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Antioxidant and Cyclooxygenase Activities of Fatty Acids Found in Food

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Several commercially available C-8 to C-24 saturated and unsaturated fatty acids (**1**−**29**) were assayed for cyclooxygenase-I (COX-I) and cyclooxygenase-II (COX-II) inhibitory and antioxidant activities. Among the saturated fatty acids tested at 60 μg mL^{-1}, there was an increase in antioxidant activity with increasing chain length from octanoic acid to myristic acid (C-8−C-14) and a decrease thereafter. All unsaturated fatty acids tested at 60 μg mL^{-1} showed good antioxidant activity except for undecylenic acid (**12**), *cis*-5-dodecenoic acid (**13**), and nervonic acid (**29**). The highest inhibitory activities among the saturated fatty acids tested on cyclooxygenase enzymes COX-I and COX-II were observed for decanoic acid to lauric acid (**3**−**5**) at 100 μg mL^{-1}. Similarly, among the unsaturated fatty acids tested, the highest activities were observed for *cis*-8,11,14-eicosatrienoic acid (**25**) and *cis*-13,16-docosadienoic acid (**27**) at 100 μg mL^{-1}.

KEYWORDS: Fatty acids; antioxidant; cyclooxygenase; antiinflammatory; saturated; unsaturated

INTRODUCTION

Fatty acids are recognized as essential nutrients in both human and animal diets, and are implicated to possess numerous health benefits (*1, 2*). Their use in the pharmaceutical industry has also been well documented (*3*). Saturated "bad" fatty acids are known to contribute to cardiovascular disease, whereas the unsaturated "good" fatty acids are reported to help cellular function and promote a healthy heart. The essential fatty acids are also implicated to have antiinflammatory effects in the body, and are used in the nutritional treatment of arthritis, asthma, allergies, and skin conditions (*4*−*7*). These essential fatty acids are considered to lower the incidence of immune system disorders such as cancer, multiple sclerosis, and lupus (*8, 9*). Several reports have suggested that dietary supplementation of essential fatty acids improved skin condition, diabetes, depression, menopausal problems, obesity, memory and learning disabilities, eye problems, and digestive disorders in male and female adults (*10*−*12*).

Fatty acids are implicated to control inflammation in vivo and in vitro. Cyclooxygenase enzymes (COX-I and COX-II) catalyze the conversion of arachidonic acid to prostaglandins, the hormone-like substances responsible for inflammation in the body (*13*, *14*). It has been determined that inhibition of cyclooxygenase isoform, COX-I, which reduces the production of prostaglandins in the stomach, causes gastric ulceration and other side effects in the body. The inhibition of COX-II isoform, which is observed in inflamed tissues, can reduce the inflammation with minimal side effects (*13*, *14*). Therefore, complete inhibition of COX-I enzyme is not preferred, and drugs that selectively inhibit the COX-II enzyme are better antiinflammatory products.

Natural antioxidants have the ability to reduce cancer, heart disease, and other degenerative problems associated with aging. Cell damage is caused by free radicals, which are atoms or molecules with one or more unpaired electrons. Oxygen radicals and lipid peroxidation are involved in several pathogenic conditions. Also, many antioxidants play an important role in the food industry, and may help to replace synthetic additives to increase the shelf life of food. Many fatty acids have the ability to act as antioxidants or prooxidants.

The primary sources of essential fatty acids in human diet are from plants. For example, linoleic acid is found primarily in seeds, nuts, grains, and legumes (*15*). Alpha-linolenic acid is found in the green leaves of plants, including phytoplankton and algae, and in selected seeds, nuts, and legumes (flax, canola, walnuts, and soy) (*15*). Because fatty acids are an integral component of many dietary supplements, vegetables, seeds, and nuts, and there is an increased consumer preference for these products, we were prompted to evaluate the antioxidant and antiinflammatory activities of several saturated and unsaturated fatty acids. Therefore, this paper presents results from antioxidant and COX-I and COX-II enzyme inhibitory experiments conducted for a series of commonly available saturated and unsaturated fatty acids in foods. The fatty acids tested were pure and purchased commercially.

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Table 1. Saturated and Unsaturated Fatty Acids Tested for Antioxidant and COX-I and COX-II Enzymes Inhibitory Activities

saturated fatty acids	unsaturated fatty acids
C8 octanoic acid (**1**)	C11:1 undecylenic acid (**12**)
C9 nonanoic acid (**2**)	C12:1 *cis*-5-dodecenoic acid (**13**)
C10 decanoic acid (**3**)	C14:1 myristoleic acid (*cis*-9) (**14**)
C11 undecanoic acid (**4**)	C15:1 *cis*-10-pentadecenoic acid (**15**)
C12 lauric acid (**5**)	C16:1 palmitoleic acid (*cis*-9) (**16**)
C13 tridecanoic acid (**6**)	C17:1 *cis*-10-heptadecenoic acid (**17**)
C14 myristic acid (**7**)	C18:1 oleic acid (*cis*-9) (**18**)
C15 pentadecanoic acid (**8**)	C18:1 *cis*-11-vaccenic acid (**19**)
C16 palmitic acid (**9**)	C18:2 linoleic acid (*cis*-9,12) (**20**)
C17 heptadecanoic acid (**10**)	C18:3 linolenic acid (*cis*-9,12,15) (**21**)
C18 stearic acid (**11**)	C19:1 *cis*-10-noanadecenoic acid (**22**)
	C20:1 *cis*-11-eicosenoic acid (**23**)
	C20:2 *cis*-11,14-eicosadienoic acid (**24**)
	C20:3 *cis*-8,11,14-eicosatrienoic acid (**25**)
	C22:1 *cis*-13-erucic acid (**26**)
	C22:2 *cis*-13,16-docosadienoic acid (**27**)
	C22:3 *cis*-13,16,19-docosatrienoic acid (**28**)
	C24:1 nervonic acid (*cis*-15) (**29**)

EXPERIMENTAL PROCEDURES

Materials. All solvents were ACS reagent grade and were purchased from Aldrich Chemical Co. (Milwaukee, WI). Positive controls used in the antioxidant (*tert*-butylhydroquinone, TBHQ; butylated hydroxyanisole, BHA; butylated hydroxytoluene, BHT; and α-tocopherol) and cyclooxygenase inhibitory (ibuprofen and naproxen) bioassays, as well as fatty acids (**Table 1**) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). The purity of free fatty acids purchased was >99%. Celebrex capsules and vioxx tablets were physician's professional samples provided by Dr. Subhash Gupta, Sparrow Pain Center, Lansing, MI.

Antioxidant Assay. Antioxidant activity of samples was evaluated by analysis of model liposome oxidation using fluorescence spectroscopy. A mixture containing 5 μmol of 1-stearoyl-2-linoleoyl-*sn*-glycerol-3-phosphocholine (Avanti Polar Lipids, Inc., Alabaster, AL) and 5 μmol of the fluorescence probe, 3-[*p*-(6-phenyl)-1,3,5-hexatrienyl]-phenylpropionic acid (Molecular Probes, Inc., Eugene, OR) was dried in vacuo at room temperature. The resulting lipid film was suspended in 1 mL of a buffer solution containing 0.15 M NaCl, 0.1 mM EDTA, and 0.01 M MOPS and subjected to 10 freeze−thaw cycles using a dry ice−EtOH bath. Prior to the assay, all buffer solutions were treated with chelating resin Chelex 100 to remove any trace metal ion impurities. The lipid−buffer suspension was then extruded at least 29 times through a LiposoFast extruder (Avestin, Inc., Ottawa, ON) containing a polycarbonate membrane with a pore size of 100 nm to yield large unilamellar liposomes (LUVs). The total volume of assay mixture was 2 mL, consisting of 100 μL of HEPES buffer (50 mM HEPES and 50 mM Tris), 200 μL of 1 M NaCl, 1.64 mL of nitrogen-sparged Millipore purified water, 20 μL of test sample or DMSO control, and 20 μL of an aliquot of the liposome suspension. The oxidation of the lipid containing the probe was initiated by the addition of 20 μL of a 0.5 mM freshly prepared $FeCl_2$ solution to achieve a final concentration of 50 μM of Fe^{2+}. The DMSO control did not contain either Fe^{2+} or the test compounds. The positive controls BHA, BHT, TBHQ, and α-tocopherol (vitamin E) were tested at a final concentration of 10 μM. The fatty acid samples were tested at 60 ppm. Fluorescence intensities of these liposome solutions were measured at 384 nm and monitored every 3 min up to 21 min using a digital fluorometer (Turner model 450, Barnstead Thermolyne, Dubuque, IA). The decrease of relative fluorescence intensity with time, as indicated by the rate of peroxidation, was recorded (*16*).

Cyclooxygenase Inhibitory Assay. COX-I enzyme inhibitory activity was measured using an enzyme preparation from ram seminal vesicles, purchased from Oxford Biomedical Research, Inc., Oxford, MI. Cyclooxygenase-II (COX-II) activity was recorded using an enzyme preparation of insect cell lysate prepared in our laboratory. Both COX-I and -II enzymes were diluted with Tris buffer (pH 7), and assays were performed at 37 °C by monitoring the initial rate of oxygen uptake using an Instech micro oxygen chamber and electrode (Instech Laboratories, Plymouth Meeting, PA) attached to a YSI model 5300 biological oxygen monitor (Yellow Springs Instrument, Inc., Yellow Springs, OH). Each assay mixture contained 0.6 mL of 0.1 M Tris buffer (pH 7.0), 1 mmol phenol, and 85 μg of hemoglobin. The test compound (100 ppm) or standards in DMSO (10 μL) and 20−30 μL of enzyme (COX-I or II) were injected into the assay chamber, and the mixture was allowed to incubate for two min. Ibuprofen (2.1 ppm), naproxen (2.5 ppm), celebrex, (1.7 ppm), and vioxx, (1.7 ppm) were used as positive controls in this assay. The reaction was initiated by adding arachidonic acid (10 μL of a 1.64 μM solution). Data were recorded using Quicklog for Windows data acquisition (Strawberry Tree Inc. Sunnyvale, CA) (*13, 14*).

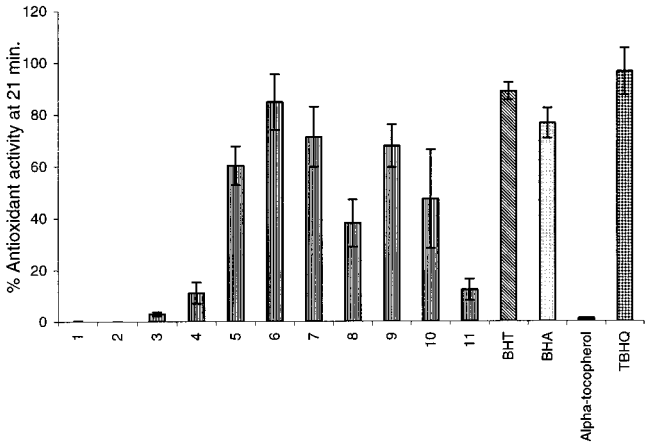

Figure 1. Antioxidant activities of saturated fatty acids at 60 ppm. **1**, octanoic acid; **2**, nonanoic acid; **3**, decanoic acid; **4**, undecanoic acid; **5**, lauric acid; **6**, tridecanoic acid; **7**, myristic acid; **8**, pentadecanoic acid; **9**, palmitic acid; **10**, heptadecanoic acid; **11**, stearic acid. The positive controls BHA, BHT, TBHQ, and α-tocopherol (Vitamin E) were tested at final concentrations of 1.8, 2.2, 1.67, and 4.31 ppm, respectively. Vertical bars represent the standard deviation of each data point ($n = 3$).

RESULTS

Saturated fatty acids of chain lengths C-8 to C-18 (**1**−**11**) and unsaturated fatty acids of chain length C-11 to C-24 (**12**−**29**) were assayed at 60 ppm concentrations in antioxidant assay (**Table 1**). The positive controls used in the experiment were butylated hydroxyanisole (BHA, 1.8 ppm), butylated hydroxytoluene (BHT, 2.2 ppm), *tert*-butylhydroquinone (TBHQ, 1.66 ppm), and α-tocopherol (vitamin E, 4.3 ppm). The saturated fatty acids, octanoic (C-8) to undecanoic acid (C-11) (**1**−**4**) did not exhibit antioxidant activity. However, lauric (**5**), tridecanoic (**6**), and myristic, (**7**) acids displayed 60, 85, and 71% antioxidant activities, respectively. When the chain length was greater than 14, such as in the case of pentadecanoic acid (**8**), the antioxidant activity was diminished significantly. In the case of C16−C18 fatty acids, palmitic (**9**) and heptadecanoic (**10**) acids gave 68 and 48% antioxidant activities, respectively, but stearic acid (**11**) was inactive (**Figure 1**).

With the exceptions of undecylenic (C-11) (**12**), *cis*-5-dodecenoic (C-12) (**13**), and nervonic (C-24) (**29**) acids, all the unsaturated fatty acids tested showed good antioxidant activities (**Figure 2**). The C-18 fatty acids, *cis*-10-heptadecenoic, oleic (*cis*-9), *cis*-11-vaccenic, linoleic (*cis*-9,12), and linolenic acids (*cis*-9,12,15) (**18**−**21**) were further studied at lower concentrations and showed antioxidant activities at 15 ppm concentration compared to BHA, BHT, and TBHQ at 1.8, 2.2, and 1.66 ppm concentrations, respectively.

Both saturated and unsaturated fatty acids were evaluated for COX-I and COX-II inhibitory activities at 100 ppm. The positive

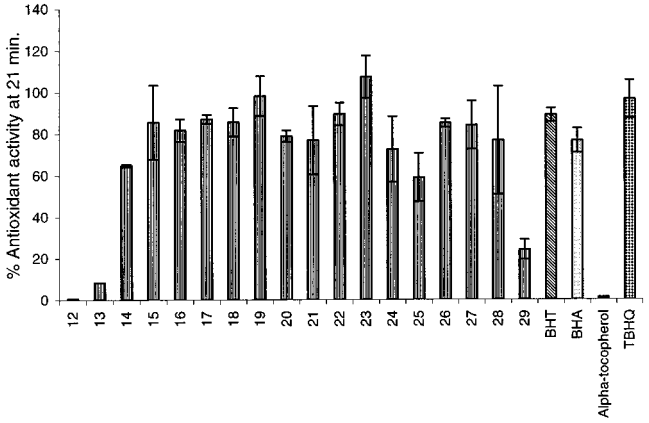

Figure 2. Antioxidant activities of unsaturated fatty acids at 60 ppm. **12**, undecylenic acid; **13**, *cis*-5-dodecenoic acid; **14**, myristoleic acid (*cis*-9); **15**, *cis*-10-pentadecenoic acid; **16**, palmitoleic acid (*cis*-9); **17**, *cis*-10-heptadecenoic acid; **18**, oleic acid (*cis*-9); **19**, *cis*-11-vaccenic acid; **20**, linoleic acid (*cis*-9,12); **21**, linolenic acid (*cis*-9,12,15); **22**, *cis*-10-noanadecenoic acid; **23**, *cis*-11-eicosenoic acid; **24**, *cis*-11,14-eicosadienoic acid; **25**, *cis*-8,11,14-eicosatrienoic acid; **26**, *cis*-13-erucic aci; **27**, *cis*-13,16-docosadienoic acid; **28**, *cis*-13,16,19-docosatrienoic acid; **29**, nervonic acid (*cis*-15). The positive controls BHA, BHT, TBHQ, and α-tocopherol (Vitamin E) were tested at final concentrations of 1.8, 2.2, 1.67, and 4.31 ppm, respectively. Vertical bars represent the standard deviation of each data point ($n = 3$).

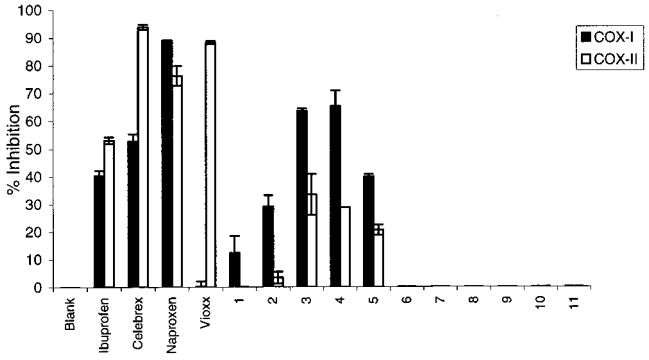

Figure 3. In vitro COX-I and COX-II inhibitory activities of saturated fatty acids (**1**–**11**) (100 ppm). **1**, octanoic acid; **2**, nonanoic acid; **3**, decanoic acid; **4**, undecanoic acid; **5**, lauric acid; **6**, tridecanoic acid; **7**, myristic acid; **8**, pentadecanoic acid; **9**, palmitic acid; **10**, heptadecanoic acid; **11**, stearic acid. Positive controls ibuprofen, naproxen, celebrex, and vioxx were tested at 2.1, 2.5, 1.7, and 1.7 ppm, respectively. Vertical bars represent the standard deviation of each data point ($n = 3$).

controls used in the experiment were vioxx (1.67 ppm), celebrex (1.66 ppm), naproxen (2.5 ppm), and ibuprofen (2.1 ppm). The saturated fatty acids octanoic (**1**), nonanoic (**2**), decanoic (**3**), undecanoic (**4**), and lauric acid (**5**) showed COX-I inhibitory activity at 12, 29, 63, 65, and 40%, respectively. Tridecanoic acid to stearic acids (**6** to **11**) did not give any COX-I enzyme inhibition. In the COX-II assay, decanoic (**3**), undecanoic (**4**), and lauric acid (**5**) gave 33, 29, and 20% COX-II inhibition, whereas the other saturated fatty acids tested as shown in **Table 1** showed little or no activity (**Figure 3**).

Among the studied unsaturated fatty acids containing one double bond, undecylenic acid (**12**) inhibited COX-I and COX-II enzymes at 54 and 38%, respectively. Dodecenoic acid (**13**) showed COX-I and COX-II activities at 35 and 32%, respectively. With the exception of oleic acid (**18**), which showed 25% of COX-I inhibition, the unsaturated fatty acids tested up to 20-carbon chain lengths did not show COX activity. Similarly,

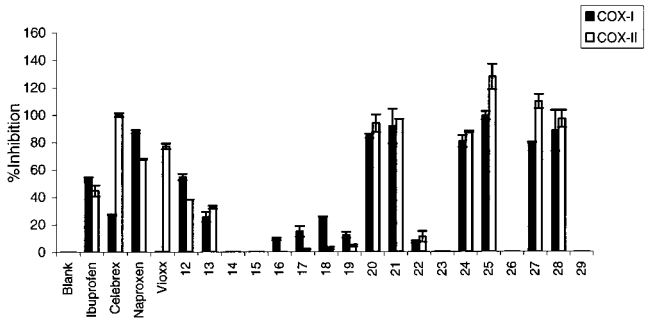

Figure 4. In vitro COX-I and COX-II inhibitory activities of unsaturated fatty acids (**12**–**29**) (100 ppm). **12**, Undecylenic acid; **13**, *cis*-5-dodecenoic acid; **14**, myristoleic acid (*cis*-9); **15**, *cis*-10-pentadecenoic acid; **16**, palmitoleic acid (*cis*-9); **17**, *cis*-10-heptadecenoic acid; **18**, oleic acid (*cis*-9); **19**, *cis*-11-vaccenic acid; **20**, linoleic acid (*cis*-9,12); **21**, linolenic acid (*cis*-9,12,15); **22**, cis-10-noanadecenoic acid; **23**, *cis*-11-eicosenoic acid; **24**, *cis*-11,14-eicosadienoic acid; **25**, *cis*-8,11,14-eicosatrienoic acid; **26**, *cis*-13-erucic acid; **27**, cis-13,16-docosadienoic acid; **28**, *cis*-13,16,19-docosatrienoic acid; **29**, nervonic acid (*cis*-15). Ibuprofen, naproxen, celebrex, and vioxx were tested at 2.1, 2.5, 1.7, and 1.7 ppm, respectively, as positive controls in this assay. Vertical bars represent the standard deviation of each data point ($n = 3$).

fatty acids above chain length of 20 carbons with one double bond did not show COX activity (**Figure 4**). However, fatty acids with two or more double bonds and with chain lengths higher than 20 carbons showed very high COX-I or COX-II inhibitions (**Figure 4**).

DISCUSSION

The saturated fatty acids evaluated in this study exhibited an increase in antioxidant activity with increasing chain length. This was evidenced by the increasing activity from nonanoic acid with the highest activity exhibited by tridecanoic acid (**Figure 1**). Octanoic acid to undecanoic acid (C-8−C-11) were virtually inactive in the antioxidant assay, but a trend toward increasing activity with increasing chain length could nonetheless be observed. Lauric acid was only moderately active, while tridecanoic and myristic acids displayed the highest activities. Thereafter, a decrease in antioxidant activity was observed with increasing chain length, with the notable exception of pentadecanoic acid, which showed a decrease in activity. It was interesting to note that the antioxidant activity of pentadecanoic acid was even lower than that of heptadecanoic acid.

With the exceptions of undecylenic, *cis*-5-dodecenoic, and nervonic acids, all the unsaturated fatty acids tested showed superior antioxidant activities compared to those of the positive controls at test concentrations (**Figure 2**). Within the C-18 series, *cis*-11-vaccenic acid was the most active, with oleic, linoleic, and linolenic acids showing comparable activities (**Figure 2**). The C-18 fatty acids also showed good antioxidant activity at 15 ppm. For the C-20 series, *cis*-11-eicosenoic acid was the most active. *Cis*-11,14-eicosadienoic and *cis*-8,11,14-eicosatrienoic acids also showed similar activities. Among the C-22 series, a decreasing order of activity was observed for *cis*-13,16,19-docosatrienoic, *cis*-13,16-docosadienoic, and *cis*-13-erucic acids, with the activity of *cis*-13-erucic acid being significantly less than that of the other two (**Figure 2**). Unsaturated fatty acids containing a *cis*-11-double bond showed enhanced activity with respect to the other fatty acids tested containing the same number of carbons.

Both saturated and unsaturated fatty acids tested for COX-I and COX-II assays were at 100 ppm concentration. For the

saturated fatty acids, there was an increase in COX-I activity from octanoic acid to undecanoic acid. The activity decreased for lauric acid, and thereafter no activity was observed. A similar trend was observed for the COX-II enzyme activity. Octanoic acid showed no COX-II inhibitory activity. However, there was an increase in activity from nonanoic acid to decanoic acid. Undecanoic acid and lauric acid showed decreased activity with respect to decanoic acid, and thereafter no activity was observed (**Figure 3**).

Among the unsaturated fatty acids containing one double bond, undecylenic acid showed good COX-I activity at 53%. Dodecenoic and oleic acids showed moderate activity, whereas palmitoleic, heptadecenoic, vaccenic, and nonadecenoic acids showed marginal COX-I inhibitory activity. All the other fatty acids tested were inactive in COX-I inhibitory assay. In COX-II enzyme inhibitory assay undecylenic and dodecenoic acids showed 38 and 31% of inhibition, respectively (**Figure 4**).

The highest COX-I and COX-II inhibitory activities were observed for C-10, C-11, and C-12 saturated fatty acids. Fatty acids with two or more double bonds showed the best COX-I and COX-II inhibitory activities (**Figure 4**). For the unsaturated fatty acids, *cis*-8,11,14-eicosatrienoic acid (**25**) showed the highest COX-II activity, followed by *cis*-13,16-docosadienoic acid (**27**) and *cis*-13,16,19-docosatrienoic acid (**28**). In addition, linoleic (**20**) and linolenic (**21**) acids showed appreciable COX-I and COX-II inhibitory activities.

Various saturated and unsaturated fatty acids evaluated for antioxidant and COX inhibitory activities are present in numerous plant-derived food products and showed substantial antioxidant and good COX inhibitory activities compared to positive controls tested at their respective concentrations. Saturated fatty acids such as myristic, palmitic, and lauric acids, which showed high antioxidant activity, are present in palm, babassu, coconut, corn, soybean, cottonseed, and many other food products (*13*). The unsaturated fatty acids known as "good" fatty acids present in castor, sunflower, safflower, parsley, soybean, linseed, perilla seed, and celery seed (*13*) also showed good COX inhibitory activities. Unsaturated fatty acids with two or more double bonds and chain lengths higher than 20 carbons exhibited COX-II enzyme inhibitory activities similar to over the counter nonsteroidal antiinflammatory drugs (NSAIDs) at the concentrations tested. Although the fatty acid chemistry is not complex compared to that of NSAIDs, they showed potent inhibitory activity against inflammation-causing enzymes. Our results, therefore, suggest that consumption of these fatty acids as a dietary supplement or as a food ingredient has the potential to provide health benefits. This may include benefits such as reducing pain related to inflammation and cardiovascular diseases by acting as strong COX inhibitors and antioxidants, respectively.

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