 13E) α-eleostearic acid, and (5b) (9Z, 11E, 13Z) punicic acid. Here, X1 represents either acid or ester (Lesot and others 2011).

than by α-LnA treatment, indicating the possible application of PA as a dietary hypolipemic constituent.

In OLETF (Otsuka Long Evans Tokushima Fatty) rats, which are a specific strain of rats for studying type II diabetes with obesity, it was observed that these rats remained relatively lean when PA was added to the diet and the fat cells could undergo programmed cell death when exposed to PA (Arao and others 2004b; Nishimura and others 2007). PA has also been shown to reduce fasting blood sugar levels in streptozotocin (STZ)-induced (Banihani and others 2013) but not in Alloxan-induced type-II diabetic rats (Jelodar and others 2007), and to prevent diet-induced obesity and insulin resistance. In mice fed with a high-fat diet (HFD) with or without 1% PSO for 12 wk, to induce obesity and insulin resistance, PSO-fed mice were not only found leaner than those without PSO, but they had improved insulin sensitivity (http://www.healthhabits.ca/2011/03/30/punicic-acid-prevents-obesity-insulin-resistance/).

The protection offered by PSO against diet-induced obesity and insulin resistance was shown to be independent of changes in food intake or energy expenditure. By employing hyper-insulinemic euglycemic clamp technology, it was also shown that PSO did not affect liver insulin sensitivity but improved peripheral neuropathy sensitivity (Vroegrijk and others 2011).

Peroxisome proliferator-activated receptor (PPAR)γ is a nuclear receptor, mainly present in adipose tissues, regulating the storage of fatty acids, metabolism of glucose, and molecules targeted by many antidiabetic agents. Bassaganya-Riera and others (2011a) described PPARs as molecular targets for obesity-related diseases and disorders and showed the need for the search for natural PPAR agonists for obesity management, in view of the side effects of available PPARγ agonistic drugs (such as Avandia). As compared to the synthetic analogs, plant-derived compounds possess better safety aspects and act through multiple molecular targets, thus PA could be a candidate for regulating blood sugar levels and controlling intestinal inflammation. PA has also been shown to improve immune system development, helps in maintaining or enhancing the levels of $CD4^+$ and $CD8^+$ T lymphocyte, increasing immune response against viruses, and preventing or ameliorate type II diabetes and obesity (Bassaganya-Riera 2011; Yuan and others 2015). Hence PA, by virtue of its PPARγ agonist characteristics, could be promoted as a prophylactic and therapeutic molecule (Anusree and others 2014).

In wild-type CD-1 mice, McFarlin and others (2009) showed that feeding PSO along with a HFD could reduce the concentration of leptin and enhance the concentration of adiponectin more than the HFD controls. The body weight, percentage weight gain, and insulin were also shown to be reduced by PSO. Possibly, PSO may reduce weight gain via the pathway mediated by leptin/adiponectin and insulin. Hence, PSO consumption may reduce the risk of type II diabetes, but risk for cardiovascular diseases (CVD) did not change as it does not affect energy intake, cholesterol profile, or biomarkers of systemic inflammation.

In STZ-induced diabetes, Saha and Ghosh (2012) studied the effect of PA and eleostearic acid (ESA) against oxidative stress, inflammatory challenge, and aberration in erythrocyte morphology. Both isomers showed potent antioxidant and anti-inflammatory activity against STZ-induced stress, significantly reduced lipid peroxidation, and restored levels of superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx), and reduced glutathione (GSH) and nitric oxide (NO) synthase level in pancreas, blood, and erythrocyte lysate. In addition, CLnA treatment reversed the STZ-induced changes of ferric-reducing antioxidant power of plasma,

restored the levels of tumor necrosis factor (TNF)-α and interleukin (IL)-6 in blood and expression of hepatic NF-kappa B (p65). PSO has also been shown to inhibit the expression of pro-inflammatory cytokines (such as IL-6, IL-8, IL-23, IL-12, and TNF-α) through PPARγ and δ modulation (Colombo and others 2013). In STZ- and nicotinamide-induced diabetic male Sprague–Dawley rats, PA exhibited antidiabetic properties by increasing serum insulin levels; however, the high glucose, TAG, LDL, and total cholesterol (TC) levels were unaffected (Nekooeian and others 2014). PA did not affect lipid peroxidation, but it could reduce the diabetes-induced oxidative stress by increasing the levels of GPx. Thus, in this model of type-II diabetes, PA could increase insulin secretion, but did not affect the fasting glucose levels. The authors assign this phenomenon to insulin resistance which plays a crucial role in the progression of type-II diabetes and abnormal functioning of mitochondria (Anusree and others 2015).

Hypolipidemic properties

The low content of PA in pomegranate and *T. kirilowii* seeds led Koba and others (2007) to attempt the production of PA-containing transgenic oil. The cDNA encoding a conjugase from *T. kirilowii* that converts linoleic acid to PA was isolated and introduced into *Brassica napus*. Even though the content of PA was very low in the resulting GM rapeseed oil (GMRO), it reduced the adipose tissue weight in mice. Both PSO and GMRO increased carnitine-palmitoyl transferase activity in hepatic and brown adipose tissues. Thus, dietary GMRO, with 0.25% PA by wt in the diet, reduced body fat mass, and altered hepatic lipid metabolism in mice. The effect of GMRO was shown to be higher than that of PSO.

Yuan and others (2009a) studied the effect of α-ESA and PA on lipid metabolism and body fat in ICR mice. A 6-wk feeding trial with the diet supplemented with 1% α-ESA, PA, a mixture of CLA isomers (predominantly c9, t11- and t10, c12-CLA), or α-LnA (as TAG) showed that the CLA-containing diet significantly decreased the perirenal and epididymal adipose tissue weight and significantly increased hepatic tissue wt. But none of the diets showed any lipemic effects with unaltered total-, HDL-, and LDL-cholesterol (LDL-C) and TAGs level. Mice fed on α-ESA or PA showed a significantly decreased hepatic TAGs content, while this parameter was increased significantly in mice given dietary CLA. Thus, body fat weight and hepatic TAG level are affected differently by CLA and CLnA.

Elbandy and Ashoush (2012) reported the hypolipidemic and antioxidative effects of PSO and defatted seed residues. The increased levels of TC, TAG, LDL-C, risk ratio, atherogenic index, and lipid peroxidation induced by feeding an atherogenic diet to animals were reversed with a diet supplemented with PSO, seed residue, and their mixture. Improved lipid profile of plasma, an increase in level of reduced glutathione, a reduction in malondialdehyde level (MDA) and reduced TC, TG, LDL-C levels were also observed in this group of animals.

Interventional studies with 3 g of PA fed to healthy young human subjects for 28 d did not result in any effect on weight reduction or serum lipid profile. The urinary 8-iso-prostaglandin F2α level increased significantly, but there was no significant effect on cholesterol-reactive proteins, insulin, glucose, and insulin resistance (Yuan and others 2009b). However, in a double-placebo clinical trial with 400 mg of PSO, given twice per day for 4 wk to hyperlipidemic subjects, it showed its anti-atherogenic effect on lipid profiles including TAG and the TAG: HDL-C ratio (Mirmiran and others 2010). Effects of PA was studied on fat accumulation and glycemic control in rats fed on an obesogenic diet by Miranda and others (2013). Rats fed with 0.5% PA for 6 wk on a moderately HFD did not show any significant change in adipose tissue weights, but glycemic value and fructosamine (product from the degradation of advanced glycation end products) levels in the PA group decreased significantly. No changes were observed in the liver, skeletal muscle composition, insulin resistance, or PPAR activation. Thus, dietary supplementation of PA did not lead to decreased fat accumulation in adipose tissue, liver, or skeletal muscle, or to improved glycemic control. The authors posed an alert on PA supplementation-induced hypoplasia due to the antiproliferative effect on hepatocytes and cautioned for proposing PA as a functional ingredient.

Anti-inflammatory and related activities

PA exerts anti-inflammatory effect through inhibition of TNF-α-induced priming of NADPH oxidase by targeting the p38MAPKinase/Ser345-p47phox-axis and myeloperoxidase release. Thus, PA could be a novel alternative therapeutic strategy in inflammatory diseases, such as inflammatory bowel diseases (IBD) (Boussetta and others 2009). For IBD, absence of effective treatment necessitates the need for alternative therapeutic options. PPARγ has been chosen as a potential target for novel therapeutics against IBD, and various compounds were tested to identify natural PPARγ agonists using computational docking for assessing the binding between compounds and PPAR. By these techniques, α-ESA was identified as a natural PPAR agonist. In a mouse model, ESA reduced macrophage infiltration and significantly hampered the progression of IBD through PPAR-dependent and PPAR-independent mechanisms and the study provided a noninvasive tool for the screening of PPARc (Lewis and others 2011). Bassaganya-Riera and others (2011b) studied the mechanism of immunoregulation in the amelioration of experimental IBD in immune cell-specific PPARγ null, PPARδ knockout, and wild-type mice and concluded that PA regulates macrophage and T-cell function through PPARγ- and δ-dependent mechanisms to ameliorate IBD and could be helpful in treatment of IBD (Yuan and others 2015).

In a rat model of necrotizing enterocolitis (NES), PSO was able to suppress the occurrence of NES and normalizing inflammatory biomarkers (TNF-α and ILs) in the ileum, at 1.5% of the diet (Coursodon-Boyiddle and others 2012). Spilmont and others (2013) demonstrated effective anti-inflammatory and anti-oxidative properties of PA and showed that the consumption of PSO at 5% of the diet improved bone mineral density and prevented trabecular micro-architecture impairment in mice by osteoclastogenesis inhibition and osteoblastogenesis improvement. PSO also downregulated the expression of specific osteoclast differentiation markers and RANK-RANKL downstream signaling targets in osteoclast-like cells. PA also elicited significant increase in alkaline phosphatase, matrix mineralization, and transcriptional levels of major osteoblast lineage markers involving the Wnt/β-catenin signaling pathways in osteoblast-like cells. PSO also inhibited pro-inflammatory factors expression and stimulated anti-inflammatory ones, and thus offers promising alternatives in nutritional management of age-related bone complications (Spilmont and others 2013).

Calder (2013) reported that saturated fatty acids promote gene expression in relation to inflammation and immune responses, while n-3 fatty acids (eicosapentaenoic and docosahexaenoic acids), isomers of CAL, and PA suppress the expression of inflammatory genes (encoding cytokines, chemokines, cyclooxygenase,

NO synthase, and metalloproteinases). The fatty acids exert anti-inflammatory actions by inhibiting the activation of NF-kappa-B, and PPARα and γ. Thus, a number of fatty acids modify expression of genes involved in development, differentiation, and function of cells involved in inflammation and immunity.

Viladomiu and others (2013) explored the activation of PPARγ by CLnA, PA, EA, and abscisic acid and other natural compounds to suppress colitis by directly modulating the host immune response. As the pathogenesis of IBD is a consequence of interactions among the immune system, the commensal microbiota and the host genotype manipulation of gut microbiota and local production of microbial-derived metabolites by pre- and probiotics and dietary fibers could be a promising means of prophylactic and therapeutic intervention against gut inflammation. Modification of the gut microbiome by diet can modify the induction of regulatory compared with effector immune responses at the mucosa and improve health outcomes. Short-chain fatty acids produced in the colon by the gut microflora activate PPARγ and thus modulate the complications of IBD.

Yamasaki and others (2006) studied the influence of PSO on immune response and lipid metabolism in C58BL/6N mice and showed that PSO promoted the production of immunoglobulins (Ig) G and M, but not IgA, in mouse splenocytes without affecting the balance of B- and T-cell populations. PSO also increased the levels of 9cis, 11trans CLA in serum, liver, and adipose tissue of experimental animals in a dose-dependent manner.

In genetically obese db/db mice and in a model of diet-induced obesity in PPARγ expressing and tissue-specific PPARγ null mice, PA caused a dose-dependent increase in PPARα and γ receptor activity in 3T3-L1 cells and bound weakly to the ligand-binding domain of human PPARγ. Dietary PA also decreased fasting plasma glucose levels, improved the glucose-normalizing ability, suppressed NF-kappa-B activation and TNF-α expression, and upregulated PPARα- and γ-responsive genes in skeletal muscle and adipose tissue. Loss of PPARγ impaired the ability of dietary PA to improve glucose homeostasis and to suppress inflammation. Thus, PA can bind and activate PPARγ levels and increase PPARγ-responsive gene expression. The loss of PPARγ in immune cells impairs its ability to ameliorate diabetes and inflammation (Hontecillas and others 2009).

Anticancer properties

Various forms of cancer affect a large population in the world, out of which colon cancer is one of the major causes of death. Kohno and others (2004) described that administration of PSO inhibits progression of azoxymethane-induced colonic adenocarcinomas and reduction of multiplicity of carcinomas. Prostate cancer is the second highest cancer affecting the world population. While studying the effect of CLnA and CLA, CLnAs have been shown to be more cytotoxic to tumor cells than CLA (Igarashi and Miyazawa 2000). Lansky and others (2005a) studied the inhibitory effects of PA and other compounds (ellagic acid, caffeic acid, and luteolin) against *in vitro* invasion of human prostate cancer (PC-3) cells or proliferation of DU 145 cells. Individually, all these compounds significantly inhibited invasion and proliferation of prostate cancer cells, but, interestingly, various combinations of active fractions of pomegranate, including PSO, exhibited synergistic effects against invasion and proliferation of prostate cancer cells (Lansky and others 2005b).

The proliferation of prostate cancer is stimulated by endogenous steroid hormone, and aromatase (CYP19) and 5α-reductase (SRD5A) are the key enzymes which help in the synthesis of these hormones. In hormone-dependent prostate cancer (LNCaP) and steroidogenesis (human adrenocortical H295R) cell models, Gasmi and Sanderson (2009) showed that PA inhibited steroidogenesis by inhibiting aromatase in H295R cells. Gasmi and Sanderson (2010) also showed the growth inhibitory, antiandrogenic, and pro-apoptotic effects of epigallocatechin gallate, delphinidin chloride, kaempferol, and PA in androgen-dependent LNCaP cells. PA and other compounds inhibited dihydrotestosterone-stimulated cell growth, androgen receptor nuclear accumulation, and the expression of the androgen receptor-dependent genes, prostate-specific antigen (PSA), and type I steroid 5 α-reductase. These compounds also induced DNA fragmentation. PA in particular induced intrinsic apoptosis through a caspase-dependent pathway, thus effectively inhibited growth in androgen-dependent LNCaP cells, which may be mediated by both antiandrogenic and pro-apoptotic mechanisms. Gasmi and Sanderson (2013) studied the cytotoxic- and apoptosis-inducing activities of 7 dietary C-18 trienoic fatty acids (including PA) against human prostate cancers, and they concluded that their cytotoxic potency was related to the degree of conformational similarity to PA and ranked these fatty acids according to cytotoxic potency. Wang and others (2014) showed that PA, in combination with luteolin and ellagic acid, inhibit the metastasis and progression of prostate cancer.

In preclinical murine models, pomegranate juice (PJ) and/or pomegranate extracts (PE) inhibited growth and angiogenesis of prostate tumors. Luteolin and ellagic acid (from PJ) and PA together exhibited inhibitory effects on prostate cancer growth, angiogenesis, and metastasis. PJ and/or PE significantly prolonged the PSA doubling time in prostate cancer patients (Wang and Green 2014).

PA also inhibited breast cancer cell proliferation in both estrogen-responsive and estrogen nonresponsive cell lines (Grossmann and others 2010). In the cell lines of MDA-MB-231 (estrogen-insensitive) and MDA-ERa7 (estrogen-sensitive, developed from MDA-MB-231), proliferation was inhibited, while apoptosis (via PKC) through a disrupted mitochondrial membrane potential was induced in these cell lines. The effects were secondary to the pro-oxidative effects of PA, as these were abolished by incubation with tocotrienol. In another study, α-EA was shown to be more effective in inhibiting proliferation of breast cancer cell lines than PA (Tran and others 2010). In another study, Costantani and others (2014) showed the cytotoxic effect of the polar compounds present in 80% aqueous methanolic extract of PSO on breast cancer cell lines (MCF-7 and MDA-MB-231) along with anti-inflammatory and antioxidant effect. Based on the data, these effects seem to be exerted in a synergistic manner.

PA inhibited the proliferation of bladder carcinoma T24 in a dose-dependent manner and induced apoptosis (Wang and others 2013). Asghari and others (2012) reported that administration of PSO in dyslipidemic patients did not affect serum TNF.

Tanaka and others (2011) showed that CLnA could inhibit colorectal tumorigenesis through modulation of apoptosis by a decrease of Bcl-2 protein in various human cancer cell lines, increase the expression of PPARγ and upregulate gene expression of p53. Thus, CLnA could have potential use as a nutraceutical and as an ingredient in functional and health-beneficial food formulations.

Hora and others (2003) demonstrated the skin cancer chemo-preventive efficacy of PSO in a CD1 mouse model against 7, 12-dimethylbenzanthracene-induced and 12-O-tetradecanoylphorbol 13-acetate (TPA)-promoted skin cancer. Overall, PSO significantly decreased the tumor incidence,

multiplicity, and TPA-induced ornithine decarboxylase activity which plays an important role in the development of skin cancer; thus, PSO could be a safe and effective chemo-preventive agent against skin cancer.

Pro- and antioxidant activities

Mukherjee and others (2002) reported the antioxidative and hypolipemic activities of PA in a rat model. PA, when fed to rats at 0.6%, 1.2%, and 2.4% for 14 wk, increased weight more with 0.6% and 1.2% PA-fed rats than the control. Plasma TC and LDL-C were significantly lower in the groups fed with 2.4% PA. The pro-oxidant and antioxidant behaviors at 1.2% and 0.6% levels of PA, respectively, was demonstrated by measuring lipoprotein oxidation susceptibility and plasma lipid peroxidation. The atherogenic index ratio [(TC/HDL-C)-1] and LDL-C/HDL-C ratio were significantly reduced at the 0.6% and 2.4% PA levels. This dual behavior of PA may probably be because of the fact that in PA isomer, the total *cis* and *trans* molecular configuration increased with an increase in the amount of PA from 0.6% to 2.4% via 1.2%. The authors proposed that inhibition of hydroperoxide formation by PA might have been either due to the lowering of free radical generation or peroxidation. Alternatively, with possible biohydrogenation (addition of free radical to one of the conjugated double bonds of PA), formation of conjugated dienes could be the possible reason for the antioxidant activity (Figure 3). Yuan and others (2009b) demonstrated that feeding PA from *T. kirilowii* seed kernels (containing 3 g PA) in healthy young humans for 28 d caused increased lipid peroxidation and this observation initiated the need to elucidate the mechanism and consequences of the increased lipid peroxidation on PA supplementation.

Using the green method of laser ablation, Sadrolhosseini and others (2014) synthesized and characterized gold nanoparticles in PSO. Mizrahi and others (2014) prepared a nano-droplet formulation of PSO (nano-PSO) to find its effect in Alzheimer's disease patient. TgMHu2ME199K mice modeling for genetic prion disease, when treated with nano-PSO, significantly delayed disease onset in asymptomatic mice and could postpone disease aggravation in the sick mice. Thus, nano-PSO possesses neuroprotective effects via its antioxidant properties.

Protection against sodium arsenite (SA) toxicity

SA causes severe oxidative stress in rats. Saha and Ghosh (2009, 2010) demonstrated the ameliorative role of α-EA and PA against SA-induced oxidative stress and DNA damage. SA caused lowering of the activities of catalase, SOD, GPx, and GSH in plasma, liver, and erythrocyte lysate, while the activity of NO synthase in plasma and liver was increased. Administration of CLnA isomers led to restoration of all the altered parameters and reduction in lipid peroxidation and leakage of transaminases from liver to blood. α-EA was a more potent antioxidant than PA against oxidative DNA damage. CLnA isomers also synergistically reversed the SA-induced oxidative stress in erythrocyte membrane disintegrity (Saha and others 2012b). The levels of various SA-induced oxidative markers and inflammatory markers expression, platelet aggregation, lipid peroxidation, protein oxidation, DNA damage, and altered expression of liver X receptor-α were restored after treatment with α-EA and PA. Morphology and fluidity of the erythrocyte membrane were significantly improved indicating the synergistic antioxidant and anti-inflammatory effects of the 2 isomers, against induced perturbations and membrane disintegrity. CLnA isomers could also synergistically ameliorate SA-induced renal oxidative stress by reducing the arachidonic acid content of renal lipids (Saha and Ghosh 2013).

Antinephrotoxic activity

In a rat model, Boroushaki and others (2010) studied the effect of PSO against hexachlorobutadiene (HCBD, a potent nephrotoxin in rodents)-induced nephrotoxicity. HCBD-induced rats showed elevation in the levels of serum creatinine and urea and urinary glucose and protein, followed by a decrease in thiol content and an increase in TBARS in kidney homogenates, which were reverted back by pretreatment with PSO. In another model, PSO exhibited nephroprotective effects against mercury-induced free radical-mediated oxidative nephrotoxicity (Boroushaki and others 2014).

Lipid peroxidation

PA has been shown to increase lipid peroxidation in young human subjects, as shown by increased urinary 8-iso-PGF2α (IP, formed from arachidonic acid) by approximately 50% without influencing C-reactive protein or IL-6 (Yuan and others 2009b). IP is further metabolized to 2, 3 dinor in peroxisomes. CLA is preferentially β-oxidized in peroxisomes and may impair IP catabolism in peroxisomes. Hence, the increase of IP as a sole result of CLA intake may indicate peroxisomal oxidation of PA and may not be a marker of lipid peroxidation (Iannone and others 2009).

Interactions with estrogen

PA has been described to be an antiestrogen on estrogen receptors (ERs; IC_{50} for Era 7.2 and 8.8 uM for ERb) with a slight preference toward the ERa subset (Tran and others 2010). However, PA seems to be exhibiting a dual behavior with activation of both ERs at lower concentrations, while at higher doses it antagonizes both receptors. Thus, PA may be exploited as a selective ER modulator.

Nutrient–nutrient interactions

Xanthigen is a mixture of fucoxanthin (from brown seaweed) and PA, which has been traditionally used for lipid-lowering effects and is shown to cause significant fat loss in human subjects (Abidov and others 2010). *In vitro*, the 2 molecules show some degree of synergism in pro-fat loss effects. Choi and others (2014) reported the possible attenuation of HFD-induced obesity by xanthigen (1%) in C57BL/6N mice. The food efficiency ratio and body weight were significantly reduced compared to the HFD-fed control, followed by a significant decrease in weights of epididymal and retroperitoneal adipose tissues and the liver and serum LDL-cholesterol. Thus, xanthigen could be considered in the development of a functional "health food" for the amelioration of obesity-related parameters. In human subjects, Ching and others (2012) described the mechanism of TAG-lowering effects of xanthigen and showed that xanthigen effectively suppressed the accumulation of lipid droplets in adipocytes in a dose-dependent manner, when compared with fucoxanthin or PA alone. It downregulates the levels of adipogenesis transcription factors PPARγ, CCAAT/enhancer-binding protein (C/EBP) β, and C/EBP δ, as well as fatty acid synthase (a key enzyme for adipogenesis). Xanthigen upregulates NAD^+-dependent histone deacetylase (SIRT1) and activates AMP-activated protein kinase (AMPK) signaling in differentiated 3T3-L1 adipocytes. Xanthigen may also activate insulin trigger signaling and result in Akt-dependent phosphorylation of forkhead/winged helix O (FoxO) 1 and FoxO3a.

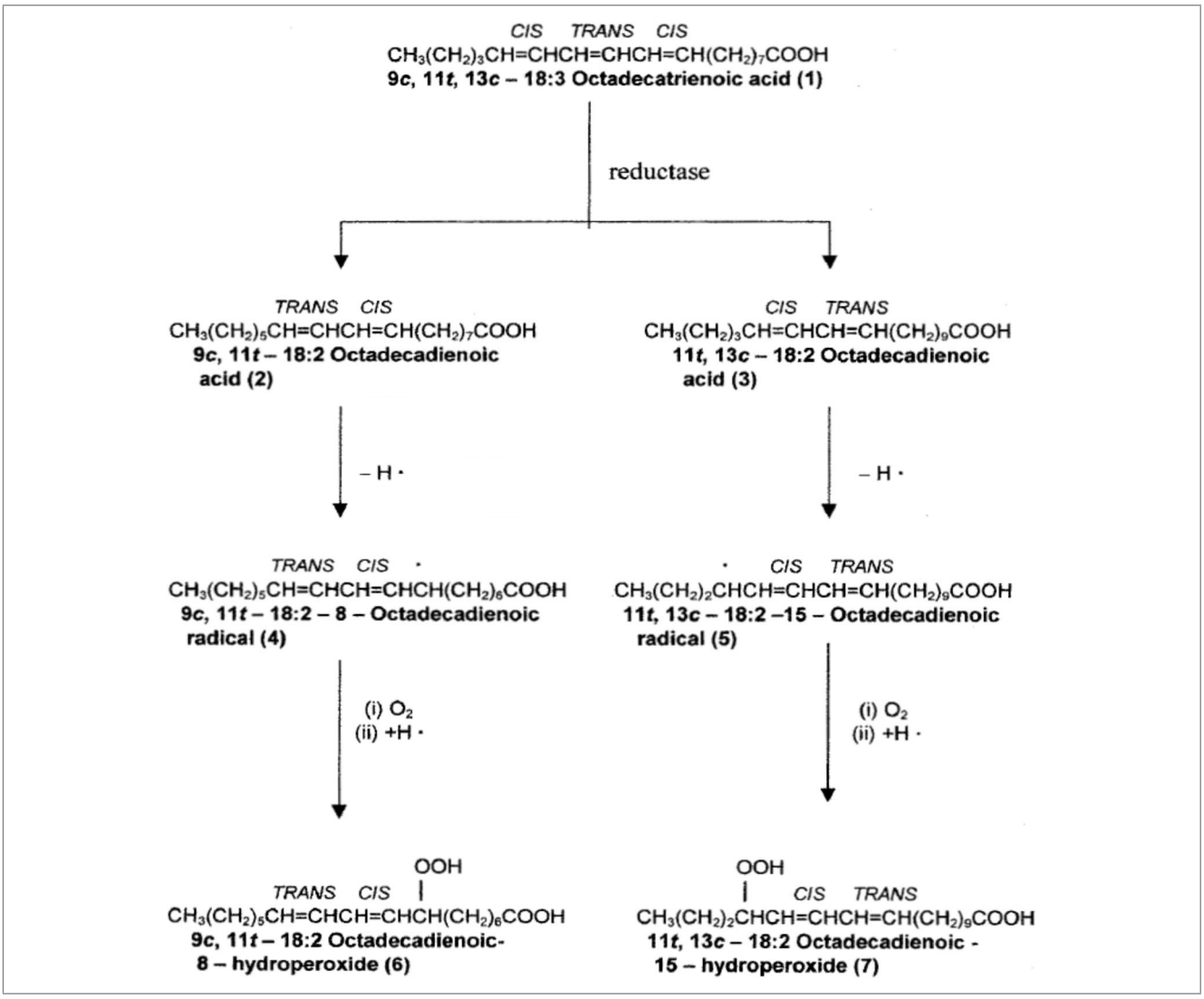

Figure 3–Possible mechanisms of antioxidant activity of PA (Mukherjee and others 2002).

Metabolism of PA

Tsuzuki and others (2004) described that PA was converted to rumenic acid (9Z11E-CLA) in a rat model, indicating that part of the bioactivity of PA could be due to c9t11 CLA. In a study with 28 d of PA intervention showed that CLA levels rose from 0.05% to 0.06% of total fatty acids (control) to 0.23% in the PA intervention group. Even PA was detected in circulation, suggesting only its incomplete metabolism into c9t11 CLA. Tsuzuki and others (2006) further demonstrated that, although CLnA is slowly absorbed in the rat intestine, it is quickly converted to CLA. Even α-EA was also shown to be converted to 9Z11E-CLA in rats through a δ13-saturation reaction, thus producing a positional and geometrical isomer. Comparatively, the rate of absorption was LnA > CLA > α-ESA = PA. However, the conversion ratio of α-ESA was higher than that of PA. In a mouse model also, Yuan and others (2009c) showed that both α-EA and PA are slowly absorbed and a fraction of these fatty acids is quickly converted to 9Z11E-CLA. In rats fed with PA, the levels of PA and CLA in liver and plasma were higher than in heart, kidney, and adipose tissue, with lowest accumulation found in the brain. Thus, the metabolite of PA, namely, 9c, 11t-CLA, by virtue of possessing an array of biological activities, makes PA a potent candidate in functional food preparations and nutraceuticals (Yuan and others 2009d).

In a mouse model, Yuan and others (2009e) demonstrated the influence of 1% each of α-ESA and PA on fatty acid composition in various tissues and was compared with a CLA mixture (cis9, trans11-, and trans10, cis12-18:2). The content of 18:2n-6 was significantly decrased in the heart and adipose tissues and total and n-6 PUFA concentrations were significantly lowered in adipose tissue by α-ESA, PA, and CLA. However, the contents of 22:6n-3 and total n-3 PUFAs were significantly increased in the liver, kidney, and heart by PA alone. On the other hand, supplementation with CLA significantly reduced 22:6n-3 in the liver, kidney, and heart and the content of 20:4n-6 was significantly decreased in the liver and kidney, but not by α-ESA and PA. Thus, α-ESA, PA, and CLA have differential effects on 22:6n-3 and 20:4n-6 contents in mouse tissues. In healthy young humans supplemented with 3 g PA per day for 28 d, Yuan and others (2009f) demonstrated that the proportion of PA was increased both in plasma (0% to 0.47%) and red blood cell membranes (RBCMs, 0% to 0.37%). The proportion of cis9, trans11-18:2 was also increased in plasma

(0.05% to 0.23%) and in RBCM (0.03% to 0.17%). Thus, PA can be incorporated into plasma and RBCM with simultaneous increase in cis9, trans11-18:2, presumably by a saturation reaction. These data provide the lead for application of CLnA as functional food ingredients, which could be strategically used for beneficial modification of the fatty acid composition of animal tissues and substantiate the possibility of making the sources of PA as a potential dietary source of CLAs.

Caco-2 cells (a validated model of the intestinal barrier), have been shown to take up various CLnA at different rates and convert them into CLA with varying efficiencies depending on the structure of the δ13 double bond (Schneider and others 2013). The distribution of CLnA between neutral lipids and phospholipids may be linked to their number of trans double bonds; the higher the number, the higher was the accumulation in the neutral lipid fraction.

Using multiparous southern Khorasan (Iran) cross-bred goat models fed with 6% and 12% of pomegranate seed pulp (PSP, dry matter basis) for 45 d, Modaresi and others (2011) showed an increase in milk fat concentration without affecting milk fat yield, protein concentration, and solids-not-fat concentration. The goats fed on 12% PSP showed a modified milk fatty acid profile, including CLA, PA, and vaccenic acids. Thus, PSP could be used as a replacement for cereals and other energy-rich dietary supplements by virtue of its high fat content.

Food Applications

Pomegranate seed and peel remain as by-products after processing the fruit into juice. Cam and others (2013) incorporated peel and seed oil into ice cream to improve its functional properties. Incorporation of the peel phenolics resulted in significant changes in the pH, total acidity, and color, with improvements in antioxidant and antidiabetic activities, and phenolic content, whereas replacement of milk fat with PSO increased the conjugated fatty acid content. Thus, it might provide the health benefits with functional properties of punicalagins and other molecules from peel and PA from PSO. Lucci and others (2015) proposed the utilization of pomegranate whole seed ethanolic extract (PSEE) as a nutraceutical/functional food ingredient with the aim of exploiting its antioxidant and antiproliferative properties against hormone-dependent prostate carcinoma and human breast cancer cell lines. Hence, PSEE could be promoted as a value-added ingredient in formulations of products to prevent diseases, especially cancer.

In order to avoid the use of synthetic antioxidants, Devatkal and others (2010) used the extracts of fruit by-products, such as kinnow rind powder (KRP) and pomegranate rind and seed powder (PRP and PSP) in goat meat patties. The overall antioxidant effect was in the order of PRP > PSP > KRP and no significant differences in sensory parameters were noted among the patties. This highlights the potential of using these fruit by-products as natural antioxidants in meat products. Keşkekoğlu and Uren (2014) incorporated 0.5% (w/w) pomegranate seed extract in beef and chicken meat balls, which were cooked using oven-roasting, pan-cooking, charcoal-grilling, and deep-fat frying. The pomegranate seed extract could reduce the formation of heterocyclic aromatic amines in both the products to varying degrees and thus may provide a safer product than the control.

PSO has been incorporated as a functional ingredient by the juice and beverage industries with PSO-in-water emulsion as base (Mohagheghi and others 2011). The effect of varying the concentration of gum Arabic with a constant oil phase content was investigated for turbidity loss rate, emulsion stability index, and droplet size distribution. The results demonstrated that the prepared beverage emulsions behaved as Newtonian fluids, and this opens up new vistas to produce comparatively stable PSO-in-water emulsions for their use in beverages as a functional agent. Goula and Adamopoulos (2012) optimized various parameters for the encapsulation of PSO using skimmed milk powder as encapsulating agent, which may provide another route for the application of PSO by food industries.

Toxicity

Meerts and others (2009) studied the safety and toxicological aspects of PSO by *in vitro* and *in vivo* tests. No mutagenicity or clastogenicity of PSO was observed in the absence and presence of metabolic activation up to 5000 μg/plate (Ames test) or 333 μg/mL (chromosome aberration test). No significant findings were revealed at 2 g PSO/kg body weight in acute oral toxicity studies. Thus, the LD50 cut-off value could be considered to be higher than 5 g/kg body weight according to the OECD 423 test guideline and no classification or labeling for oral toxicity is required for PSO. In 28-d dietary toxicity studies, PSO was administered at concentrations as high as 150000 ppm (mean intake of 1.42 and 1.37 g PSO/kg body weight per day in male and female rats, respectively). At 150000 ppm, increased liver function enzymes (aspartate and alanine aminotransferases and alkaline phosphatase) were detected in plasma followed by increased liver-to-body weight ratios. These effects might have been due to a physiological response to the very high dose of PA. Thus, toxicologically, these data may not be relevant as PA/PSO do not constitute a part of the normal diet, and also at such a higher dose. The no observable adverse effect level (NOAEL) of PA was reported at 50000 ppm as PSO, which is equivalent to 4.3 g PSO/kg body weight/day.

Conclusion

Pomegranate *per se* has attracted human attention due to its array of health benefits, including antioxidant, anticancer, anti-inflammatory, and antidiabetic and also many other properties. The peel, membranes, and seeds are inadvertently produced by the processing industries (known as "Marc"; Qu and others 2010), which becomes a burden in terms of environmental pollution, owing to its high biological oxygen demand, and it is used as either cattle feed or is discarded. The above description focuses on the health-beneficial aspects of seeds as seed oil with a high content of PA. This, in turn, could be utilized for the development of health-beneficial (including food) products, targeted for precise consumers in particular and also general consumers. A cursory survey of the literature reveals that it has not yet moved food industries to see it as a functional ingredient. The exploitation of PSO by industries would not only pave a mode for waste utilization but also toward the development of value-added products. PSO with a very high concentration of NOAEL, does not have any safety issues. Only 1 or 2 reports describing the pro- and antioxidant role of PA at different concentrations may be necessary to start its judicious incorporation in food products; and such products developed with PA may then be evaluated for their health benefits. Studies pertaining to the stability of PA in food products could be another line of future research.

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Authors' Contributions

P. Aruna collected much of the information used and prepared the draft. D. Venkataramanamma, collected much of the information used and also prepared the draft. Alok Kumar Singh, collected much of the information used. R.P. Singh, finally refined the draft and fine-tuned it for publication. The authors declare no conflict of interest statement pertaining to this manuscript.

References

Abbasi H, Rezaei K, Emamdjomeh Z, Mousavi SME. 2008. Effect of various extraction conditions on the phenolic contents of pomegranate seed oil. Eur J Lipid Sc Tech 110:435–40.

Abidov M, Ramazanov Z, Seifulla R, Grachev S. 2010. The effects of xanthigen in the weight management of obese premenopausal women with non-alcoholic fatty liver disease and normal liver fat. Diabetes Obesity Metabolism 12:72–81.

Ahangari B, Sargolzaei J. 2012. Extraction of pomegranate seed oil using subcritical propane and supercritical carbon dioxide. Theor Foundations Chem Eng 46:258–65.

Ali K, Che-Man YB, Roberts TH. 2014. Physico-chemical properties and fatty acid profile of seed oils from pomegranate (*Punica granatum L.*) extracted by cold pressing. Eur J Lipid Sc Tech 116:553–62.

Anusree SS, Priyanka A, Nisha VM, Das AA, Raghu KG. 2014. An *in vitro* study reveals the nutraceutical potential of punicic acid relevant to diabetes via enhanced GLUT4 expression and adiponectin secretion. Food Funct 5(10):2590–601.

Anusree SS, Nisha VM, Priyanka A, Raghu KG. 2015. Insulin resistance by TNF-α is associated with mitochondrial dysfunction in 3T3-L1 adipocytes and is ameliorated by punicic acid, a PPARγ agonist. Mol Cell Endocrinol 413:120–8.

Arao K, Yotsumoto H, Seo HY, Nagao K, Yanagita T. 2004a. The 9cis,11trans,13cis isomer of conjugated linolenic acid reduces apolipoprotein B100 secretion and triacylglycerol synthesis in HepG2 cells. Biosc Biotech Biochem 68:2643–5.

Arao K, Wang Y, Inoue N, Hirata J, Cha J, Nagao K, Yanagita T. 2004b. Dietary effect of pomegranate seed oil rich in 9cis, 11trans, 13cis conjugated linolenic acid on lipid metabolism in obese, hyperlipidemic OLETF rats. Lipids Health Dis 3:24–30.

Asghari G, Sheikholeslami S, Mirmiran P, Chary A, Hedayati M, Shafiee A, Azizi S. 2012. Effect of pomegranate seed oil on serum TNF- alpha level in dyslipidemic patients. International J Food Sc Nut 63:368–71.

Banihani S, Swedan S, Alguraan Z. 2013. Pomegranate and type II diabetes. Nut Res 33:341–8.

Bassaganya-Riera J. 2011. Method of using punicic acid to enhance immune response and prevent metabolic disorders. US patent No. 20110250231 A1.

Bassaganya-Riera J, Guri AJ, Hontecillas R. 2011a. Treatment of obesity-related complications with novel classes of naturally occurring PPAR agonists. J Obesity (http://dx.doi.org/10.1155/2011/897894).

Bassaganya-Riera J, DiGuardo M, Climent M, Vives C, Carbo A, Jouni ZE, EInerhand AW, O'Shea M, Hontecillas R. 2011b. Activation of PPAR gamma and delta by dietary PA ameliorates intestinal inflammation in mice. Br J Nut 106:878–86.

Boroushaki MT, Sadeghnia HR, Banihasan M, Yavari S. 2010. Protective effect of pomegranate seed oil on hexachlorobutadiene-induced nephrotoxicity in rat kidneys. Renal Failure 32:612–7.

Boroushaki MT, Mollazadeh H, Rajabian A, Dolati K, Hoseini A, Paseban M, Farzadnia M. 2014. Protective effect of pomegranate seed oil against mercuric chloride-induced nephrotoxicity in rat. Renal Failure 36: 1581–6.

Boussetta T, Raad H, Letteron P, Gougerot-Pocidalo MA, Marie JC, Fathi D, El-Benna J. 2009. Punicic acid: a conjugated linolenic acid inhibits TNFa-induced neutrophil hyperactivation and protects from experimental colon inflammation in rats. PLoS One 4(7):e6458.

Calder PC. 2013. Long chain fatty acids and gene expression in inflammation and immunity. Curr Opin Clin Nut Met Care 16:425–33.

Cam M, Erdogan F, Aslan D, Dinc M. 2013. Enrichment of functional properties of ice cream with pomegranate by-products. J Food Sci 78:C1543–50.

Ching SL, Mei LT, Badmaev V, Jimenez M, Chi TH, Min HP. 2012. Xanthigen suppresses preadipocyte differentiation and adipogenesis through down-regulation of PPAR gamma and C/EBPs and modulation of SIRT-1, AMPK, and FoxO pathways. J Agri Food Chem 60:1094–101.

Choi KM, Jeon YS, Kim W, Lee A, Gil Kim Y, Lee JH, Kang YE, Jung JC, Lee J, Min B, Kwon SH, Davis B, Lee MK, Yoo HS. 2014. Xanthigen Attenuates High-fat Diet-induced Obesity through Down-regulation of PPAR Y and Activation of the AMPK Pathway. Food Sci Biotechnol 23(3):931–35.

Colombo E, Sangiovanni E, Dell' Agli M. 2013. A review on the anti-inflammatory activity of pomegranate in the gastrointestinal tract. Evidence-Based Complementary Alt Med, Art. ID 247145, p 11 (http://dx.doi.org/10.1155/2013/247145).

Costantani S, Rusolo F, De Vito V, Moccia S, Picariello G, Capone F, Guerriero E, Castello G, Volpe MG. 2014. Potential anti-inflammatory effects of the hydrophilic fraction of pomegranate (Punica granatum L.) seed oil on breast cancer cell lines. Molecules 19(6):8644–60.

Coursodon-Boyiddle CF, Snarrenberg CL, Adkins-Rieck CK, Bassaganya-Riera J, Hontecillas R, Lawrence P, Brenna JT , Jouni ZE, Dvorak B. 2012. Pomegranate seed oil reduces intestinal damage in a rat model of necrotizing enterocolitis. Am J Physiol 303:G744–51.

Dadashi S, Mousazadeh M, Emam-Djomeh Z, Mousavi SM. 2013. Pomegranate (*Punica granatum L.*) seed: a comparative study on biochemical composition and oil physicochemical characteristics, Biochemical composition of pomegranate seed oil. Int J Adv Biol Biomed Res 1:351–63.

Devatkal SK, Narsaiah K, Borah A. 2010. Anti-oxidant effect of extracts of kinnow rind, pomegranate rind and seed powders in cooked goat meat patties. Meat Sci 85:155–9.

Elbandy MA, Ashoush IS. 2012. Phytochemicals in pomegranate seeds and their effect as hypolipidemic agent in hypercholesterolemic rats. World J Dairy Food Sci 7:85–92.

Fadavi A, Barzegar M, Azizi MH. 2006. Determination of fatty acids and total lipid content in oilseed of 25 pomegranates varieties grown in Iran. J Food Comp Anal 19:676–80.

Franzke C, Goebel R, Kroll J. 1982. Studies on the glyceride structure of fats. XIV. Composition of pomegranate fat. Nahrung 26:K1–2.

Gasmi J, Sanderson T. 2009. Anti-proliferative and anti-steroidogenic effects of pomegranate compounds in human LNCaP prostate and H295R adrenocortical cancer cells. Presented in 41st Annual Symposium of Society of Toxicology of Canada, November 29–December 1, 2009.

Gasmi J, Sanderson T. 2010. Growth inhibitory, antiandrogenic, and pro-apoptotic effects of PA in LNCaP human prostate cancer cells. J Agri Food Chem 58(23):12149–56.

Gasmi J, Sanderson T. 2013. Jacaric acid and its octadecatrienoic acid geoisomers induce apoptosis selectively in cancerous human prostate cells: a mechanistic and 3-D structure-activity study. J Phyt 20:734–42.

Goula AM, Adamopoulos KG. 2012. A method for pomegranate seed application in food industries: Seed oil encapsulation. Food Bio-products Proces 90:639–52.

Grossmann ME, Mizuno NK, Schuster T, Cleary MP. 2010. Punicic acid is an omega-5 fatty acid capable of inhibiting breast cancer proliferation. Int J Oncol 36:421–6.

Guangmin L, Xiang X, Qinfeng H, Yanxiang G. 2009. Supercritical CO_2 extraction optimization of pomegranate (*Punica granatum L.*) seed oil using response surface methodology. LWT - Food Sc Tech 42:1491–5.

Guangmin L, Xiang X, Ying G, Li H, Yanxiang G. 2012. Effects of supercritical CO_2 extraction parameters on chemical composition and free radical-scavenging activity of pomegranate (*Punica granatum L.*) seed oil. Food Bio-products Proces 90:573–8.

Habibnia M, Ghavami M, Ansaripour M, Vosough S. 2012. Chemical evaluation of oils extracted from five different varieties of Iranian pomegranate seeds. J Food Biosc Tech 2:35–40.

Hontecillas R, O'Shea M, Einerhand A, Diguardo M, Bassaganya-Riera J. 2009. Activation of PPAR gamma and alpha by punicic acid ameliorates glucose tolerance and suppresses obesity-related inflammation. J Am College Nut 28:184–95.

Hopkins CY, Chisholm MJ. 1968. A survey of the conjugated fatty acids of the seed oils. J Am Chem Soc 45:176–82.

Hora JJ, Maydew ER, Lansky EP, Dwivedi C. 2003. Chemopreventive effects of pomegranate seed oil on skin tumor development in CD1 mice. J Med Food 6:157–61.

Hornung E, Pernstich C, Feussner I. 2002. Formation of conjugated delta 11 delta13-double bonds by delta 12-linoleic acid (1,4)-acyl-lipid-desaturase in pomegranate seeds. Eur J Biochem 269:4852–9.

Iannone A, Petroni A, Murru E, Cordeddu L, Carta G, Melis MP, Bergamini S, Casa LD, Cappiello L, Carissimi R, O'Shea M, Bell D, Santis ED, Banni S. 2009. Impairment of 8-iso-PGF (2ALPHA) isoprostane metabolism by dietary conjugated linoleic acid (CLA). Prostaglandins, Leukotrienes Essential Fatty Acids 80:279–87.

Igarashi M, Miyazawa T. 2000. Newly recognized cytotoxic effect of conjugated trienoic fatty acids on cultured human tumor cells. Cancer Let 148:173–9.

Iwabuchi M, Kohno MJ, Imamura J. 2003. Delta12-oleate desaturase-related enzymes associated with formation of conjugated trans- delta11, cis- delta13 double bonds. J Biol Chem 278:4603–10.

Jelodar G, Mohsen M, Shahram S. 2007. Effect of walnut leaf, coriander and pomegranate on blood glucose and histopathology of pancreas of alloxan induced diabetic rats. Af J Trad Compl Alt Med 4:299–305.

Jing Y, Ciqin Z, Gaofeng Y, Duo L. 2012. Effects of geographical origin on the conjugated linolenic acid of Trichosanthes kirilowii Maxim seed oil. J Am Oil Chem Soc 89:401–7.

Keşkekoğlu H, Uren A. 2014. Inhibitory effects of pomegranate seed extract on the formation of heterocyclic aromatic amines in beef and chicken meatballs after cooking by four different methods. Meat Sci 96:1446–51.

Kittur MH, Mahajanshetti CS, Lakshminarayana G. 1993. Characteristics and composition of *Trichosanthes bracteata*, *Urena sinuata* and *Cappari sdivaricata* seeds and oils. J Oil Tech Ass Ind 25:39–41.

Koba K, Imamura J, Akashoshi A, Kohno-Murase J, Nishizono S, Iwabuchi M, Tanaka K, Sugano M. 2007. Genetically modified rapeseed oil containing cis-9,trans-11,cis-13-octadecatrienoic acid affects body fat mass and lipid metabolism in mice. J Agri Food Chem 55:3741–8.

Kohno H, Suzuki R, Yasui Y, Hosokawa M, Miyashita K, Tanaka T. 2004. Pomegranate seed oil rich in conjugated linolenic acid suppresses chemically induced colon carcinogenesis in rats. Cancer Sci 95:481–6.

Kyralan M, Goeluekcue M, Tokgoez H. 2009. Oil and conjugated linolenic acid contents of seeds from important pomegranate cultivars (*Punica granatum L.*) grown in Turkey. J Am Oil Chem Soc 86:985–90.

Lansky EP, Newman RA. 2007. *Punica granatum* (pomegranate) and its potential for prevention and treatment of inflammation and cancer. J Ethnopharm 109:177–206.

Lansky EP, Harrison G, Froom P, Jiang W. 2005a. Pomegranate (*Punica granatum*) pure chemicals show possible synergistic inhibition of human PC-3 prostate cancer cell invasion across Matrigel. Investig New Drugs 23:121–2.

Lansky EP, Jiang W, Mo H, Bravo L, Froom P, Yu W, Harris NM, Neeman I, Campbell MJ. 2005b. Possible synergistic prostate cancer suppression by anatomically discrete pomegranate fractions. Investig New Drugs 23:11–20.

Lesot P, Serhan Z, Billault I. 2011. Recent advances in the analysis of the site-specific isotopic fractionation of metabolites such as fatty acids using anisotropic natural-abundance ^{2}H NMR spectroscopy: application to conjugated linolenic methyl esters. Anal Bioanal Chem 399:1187–1200.

Lewis SN, Brannan L, Amir J, Guri AJ, Lu P, Hontecillas R, Bassaganya-Riera J, Bevan DR. 2011. Dietary a-eleostearic acid ameliorates experimental inflammatory bowel disease in mice by activating peroxisome proliferator-activated receptor-c. PLoS ONE 6:e24031.

Lotti G, Izzo R, Nicolini F. 1973. Influence of ripening and environment on the seed oil of *Momordica balsamina L.* Rivista-Italiana-delle-Sostanze-Grasse 50:425–33.

Lucci P, Pacetti D, Loizzo MR, Frega NG. 2015. *Punica granatum cv*. Dente di Cavallo seed ethanolic extract: Antioxidant and antiproliferative activities. Food Chem 167:475–83.

May P. 2014. Supercritical pomegranate seed extract helps maintain good health. Innovative Food Tech 62:34–6.

McFarlin BK, Strohacker KA, Kueht ML. 2009. Pomegranate seed oil consumption during a period of high-fat feeding reduces weight gain and reduces type 2 diabetes risk in CD-1 mice. Br J Nut 102:54–9.

Meerts IATM, Verspeek-Rip CM, Buskens CAF, Keizer HG, Bassaganya-Riera J, Jouni ZE, van Huygevoort AH, van Otterdijk FM, van de Waart EJ. 2009. Toxicological evaluation of pomegranate seed oil. Food Chem Toxico 47:1085–92.

Melo ILP, Carvalho EBT, Mancini-Filho J. 2014. Pomegranate seed oil (*Punica granatum L.*): a source of PA (conjugated α-linolenic acid). J Human Nut Food Sci 2:1024–34.

Miranda J, Aguirre L, Fernandez-Quintela A, Macarulla MT, Martinez-Castano MG, Bilbao AJ E, Portillo MP. 2013. Effects of pomegranate seed oil on glucose and lipid metabolism-related organs in rats fed an obesogenic diet. J Agri Food Chem 61:5089–96.

Mirmiran P, Fazeli MR, Asghari G, Shafiee A, Azizi F. 2010. Effect of pomegranate seed oil on hyperlipidaemic subjects: a double-blind placebo-controlled clinical trial. Br J Nut 104:402–6.

Mizrahi M, Friedman-Levi Y, Larush L, Frid K, Binyamin O, Dori D, Fainstein N, Ovadia H, Ben-Hur T, Magdassi S, Gabizon R. 2014. Pomegranate seed oil nanoemulsions for the prevention and treatment of neurodegenerative diseases: the case of genetic CJD. Nanomedicine 10:1353–63.

Modaresi J, Fathi-Nasri MH, Rashidi L, Dayani O, Kebreab E. 2011. Effects of supplementation with pomegranate seed pulp on concentrations of conjugated linoleic acid and punicic acid in goat milk. J Dairy Sci 94:4075–80.

Mohagheghi M, Rezaei K, Labbafi M, Mousavi M. 2011. Pomegranate seed oil as a functional ingredient in beverages. Eur J Lipid Sci Tech 113:730–6.

Mukherjee C, Bhattacharyya DK. 2006. Oxidative stability of some seed oils containing conjugated octadecatrienoic fatty acids isomers. J Lipid Sci Tech 38:175–82.

Mukherjee C, Bhattacharyya S, Ghosh S, Bhattacharyya DK. 2002. Dietary effects of punicic acid on the composition and peroxidation of rat plasma lipid. J Oleosci 51:513–22.

Nekooeian AA, Eftekhari MH, Adibi S, Rajaeifard A. 2014. Effects of pomegranate seed oil on insulin release in rats with type 2 diabetes. Iranian J Med Sci 39:130–5.

Nishimura K, Tsumagari H, Morioka A, Yamauchi Y, Miyashita K, Lu S, Jisaka M, Nagaya T, Yokota K. 2007. Regulation of apoptosis through arachidonate cascade in mammalian cells. Appl Biochem Biotech 102–103:239–50.

Pande G, Akoh CC. 2009. Antioxidant capacity and lipid characterization of six Georgia-grown pomegranate cultivars. J Agri Food Chem 57: 9427–36.

Qu WJ, Pan ZL, Ma HL. 2010. Extraction modeling and activities of antioxidants from pomegranate marc. J Food Eng 99:16–23.

Sadrolhosseini AR, Noor ASM, Husin MS, Sairi NA. 2014. Green synthesis of gold nanoparticles in pomegranate seed oil stabilized using laser ablation. J Inorg Organometallic Polymers Mate 24:1009–13.

Saha SS, Ghosh M. 2009. Comparative study of antioxidant activity of alpha-eleostearic acid and PA against oxidative stress generated by sodium arsenite. Food Chem Toxicol 47:2551–6.

Saha SS, Ghosh M. 2010. Ameliorative role of conjugated linolenic acid isomers against oxidative DNA damage induced by sodium arsenite in rat model. Food Chem Toxicol 48:3398–405.

Saha SS, Ghosh M. 2012. Antioxidant and anti-inflammatory effect of conjugated linolenic acid isomers against streptozotocin-induced diabetes. Br J Nut 108:974–83.

Saha SS, Ghosh M. 2013. Protective effect of conjugated linolenic acid isomers present in vegetable oils against arsenite-induced renal toxicity in rat model. Nutrition 29:903–10.

Saha SS, Patra M, Ghosh M. 2012a. *In vitro* antioxidant study of vegetable oils containing conjugated linolenic acid isomers. LWT Food Sci Tech 46:10–15.

Saha SS, Dasgupta P, Bandyopadhyay SS, Ghosh M. 2012b. Synergistic effect of conjugated linolenic acid isomers against induced oxidative stress, inflammation and erythrocyte membrane disintegrity in rat model. Biochim Biophys Acta 1820:1951–70.

Sargolzaei J, Moghaddam AH. 2013. Predicting the yield of pomegranate oil from supercritical extraction using artificial neural networks and an adaptive-network-based fuzzy inference system. Frontiers of Sci Eng 7:357–65.

Schneider AC, Mignolet E, Schneider YJ, Larondelle Y. 2013. Uptake of conjugated linolenic acids and conversion to cis-9, trans-11-or trans-9, trans-11-conjugated linoleic acids in Caco-2 cells. Br J Nut 109:57–64.

Soetjipto H, Pradipta M, Timotius KH. 2010. Fatty acids composition of red and purple pomegranate (*Punica granatum L*) seed oil. Indonesian J Cancer Chemoprevention 1:74–7.

Spilmont M, Leotoing L, Davicco MJ, Lebecque P, Mercier S, Miot-Noirault E, Pilet P, Rios L, Wittrant Y, Coxam V. 2013. Pomegranate seed oil prevents bone loss in a mice model of osteoporosis, through osteoblastic stimulation, osteoclastic inhibition and decreased inflammatory status. J Nut Biochem 24:1840–8.

Takagi T, Itabashi Y. 1981. Occurrence of mixtures of geometrical isomers of conjugated octadecatrienoic acids in some seed oils: analysis by open-tubular gas liquid chromatography and high performance liquid chromatography. Lipids 16:546–51.

Tanaka T, Hosokawa M, Yasui Y, Ishigamori R, Miyashita K. 2011. Cancer chemopreventive ability of conjugated linolenic acids. Int J Mol Sci 12:7495–509.

Tian Y, Xu Z, Zheng B, Martin LY. 2013. Optimization of ultrasonic-assisted extraction of pomegranate (*Punica granatum L.*) seed oil. Ultrasonics Sonochem 20:202–8.

Tran HN, Bae SY, Song BH, Lee BH, Bae YS, Kim YH, Lansky EP, Newman RA. 2010. Pomegranate (*Punica granatum*) seed linolenic acid isomers: concentration-dependent modulation of estrogen receptor activity. Endocrine Res 35:1–16.

Tsuzuki T, Tokuyama Y, Igarashi M, Nakagawa K, Ohsaki Y, Komai M, Miyazawa T. 2004. Alpha-eleostearic acid (9Z11E13E-18:3) is quickly converted to conjugated linoleic acid (9Z11E-18:2) in rats. J Nut 134:2634–9.

Tsuzuki T, Kawakami Y, Abe R. 2006. Conjugated linolenic acid is slowly absorbed in rat intestine, but quickly converted to conjugated linoleic acid. J Nut 136:2153–9.

Verardo V, Garcia-Salas P, Baldi E, Antonio SC, Alberto FG, Maria FC. 2014. Pomegranate seeds as a source of nutraceutical oil naturally rich in bioactive lipids. Food Res Int, http://dx.doi.org/10.1016/j.foodres, 2014.04.04.

Viladomiu M, Hontecillas R, Lijuan Y, Pinyi L, Bassaganya-Riera J. 2013. Nutritional protective mechanisms against gut inflammation. J Nut Biochem 24:929–39.

Vroegrijk IOCM, Diepen JAV, Berg SVD, Westbroek I, Keizer H, Gambelli L, Hontecillas R, Bassaganya-Riera J, Zondag GC, Romijn JA, Havekes LM, Voshol PJ. 2011. Pomegranate seed oil, a rich source of PA, prevents diet-induced obesity and insulin resistance in mice. Food Chem Toxicol 49:1426–30.

Wang L, Green MM. 2014. Pomegranate and its components as alternative treatment for prostate cancer. Intl J Mol Sci 15(9):14949–66.

Wang L, Li W, Lin M, Garcia M, Mulholland D, Lilly M, Green MM. 2014. Luteolin, ellagic acid and punicic acid are natural products that inhibit prostate cancer metastasis. Carcinogenesis 35(10):2321–30.

Wang W, Wang H, Wang J, Ye S, Xiao S. 2013. Induction of apoptosis by punicic acid in bladder carcinoma T24 cells. J Dalian Polytechnic Univ 32:82–5.

Yamasaki M, Kitagawa T, Koyanagi N, Chujo H, Maeda H, Kohno-Murase J, Imamura J, Tachibana H, Yamada K. 2006. Dietary effect of pomegranate seed oil on immune function and lipid metabolism in mice. Nutrition 22:54–9.

Yong GJ, Seung JK, Christie WW. 1995. The structure of the triacylglycerols, containing punicic acid, in the seed oil of *Trichosanthes kirilowii*. J Am Oil Chem Soc 72:1037–42.

Yuan GF, Hai YS, Sinclair AJ, Li D. 2009a. Effects of conjugated linolenic acid and conjugated linoleic acid on lipid metabolism in mice. Eue J Lipid Sci Tech 111:537–45.

Yuan GF, Wahlqvist ML, Yuan J, Wang Q, Li D. 2009b. Effect of PA naturally occurring in food on lipid peroxidation in healthy young humans. J Sci Food Agri 89:2331–5.

Yuan GF, Sinclair AJ, Zhou CQ, Li D. 2009c. Alpha-eleostearic acid is more effectively metabolized into conjugated linoleic acid than punicic acid in mice. J Sci Food Agri 89:1006–11.

Yuan GF, Yuan JQ, Li D. 2009d. Punicic acid from *Trichosanthes kirilowii* seed oil is rapidly metabolized to conjugated linoleic acid in rats. J Med Food 12:416–22.

Yuan GF, Sinclair AJ, Hai YS, Li D. 2009e. Fatty acid composition in tissues of mice fed diets containing conjugated linolenic acid and conjugated linoleic acid. J Food Lipids 16:148–63.

Yuan GF, Sinclair AJ, Xu C, Li D. 2009f. Incorporation and metabolism of punicic acid in healthy young humans. Mol Nut Food Res 53:1336–42.

Yuan GF, Sun HY, Li D. 2011. Analysis of conjugated linolenic acid from plant seeds. J Zhejiang Univ (Agriculture and Life Sciences) 37:326–31.

Yuan Y, Li MM, Li CB, Chen Q. 2013. Optimization of ultrasound-assisted extraction of pomegranate seed oil using response surface methodology and the composition analysis. Food Sc Tech 38:179–84.

Yuan G, Chen X, Li D. 2015. Modulation of peroxisome proliferator-activated receptor gamma(PPAR γ) by conjugated fatty acid in obesity and inflammatory bowel disease. J Agric Food Chem 25:1883–95.

Web References

http://www.healthhabits.ca/2011/03/30/punicic-acid-prevents-obesity-insulin-resistance/. Accessed on 2014 October 31.

Pomegranate . . . The Fabled Fruit™, www.morretec.com/img/Pomegranate_Brochure.pdf. Accessed on 2014 November 21.

http://extract-herb.com/html_products/Punicic-acids-oleic-acid-Linolenic-acid-Palmitic-acid-Pomegranate-Seed-Oil-157.html. Accessed on 2014 November 21.

http://www.metacyc.org/META/new-image?type=PATHWAY&object=PWY-5374. Accessed on 2014 November 17.