07/02/12

Review:
S-Methyl Cysteine Sulphoxide: the Cinderella Phytochemical.

Edmands WMB, Gooderham NJ, Holmes E, Mitchell SC.

Biomolecular Medicine, Department of Surgery and Cancer, Faculty of Medicine, Imperial College London, South Kensington, London SW7 2AZ, United Kingdom.

Introduction

One noticeable feature that is shared by cabbages, leeks, garlic and onions, amongst others, is their strikingly pungent aroma and distinctive taste, mostly attributable to sulphur-containing chemicals. Indeed, it has been appreciated for many years that these vegetables contain large amounts of sulphurous materials that release volatile compounds, especially during the processes involved in food preparation.[1] [2] In alignment with this, workers have shown that the greatest loss of sulphur from soils was found after harvesting peas and cabbage.[3] Many of these plants, and others belonging to the *Alliceae* (*~Liliaceae;* now sub-family *Alloideae), Brassicaceae (Cruciferae)* and *Fabaceae (Leguminosae)* families, are considered beneficial to human health and sulphur compounds have been advocated as being responsible.[4-7]

One group of chemicals in particular, known collectively as glucosinolates (thioglucosides), have been and still are the prime focus of research in this area. Their diversity of structure, with aliphatic, aromatic or heterocyclic side-chains derived from amino acids, together with their hydrolysis products (isothiocyanates, thiocyanates, nitriles), contribute undoubtedly to the wealth of biological effects attributed to these vegetables.[8, 9] However, another compound, S-methyl-L-cysteine sulphoxide (3-(methylsulphinyl)alanine; methiin; SMCSO), which is found in greater concentrations in *Brassica* vegetables (1-2% dry weight) than all glucosinalates combined (0.1-0.6% dry weight) (Seigler 1998), may have a significant, but largely unrecognised role to play. Although studies examining a mixture of S-alk(en)yl cysteine sulphoxide compounds derived from *Alliceae* species have reported promising results, few investigations have explored SMCSO in isolation.[10]

This review aims to summarise available information regarding SMCSO and hopefully to act as a catalyst to stimulate further scrutiny of this important amino acid derivative. Owing to its ubiquity and abundance in many plant foods it would be, perhaps, surprising if this phytochemical was found to be without biological activity in man.

Occurrence, concentration and distribution in commonly consumed vegetables

S-Methyl-L-cysteine appears to have been first prepared during the 1930's amidst enquiries into the metabolism and interconversion of the sulphur-containing amino acids (cysteine and methionine) and during the exploration of cysteine-deficient diets.[11-14] In these studies, although the exhaustive oxidation of the sulphur moiety to

yield sulphate was measured, the possibility of limited oxidation to yield the stable S-oxide seemingly was ignored. Mention of the synthesis of SMCSO surfaced in the literature in the early 1950's.[15, 16] Proof of its existence in nature was revealed during 1955 in publications that showed that SMCSO could be extracted from cabbage leaves and turnip roots[17-19] and fastidious experimentation demonstrated that this sulphoxide did not arise from S-methylcysteine by 'accidental' oxidation during the isolation procedures.[19]

There was an acknowledged, but now forgotten, precedent to this where a Japanese paper published a year earlier (1954) had reported the chromatographic identification of SMCSO in extracts from garlic (*Allium sativum*) and other *Allium* species. The author cited in his English abstract that, '*the presence of S-methyl- and S-propyl-L-cysteine sulfoxide species can be assumed in Allium sativum L., besides alliin*'.[16] A few years later, in a text concerning the organic chemistry of sulphur, the writer remarks pointedly upon the discovery of SMCSO in plant materials; '*When the results were announced they afforded the first instance of the occurrence of a derivative of S-methylcysteine in nature, nor had the amino acid itself been recognised as a natural product. This had always seemed rather surprising in view of the wide occurrence of cysteine and cystine and the closely related homologue*.[20] This statement appears to ratify the discovery date as the mid 1950's.

Following these widely publicised key discoveries in vegetables of the *Brassicaceae* family, a series of publications followed rapidly identifying the compound within the plant tissues of many members of the *Fabaceae* (*Leguminosae*) and *Alliaceae* (*~Liliaceae*) families.[21-26] However, it appeared to be most highly concentrated within members of the *Brassicaceae*, and especially within those of the genus, *Brassica*. Indeed, the initial isolators of this compound remarked that, '*our experience with non-crucifers indicates the sulfoxide to be a minor constituent of the non-protein fraction if it is present at all*'.[27] As analytical technologies progressed (from paper chromatography) SMCSO was identified in many more species. It was also recognised in combination with glutamic acid as a dipeptide (γ-glutamyl-S-methylcysteine sulphoxide) in these vegetables and also in many types of legumes such as Lima beans (*Phaseolus lunatus*), kidney beans (*Phaseolus vulgaris*) and mung beans (*Phaseolus aureus*). Of particular interest is a relatively high concentration in plants of the genus *Astragalus (<3000 species)* of herbs and shrubs such as *Astragalus propinquus* that is considered one of the 50 fundamental herbs employed in traditional Chinese medicine, also known as *huáng qí (yellow leader)*.[28-32]

Perhaps it is surprising that SMCSO has not been found to be a normal constituent of proteins or in its free form in animals. It is believed generally that this methylated cysteine does not form part of the free amino acid pool in mammals by enzymatic conversion to either cysteine or methionine.

Biosynthesis and potential role within the plant

The pathways involved in the biosynthesis of cysteine and substituted cysteine molecules within plants have been explored by chemical analysis and radiolabel feeding and tracer studies.[5, 6, 28, 29] Inorganic sulphate is the primary source of sulphur in plants and is accumulated in the root cells where it is initially converted to 5-

adenylsulphate (APS; adenosine phosphosulphate) by the enzyme ATP sulphurylase. Two enzymes, APS reductase and sulphide reductase, then sequentially reduce the APS to sulphite and sulphide. Cysteine and acetate are formed by reaction of the sulphide with *O*-acetylserine, catalysed by the enzyme, *O*-acetylserine-thiol-lyase (*O*-acetylserine sulphydrylase or transsulphurylase; cysteine synthase) Interestingly, this latter reaction, forming cysteine from serine and sulphide, also occurs in mammals, but is deemed of little importance.[33, 34] The above sequence is considered to be the main pathway of cysteine biosynthesis in plants (and microbes).[35]

Some workers have proposed that the direct methylation of cysteine, to form S-methyl-L-cysteine followed by sulphur oxygenation, is the route of synthesis of SMCSO.[36] Others have suggested the S-methyl-L-cysteine is produced by reaction of serine with methyl mercaptan, employing the thiomethyl moiety of methionine, and again followed by sulphur oxygenation.[37-41]

When $^{35}SO_4$ was fed to garlic and onions, radiolabelled SMCSO could be isolated from their leaves. In similar plants, the radiolabel from [^{14}C]-serine was rapidly assimilated into [^{14}C]-SMCSO, but when their leaves were exposed to an atmosphere containing hydrogen sulphide (presumed to rapidly form cysteine from serine), the radiolabel was still incorporated, even though [^{14}C]-cysteine (isolated) was evidently produced as an intermediate.[42] The feeding of [^{14}C]-DL-cystine led to appreciable amounts of radiolabel being present as [^{14}C]-SMCSO in crucifers but little if any when the kidney bean was examined. The lack of rapid metabolism of SMCSO in cruciferous vegetables was proffered to help explain these latter findings.[36] The radiolabelled methyl group from [^{14}C]-methionine was transferred to [^{14}C]-SMCSO either via a [^{14}C]-$CH_3\cdot$ moiety to cysteine or via a [^{14}C]-$CH_3S\cdot$ moiety to serine. Employing [^{35}S]-methionine, radiolabel was integrated into [^{35}S]-SMCSO and it was postulated that this was a direct thiomethyl transfer reaction, or even via the production of free methyl mercaptan, both involving serine. However, this latter observation could also be explained by evoking a trans-sulphuration via cystathionine and then utilizing cysteine.[39, 42]

To reconcile these observations, it has been suggested that both routes (via cysteine and via serine) underlie the synthesis of SMCSO, but other homologues such as S-allyl-L-cysteine sulphoxide (alliin) and S-propyl-L-cysteine sulphoxide (dihydroalliin) only arise via the serine pathway, at least in *Allium* species.[42]

Apart from the fact that SMCSO appears universally present, the actual content within different, and even the same, *Brassica* crops displays great variations. Many factors, including species, varietal and cultivar differences, growing, nutriment supplementation and environmental conditions, and times of harvest and subsequent storage have great influence on SMCSO concentrations.[8, 43-47] Only small amounts of SMCSO are found in seeds. However, there is an initiation of its synthesis following germination and during the plant's sexual development and secondary growth. These developmentally influenced increases in SMCSO concentrations support the idea that the compound functions as a phytoalexin at key growth stages. Initially to prevent soil microbial attack and consequent pathogenesis of the hypocotyl and root at germination and later to offset or minimize damage owing to herbivorous attack during secondary growth and flowering. Not surprisingly, the highest concentrations

of SMCSO are usually found in younger leaves when compared to the more mature leaves, such as the outer layers of the common onion (*Allium cepa*).[48]

Following the mechanical disruption of vegetable tissue, cysteine sulphoxide lyases that are normally kept safely within plant vacuoles are released and break down SMCSO into ammonia, pyruvate and methanesulphenic acid.[49-51] Dependent upon prevailing conditions, the highly reactive methanesulphenic acid undergoes immediate chemical disproportionation to form methane thiol and methane sulphinate. Other products formed from this reactive intermediate via dimerization and redox procedures, amongst others, include S-methyl methanethiosulphinate (dimethyl disulphide sulphoxide; MMTSI), S-methyl methanethiosulfonate (dimethyl disulphide sulphone; MMTSO), dimethyl disulphide and methanethiol (Fig. X).[52, 53] Indeed, dimethyl disulphide has been detected in the air above fields of *Brassicas* during the growing and flowering periods.[54, 55] The major end products of this lyase-mediated reaction sequence, MMTSI and MMTSO, are regarded as phytoalexins that, on plant tissue wounding, prevent bacterial or yeast infections from taking hold.[51, 56] Interestingly, during germination the concentration of the cysteine-lyases increases linearly with that of its substrate, SMCSO, suggesting that the enzymatic release of these volatile sulphur compounds almost certainly functions to help establish the plant in the soil at this crucial stage. Some workers have intimated the lyase enzymes within *Brassica* vegetables are not very active *in vivo*, presumably not being required until tissue damage and cellular disruption occurs.[36]

Mammalian Metabolism

A near complete recovery of radioactivity was achieved within 14 days following the oral administration of [^{35}S]-SMCSO (200mg) to male volunteers. Excellent absorption from the gastrointestinal tract was evidenced by primarily urinary excretion (*c*.96% dose) and a consequent low faecal contribution. Over half of the radioactivity (*c*.60% dose) was recovered during the first day with a large proportion (*c*.33% dose) excreted between 3-9 hours post ingestion. This was in contrast to a comparable human study where [^{35}S]-S-methyl-L-cysteine (150mg) was swallowed and a lower 0-24 hour recovery (*c*. 41%dose) was obtained.[57, 58] The difference in clearance rates between these two cysteine analogues was most probably attributable to steric factors in the initial stages of metabolism coupled with potential transporter difficulties.

Although the metabolic fate of [^{35}S]-SMCSO was not determined in this study, it was apparent that a substantial amount of the urinary radioactivity was present in the form of inorganic sulphate, with up to 20% excreted in this form during the first day and a total of 35-40% over the course of the 14 day study.[57, 58] It was also noticed, incidentally, that no sulphoxide reduction to yield S-methyl-L-cysteine had occurred (Waring and Mitchell, unpublished observations).

Studies in man employing three S-methyl-L-cysteine preparations containing radiolabels in different positions ([^{35}S]-; [thiomethyl-^{14}C]-; [backbone-^{14}C]-), enabled a thorough investigation of its metabolic fate to be undertaken .[57, 58]The most striking observation was the extensive degradation of the molecule (*c*.50% dose) to yield inorganic sulphate, urea and carbon dioxide. Earlier workers had also seen an increase in urinary sulphate that continued to be excreted over several days.[11, 13, 14] The

terminal methyl group has also been shown previously to be removed and oxidised to carbon dioxide.[59, 60] However, this terminal methyl group cannot be removed intact, thereby producing cysteine, as S-methyl-L-cysteine has been shown to be unable to replace cysteine in deficient diets.[11, 13] Also, no demethylation was observed when S-methyl-L-cysteine was incubated with kidney slices.[11] It has been suggested that, like the example of methanol, the methyl group, '*is handled by the processes of oxidation and reduction before incorporation*' as opposed to a straight transmethylation reaction.[59] It is probable that the molecule is cleaved at the β-carbon (β-lyase activity) position on the amino acid side of the sulphur and only then further degraded or desulphurated and reassigned.

Other reactions to form metabolites, as opposed to degradation products, included oxygenation of the sulphur moiety and N-acetylation and deamination/transamination of the amino acid chain. These pathways have been demonstrated previously for S-methyl-L-cysteine and other S-alkyl-L-cysteines and are expected and common routes within amino acid catabolism.[61, 62]

From this data, and owing to the similarity in structures, a tentative but probable metabolic scheme for SMCSO may be proposed (Fig Y). A point arising from these limited studies is that the near complete recovery of radioactivity suggests that the plant derived amino acids, SMCSO and S-methyl-L-cysteine, do not appear to enter the mammalian free amino acid pool and are not incorporated into any sulphur-containing biomolecules for any considerable time period. However, one must consider acute versus chronic dosage. The results obtained from this single acute ingestion of SMCSO may not reflect the metabolic fate and disposition of SMCSO ingested in lower concentrations over longer periods of time. Only further studies could resolve this irksome issue.

It is generally thought that SMCSO observed in human biofuids originate entirely from plant dietary sources[63, 64] but it has been shown that methylating agents such as methyl chloride and dimethyl nitrosamine may also engender low level SMCSO excretion via methylation of cysteine within endogenous glutathione, haemoglobin and other cysteine residues followed by subsequent cleavage and sulphur oxygenation.[65-67]

It is apparent that cleaving the β-C-S bond of S-methyl-L-cysteine produces methanethiol that, like most thiols, is a relatively reactive compound, capable of dimerization and thiol-disulphide disruptive activity if the surrounding conditions are favourable. However, breaking this bond in SMCSO liberates methanesulphenic acid, a 'transient species' that is extremely reactive. Unlike the few known stable sulphenic acids, methanesulphenic acid has neither a polar or bulky group adjacent to the sulphenic acid moiety nor does it entertain intramolecular hydrogen bonding, all properties able to bestow stability. The highly nucleophilic/electrophilic nature of methanesulphenic acid encourages intermolecular hydrogen bonding and facilitates its self-condensation, leading to a variety of end products.[68] Hence, it is fortuitous that the SMCSO is available as the substrate for plant cysteine lyase and not the sulphide, as its hydrolysis product is more reactive and able to initiate a stream of adept molecules able to defend the plant against attack.

Microorganism metabolism

It has been reported that many bacteria of the human microbiota exhibit a cysteine β-lyase activity that is able to cleave the C-S bonds of many S-alkyl-cysteine molecules, liberating ammonia, pyruvate and the corresponding thiol, in a similar fashion to the cysteine lyases found in plant materials. Such activity has been shown to be widely distributed throughout various microorganisms, many of which reside within the gastroinestinal tract, including, *Anaerovibrio lipolytica*, *Bacillus subtilis*, *Bacteroides* spp., *Escherichia coli*, *Eubacterium limosum*, *Fusobacterium necrophorum* and *varium*, *Lactobacillus* spp., *Megasphaera elsdenii*, *Pseudomonas cruciviae* and *Veillonella alcalescens*.[69-76] These cysteine β-lyases located within the gut microbiota demonstrated wide substrate specificity when compared to their mammalian counterparts and undoubtedly perform an important role in the metabolism of alkyl-cysteine conjugates in the diet. Assuming the cysteine β-lyases found within the plant matrix are denatured due to high temperature/microwave cooking the gut microbiota offer the first point of contact and are almost certainly responsible for the further metabolism of SMCSO, being the major site of cleavage of dietary SMCSO to its reactive metabolic end-products.

A cysteine conjugate β-lyase activity, purified from rat liver, has been identified to which L-cysteine conjugates of aromatic compounds were good substrates, but those molecules with aliphatic or alicyclic S-substituents were stated as being virtually unaltered. S-Methyl-L-cysteine was shown not to be a substrate.[77] Other investigations have found that although a variety of alkyl-cysteine conjugates had high substrate specificity for another hepatic extract, SMCSO was not cleaved.[78] However, there are reports of partially purified mammalian enzyme ('thionase') activity that was able to liberate methanethiol from S-methyl-L-cysteine, and also the corresponding thiols from other S-alkyl cysteines, but no studies have involved SMCSO.[79] This activity has been taken as being a crude mix of cystathionine-cleaving enzymes. Since that time, it has been demonstrated that cystathionase purified from rat liver was highly substrate specific to various S-alkyl-L-cysteine conjugates and amino acids such as L-cysteine, L-homoserine and L-cystathionine. However, no cleavage of the C-S bond of S-methylcysteine or S-ethylcysteine was observed.[80] On balance, it is probable that these enzymes within mammalian tissue add little to the overall biotransformation of SMCSO derived from the diet, or even ingested as the pure material; the gut microflora, if reached, are presumably quite capable of metabolising the compound.

Janus properties: Ruminant Toxicity and Chemoprotective Activity

Ruminant Toxicity

It was over seventy years ago that the agricultural world was alerted to health problems that may occur in animals consuming a diet of kale,[81, 82] and with ensuing reports from several countries it soon became common knowledge that when ruminant animals were fed mainly or exclusively on kale or on a variety of *Brassica* crops, it was probable that illness would follow. (The term 'kale' was usually taken as a generic name for various edible plants of the genus *Brassica*). In the majority of cases, a severe haemolytic anaemia would develop within 7 to 21 days after feeding

commenced. A fall in blood haemoglobin levels, haemoglobinuria, tachycardia, jaundice, loss of appetite, growth stasis, decreased milk production and a decreased conception rate may follow, as may liver and kidney damage.[83, 84] Cattle that survived the haemolytic crisis showed a gradual recovery in haemoglobin content despite continued kale feeding, although further cycles of haemolysis and partial recovery were observed. This cyclic nature of haemolysis lent some insight into the mechanism of the problem; it has been suggested that increased glutathione levels seen in young red cell populations (replacing damaged erythrocytes) offered a temporary, although eventually futile, ability to resist haemolysis.[85, 86] The observation of dense particles and clumps (Heinz-Ehrlich bodies) within erythrocytes had been known for many years to be indicative of some type of poison or toxic chemical within the circulation. These Heinz-Ehrlich bodies were an easily detectable marker during the examination of a blood smear and consist of dark-staining refractile granules of denatured haemoglobin that are apparently attached to the inner surface of the red cell plasma membrane thereby distorting the shape of the erythrocyte. This deformation makes them more susceptible to rupture and signals their impending destruction by the spleen and other segments of the reticuloendothelial system.[87]

Armed with this knowledge, the search for the agent(s) responsible for this kale-related haemocytolysis was underway. Mineral and vitamin deficiencies were considered initially as being responsible for the anaemia but experimentation could not confirm this hypothesis.[88, 89] However, although not the primary causation, a generalised poor nutritional status should not be overlooked as a contributing factor to such problems, especially when the majority of cases surfaced during the winter months when feeding may have been almost exclusively restricted to stored kale and related crops. Nitrates, hydroxylamines and various glucosinolates and their hydrolysis products, were also excluded from the list of culpable chemicals.[83, 90, 91]

In 1973, SMCSO was implicated. Studies with goats showed that irrespective of the source of the sulphoxide, whether it was derived from kale or dosed as the pure synthetic compound, similar daily intakes gave comparable haemolytic responses.[75] Another study cited that ingestion of feed mixed with SMCSO (2-4% w/w) led to anaemia in rats, the condition being reversible after about 14 days.[91, 92] However, the compound has been administered to man without obvious untoward effect[58] but this was at a much lower dose level (*c*. 2.3mg/kg body weight), probably a thousand times less than that consumed by the rats (*c*. 2-4g/kg body weight, estimated). Interestingly, MMTSO has been fed to rats (1g/kg body weight) for 7 days with no signs of toxicity.[93]

Although normally quoted as between 1-2% (w/w) dry weight[25, 94-97] some *Brassica* crops may contain up to 5% (w/w) dry matter as SMCSO,[84, 85, 91] and it has been suggested that levels as low as 0.35% (w/w) dry weight may lead to production of Heinz-Ehrlich bodies.[98] As a rough guide, daily intakes of 15 to 20g SMCSO per 100kg live weight are sufficient to elicit a haemolytic response. Although difficult to generalise, a bull may weigh in the region of 1000kg or more meaning that this toxic level corresponds to a daily sulphoxide intake of 150 to 200g. Lower intakes (10-15g/100kg) may give rise to mild disturbances.[85]

However, when SMCSO is incubated with erythrocytes no haemolysis is observed. It appears that species differences play a part in kale-associated anaemia and that the

phenomenon is restricted mainly to ruminants. Cattle, goats and to a lesser extent sheep, together with rats and fowl, have been found to be susceptible whereas guinea pigs, hamsters, mice and rabbits did not become anaemic when fed fresh or dried kale.[91, 92, 99-103] Amongst several possibilities lies the suggestion that the toxic haemolysin is produced via fermentation in contact with the rumen microorganisms. This notion was further supported by the observation that SMCSO was inactive in germ-free (gnotobiotic) lambs.[91]

Hydrolytic activity has been associated with gastrointestinal microbes since heat-sterilized rapeseed meal was not toxic when given to germ-free animals.[104-106] Incubation of SMCSO with fresh rumen contents afforded, amongst other products, large amounts of dimethyl disulphide and when this compound was administered to a young goat it gave a haemolytic response characteristic of kale or SMCSO poisoning.[75, 85] Additionally, the ingestion of dimethyl disulphide by chickens for 12 days produced Heinz bodies in their erythrocytes followed by a generalised leg weakness, feather ruffling and lethargy.[107]

Other studies in cattle showed that blood levels of dimethyl disulphide increased as the SMCSO intake from *Brassica* crops progressed. These levels were maximal at the haemolytic crisis (20-50μM; 1.8-4.7mg/l) but declined with the emergence of young erythrocytes, to increase again as the new red blood cells matured.[84, 108] In contrast, plasma SMCSO levels did not rise with increasing sulphoxide intake in sheep fed fresh kale plus sulphoxide supplement, indicating negligible absorption of the unchanged compound (or its lack of availability owing to degradation) from the ruminant digestive system.[95]

It appears that the particular microorganisms within the ruminant gastrointestinal tract undertake effectively two reactions; firstly an initial lyase procedure to produce MMTSI followed by a removal of oxygen (reduction) to dimethyl disulphide, the active haemolytic agent.[90, 109] In the reducing environment of the bowel the disulphide may also be cleaved to the free sulphydryl structure. Both of these compounds, in equilibrium, appear to be rapidly absorbed and participate insidiously in thiol-disulphide exchange reactions, disrupting thiol-containing enzymes such as gluathione reductase within erythrocytes thereby depleting protective glutathione levels. The reduction of antioxidant potential and initiation of free radical activity precipitates oxidative stress, altering membrane permeability, causing intracellular disruption and leading to haemolytic sequelae.[84, 110]

In summary, SMCSO is effectively innocuous, but becomes toxic when given in relatively large doses and also finds itself in an environment where the resident microorganisms are able to chemically degrade it to release a reactive sulphydryl grouping. Also, and in part owing to specific ruminant microflora and differences in erythrocyte fragility, certain animal species are more susceptible to its effects than others. We thus have an almost perfect example of a compound that illustrates the three principles that usually pervade a toxic response; dose matters, things change, people (species) differ.[111]

Fortunately, for cattle and the agricultural industry, the incidence of kale-associated anaemia has dropped dramatically in the past few decades. This is due mainly to the creation of new cultivars that have drastically reduced the concentrations of SMCSO

present in *Brassica* species employed for cattle fodder thereby effectively abolishing potential problems.[43, 112] However, the cysteine sulphoxide lyase activity responsible for the production of dimethyl disulphide (and hence methanethiol) from ingested SMCSO has been shown to be present in several microorganisms that inhabit the human gastrointestinal tract[113-115] and hence could equally well liberate potentially toxic dimethyl disulphide within the human bowel. A single meal (150g) of Brussel's sprouts can provide 0.25g SMCSO[116] and broccoli and cauliflower florets up to 0.36g.[27] For a 70kg human this is a dose rate of about 0.5g/100kg body weight. Should we be considering low-grade anaemia as a consequence of *Brassica* ingestion? Do certain individuals within the population possess a gut microbiota that would make them more susceptible to these problems? Should we be restricting, not encouraging, *Brassica* intake?

One must keep this in perspective; humans tend to cook their food. Boiling tends to reduce the overall sulphur content of *Brassicas*.[117] Acid catalysed thermal hydrolysis of SMCSO to MMTSO and dimethyl disulphide has been shown to occur in the laboratory.[118] Degradation of SMCSO within plant material would undoubtedly occur during food processing. Cutting and chopping during the initial preparation liberates cysteine sulfoxide lyase from *Brassica* tissue (similar to alliinase purified from garlic; Stoll and Seebeck 1949)[119] with the consequent release of volatile materials.[49, 51, 53] It has been reported that the concentration of SMCSO was higher in the juice obtained from heated cabbage than from unheated vegetable and it was proposed that the heating process may have facilitated the release of SMCSO by cellular disruption, assisted in its liberation from a bound form or thermally degraded a precursor compound.[49, 51, 53] In general agreement with this, measurement of SMCSO levels in plant tissue both before and after cooking showed that virtually all the material had been removed, with considerable amounts being extracted into the cooking water (Waring and Mitchell, unpublished results).

Chemoprotective Activity

Anti-carcinogenic effects

As the majority of research, albeit a small amount, has concentrated upon the anti-carcinogenic activity of the glucosinolates and their hydrolysis products, the possible contribution of SMCSO to the beneficial effects of *Brassica* vegetables virtually has been overlooked. However, the anti-mutagenic activity of SMCSO in isolation has been demonstrated utilising the mouse bone marrow micronucleus assay where mice (ICR) were treated with the tobacco carcinogen, benzo[*a*]pyrene, and concurrent administration of SMCSO and its metabolite, MMTSI. Observation of resulting micronucleated polychromatic erythrocytes showed that low levels of these materials (0.05mmol/kg body weight) produced around 33% reduction in micronucleus formation.[120] This suggests that these two plant derived compounds also may contribute to the observed anticarcinogenic activity of *Brassica* plant juices in addition to the hydrolysis products of glucosinolates.

A mixture of thiosulphinates, mainly consisting of MMTSI and S-methyl-2-propene-1-thiosulphinate, isolated from the garlic chive (Chinese chive; *Allium tuberosum*) have shown promise in inhibiting the *in vitro* proliferation of human prostate and

colon cancer cell lines. They also have shown success in increasing the life expectancy of mice inoculated with a fibrosarcoma cell line. The detailed mechanism of action is uncertain but apoptosis is induced by both caspase-dependent and caspase-independent pathways.[121-124] The nucleophilic centre of the thiosulphinate also may have the ability to scavenge electrophilic carcinogenic intermediates and inhibit genotoxic initiation.[120]

Others have shown that MMTSO is an effective antimutagen *in vivo*, decreasing the incidence of mutant wing spots induced by mitomycin C in the somatic mutation and recombination test (SMART) of *Drosophila melanogaster* and also the number of micronucleated peripheral reticulocytes found after mitomycin C treatment in mice.[125]

The sulphone-containing molecule, MMTSO, has also been shown to have a restraining effect on the mutagen aflatoxin B_1, an indirectly acting carcinogen that induces clastogenic and aneugenic changes in mammalian cells. Aflatoxin B_1 chromosome aberrations were observed readily in treated rat bone marrow cells but their formation was found to be potently suppressed by MMTSO (given in the range of 1-20mg/kg body weight) particularly if injected in a window of two hours before and two hours after treatment with aflatoxin B_1. It is thought that MMTSO exhibits this anti-genotoxic effect by modulation of enzymatic sulfhydryl groups. This proposed mechanism of action was identified by a similar effect on aflatoxin B_1 induced chromosome aberrations by diphenyl disulphide that is known to modify sulphydryl groups in proteins. Juices of both cabbages and onion were also shown to produce the same effect further strengthening the idea that SMCSO is a chemoprotective compound in the human diet.[126]

In addition, MMTSO was also shown to modify the effect of azoxymethane on rat colon carcinogenesis upon dietary exposure. Over the course of five weeks, rats were given azoxymethane (15mg/kg body weight) and concurrently fed with MMTSO (5mg/kg body weight). When the rat colon was examined, MMTSO was shown to have reduced the occurrence of aberrant crypt foci, which are regarded as precursor lesions of colorectal cancer. Also several biomarkers of cell proliferation; colonic mucosal ornithine decarboxylase activity, silver stained nucleolar organiser regions per nucleus in colonic epithelium and polyamine levels in blood; were significantly reduced.[93] The incidence of intestinal neoplasms in a longer term study with azoxymethane was also shown to decrease in a dose dependent manner with MMTSO administration and MMTSO appeared not only to be able to prevent the development of aberrant crypt foci, but could also regress these lesions.[127] Further to this, a year-long study examining the suppression of intestinal neoplasms demonstrated a synergistic effect of MMTSO when administered with sulindac, a non-steroidal anti-inflammatory drug.[128]

Anti-diabetic and cardiovascular effects

Rats maintained on a special hypercholesterolaemic diet, which produced high cholesterol levels in both the blood plasma and liver tissue, showed a marked depression of these cholesterol levels, particularly LDL and VLDL, following the addition of SMCSO to their diet. The sulphide amino acid, S-methyl-L-cysteine, had a smaller but measurable effect whereas S-methyl-L-cysteine sulphone and cysteine

were without activity.[129-131] Lipid profiles in serum and tissues showed a reduced concentration of total cholesterol and triglycerides but little effect on phospholipids.[129] Total lipoprotein lipase activity in the adipose tissues was decreased with a subsequent decrease in the free fatty acid levels in serum and tissues. Increased excretion of bile acids and sterols was also observed following the SMCSO dosing regimen.[132] In alloxan-induced diabetic rats, SMCSO demonstrated both antihyperlipidemic and antidiabetic properties, significantly controlling blood lipid and glucose levels, with the authors mentioning that these effects were comparable to those observed with insulin.[133-135]

The results of these studies suggest that SMCSO causes reduction of endogenous lipogenesis, increased lipid catabolism and excretion of bile acids. This is thought to occur by interaction of the cysteine moiety of SMCSO inhibiting lipogenic enzymes within the liver as well as increasing activity of cholesterol 7α-hydroxylase, the rate-limiting enzyme of bile acid biosynthesis.[132, 133] In agreement with this, the oral administration of SMCSO was found to mimic the effects of cabbage extract in suppressing hypercholesterolemia by upregulating cholesterol catabolism, namely cholesterol 7α-hydroxylase, in hepatoma-bearing rats.[136] Also, the reduction of SMCSO contributes to oxidation of the lipogenic coenzyme NADPH that may be contributing to the hypolipidaemia observed.[137]

In biochemical analysis, MMTSO is well known as a reagent in enzyme activation and protein function studies as it reversibly blocks cysteines and other sulfhydryl groups in thiol containing molecules. MMTSO modifies thiol groups to dithiomethane (-S-S-CH_3), proximal or adjacent thiol groups within the proteins have the ability to reduce this dithiomethane group.[138, 139] This modification of thiol groups of cysteines moieties of enzymes, proteins and reduced glutathione serves as potential mechanism of the *in vivo* and *in vitro* biological effects of MMTSO but requires further investigation.

It should also be appreciated that MMTSI contains the thiosulphinate functional group *R*-S(O)-S-*R,* a commonality with the alliciin compound found in garlic which is thoroughly researched and implicated in many of the health benefits of *Alliums* found in animal studies. Alliciin is thought to be responsible for reducing atherosclerosis and fat deposition, normalising the lipoprotein balance, decreasing blood pressure, anti-thrombotic effects, anti-inflammatory activities and functions as an antioxidant.[10] It is expected that SMCSO and its products, MMTSI and MMTSO, would share many of these biological effects and this is reflected by the few studies that are, at present, available in the literature.

End Note

With the increasing number of observations indicating that SMCSO and its metabolic products exhibit biological properties that could be exploited, it is disturbing that more research has not been undertaken in this area. There exists a general agreement that vegetables containing SMCSO, consumed in moderation, are beneficial to health, but the presence of glucosinolates has dominated the scene. Any potential contribution arising from SMCSO appears to have been overlooked or disregarded as unimportant. However, this may change. Preparations from vegetables (including

broccoli and cabbage) containing SMCSO (15-400mg) have been patented already as drugs and health foods and drinks for lowering serum cholesterol levels, although this aspect still remains to be developed.[140] The prophylactic use of non-nutritive amino acids, particularly one as abundant in the human diet as SMCSO, certainly should be investigated.

Epidemiological studies involving sometimes vast cohorts and, unfortunately, usually within poorly controlled situations, are able to provide pointers to environmental and life-style factors that are deleterious of beneficial to health. In such studies that may relate to SMCSO, it is essential to confirm objectively that *Brassica* material has been eaten as part of the diet. Recent detailed work employing high-throughput ^{1}NMR coupled with multivariate chemometrics, has shown that SMCSO, and three other distinct but as yet unidentified methyl-sulphoxide proton features, provided a metabolic finger-print in human urine acting as a biomarker of previous *Brassica* vegetable consumption. Refinement of this technique to enable quantitative measurements, followed by specific targeted research, may help to uncover hitherto unappreciated beneficial aspects of SMCSO intake. Hopefully, this relatively simple derivative of cysteine, with its oxygenated sulphur moiety, will prove to be useful as adjunctive therapy in a wide range of harmful human conditions.

****

References

1. E. Salkowski, Hoppe-Seyler's, Z. Physiol. Chem., Editon edn., vol. 92, pp. 89-103.
2. M. Shankaranarayana, B. Raghavan, K. Abraham and C. Natarajan, Elsevier Scientific Publishing Company, Oxford, Food Flavours, Editon edn., 1982, pp. 169-281.
3. A. RK., Agrokhimiya, Editon edn., 1964, vol. 10, pp. 3-16.
4. A. Crozier, M. N. Clifford and H. Ashihara, *Plant secondary metabolites: occurrence, structure and role in the human diet*, Blackwell Pub., 2006.
5. R. Mithen, in *Plant Secondary Metabolites*, Blackwell Publishing Ltd, Editon edn., 2006, pp. 25-46.
6. D. Selmar, in *Annual Plant Reviews Volume 40: Biochemistry of Plant Secondary Metabolism*, Wiley-Blackwell, Editon edn., 2010, pp. 92-181.
7. H. Tapiero, *Biomedicine & pharmacotherapy*, 2004, **58**, 183.
8. M. Björkman, I. Klingen, A. N. E. Birch, A. M. Bones, T. J. A. Bruce, T. J. Johansen, R. Meadow, J. Mølmann, R. Seljåsen, L. E. Smart and D. Stewart, *Phytochemistry*, 2011, **72**, 538-556.
9. I. Johnson, *International journal for vitamin and nutrition research*, 2002, **72**, 26-31.
10. P. Rose, M. Whiteman, P. K. Moore and Y. Z. Zhu, *ChemInform*, 2005, **36**.
11. E. Brand, R. J. Block and G. F. Cahill, *Journal of Biological Chemistry*, 1937, **119**, 689-696.
12. R. W. Jackson, *Journal of biological chemistry*, 1932, **98**, 465.
13. V. du Vigneaud, *Journal of biological chemistry*, 1934, **105**, 481.

14. N. W. Pirie, *Biochemical journal*, 1932, **26**, 2041.
15. H. R. Bentley, E. E. McDermott and J. K. Whitehead, *Proceedings of the Royal Society of London. Series B, Biological Sciences*, 1951, **138**, 265-272.
16. S. Yurugi, *薬学雑誌*, 1954, **74**, 511.
17. C. Morris and J. Thompson, Chemistry and Industry, Editon edn., 1955, p. 951.
18. R. L. Synge and J. C. Wood, *Biochemical journal*, 1955, **60**, xv-xvi.
19. R. L. Synge and J. C. Wood, *Biochem J*, 1956, **64**, 252-259.
20. E. Kühle, *Angewandte Chemie*, 1961, **73**, 744-744.
21. B. J. PS, Food Sci Tech Abstr, Editon edn., 1973, vol. 6, p. 1468.
22. J. F. Carson, *Journal of organic chemistry*, 1961, **26**, 4997.
23. R. Hegnauer, *Chemotaxonomie der Pflanzen: Leguminosae: Teil 3, Papilionoideae*, 2001.
24. A. Virtanen and E. Matikkala, Acta. Chem. Scand., Editon edn., 1959, vol. 13, pp. 1898-1900.
25. P. J. Whittle, R. H. Smith and A. McIntosh, *Journal of the Science of Food and Agriculture*, 1976, **27**, 633-642.
26. M. Yoshimura, Vitamins (Kyoto), Editon edn., 1958, vol. 14, pp. 633-640.
27. C. J. Morris and J. F. Thompson, *Journal of the American Chemical Society*, 1956, **78**, 1605-1608.
28. D. M. Chen, S. N. Nigam and W. B. McConnell, *Canadian Journal of Biochemistry*, 1970, **48**, 1278-1283.
29. C. M. Chow, S. N. Nigam and W. B. McConnell, *Biochimica et Biophysica Acta (BBA) - General Subjects*, 1972, **273**, 91-96.
30. P. M. Dunnill and L. Fowden, *Phytochemistry*, 1967, **6**, 1659-1663.
31. M. d. L. R. Giada, M. T. M. Miranda and U. M. L. Marquez, *Food Chemistry*, 1998, **61**, 177-184.
32. S. N. Nigam, J.-I. Tu and W. B. McConnell, *Phytochemistry*, 1969, **8**, 1161-1165.
33. G. DM, Academic Press, New York, Metabolism of Sulfur Compounds, Editon edn., 1975, pp. 505-528.
34. R. J. Huxtable, *Biochemistry of sulfur*, 1986.
35. J. F. Thompson, *Annual review of plant physiology*, 1967, **18**, 59.
36. J. F. Thompson, *Plant physiology*, 1966, **41**, 1301.
37. W. N. Arnold and J. F. Thompson, *Biochimica et biophysica acta*, 1962, **57**, 604-606.
38. J. Giovanelli, *Biochemical and biophysical research communications*, 1968, **31**, 275.
39. M. Sugii, S. Nagasawa and T. Suzuki, *Chemical & Pharmaceutical Bulletin*, 1963, **11**, 135-136.
40. J. F. Thompson and D. P. Moore, *Biochemical and biophysical research communications*, 1968, **31**, 281-286.
41. E. C. Wolff, *Journal of the American Chemical Society*, 1956, **78**, 5958.
42. B. Granroth, Hki : Suomalainen tiedeakatemia, Editon edn., 1970.
43. J. D. Phillipson, *Flavour and Fragrance Journal*, 1986, **1**, 87-87.
44. D. W. Griffiths and W. H. M. Smith, *Journal of the Science of Food and Agriculture*, 1989, **47**, 249-252.
45. D. W. Griffiths, W. H. Macfarlane-Smith and B. Boag, *Journal of the Science of Food and Agriculture*, 1994, **64**, 283-288.
46. D. Kopsell, W. Randle and M. Eitman, J. Amer. Soc. Hort. Sci., Editon edn., 1999, vol. 124, pp. 177-183.

47. J. E. Lancaster, B. J. McCallion and M. L. Shaw, *Journal of the Science of Food and Agriculture*, 1984, **35**, 415-420.
48. R. Kubec, *Journal of agricultural and food chemistry*, 2000, **48**, 428.
49. M. Mazelis, *Phytochemistry*, 1963, **2**, 15.
50. A. Hamamoto, *Plant physiology*, 1986, **80**, 702.
51. K. H. Kyung and H. P. Fleming, *J Food Prot*, 1997, **60**, 67-71.
52. H.-W. Chin and R. C. Lindsay, *Journal of Agricultural and Food Chemistry*, 1994, **42**, 1529-1536.
53. H. S. Marks, *Journal of agricultural and food chemistry*, 1992, **40**, 2098.
54. R. D. Butcher, *Clinical and experimental allergy*, 1994, **24**, 1105.
55. W. H. Macfarlane Smith and M. McEwan, *Clinical and experimental allergy*, 1998, **28**, 332-338.
56. A. Z. Tulio, Jr., H. Yamanaka, Y. Ueda and Y. Imahori, *Journal of Agricultural and Food Chemistry*, 2002, **50**, 1502-1507.
57. S. C. Mitchell, R. L. Smith, R. H. Waring and G. F. Aldington, *Xenobiotica*, 1984, **14**, 767-779.
58. R. H. Waring, R. M. Harris, G. B. Steventon and S. C. Mitchell, *Drug Metabol Drug Interact*, 2003, **19**, 241-255.
59. W. H. Horner, *Journal of biological chemistry*, 1959, **234**, 2935.
60. K. Edward J, *Archives of Biochemistry and Biophysics*, 1965, **112**, 605-609.
61. E. A. Barnsley, *Biochimica et biophysica acta. G, General subjects*, 1964, **90**, 24.
62. N. M. Sklan, *Biochemical journal*, 1968, **107**, 217.
63. W. M. Edmands, O. Beckonert, C. Stella, A. Campbell, B. Lake, J. C. Lindon, E. Holmes and N. J. Gooderham, *J Proteome Res*, 2011, 110719101604034-110719101604034.
64. F. Tominaga, *Journal of Biochemistry*, 1965, **57**, 717.
65. E. Bailey, *Cancer research*, 1981, **41**, 2514.
66. M. Törnqvist, *Mutation research. Genetic toxicology testing*, 1988, **204**, 521.
67. R. Doorn, *International archives of occupational and environmental health*, 1980, **46**, 99.
68. B. GC, John Wiley & Sons, Chichester, The Chemistry of Sulphenic Acids and their Derivatives, Editon edn., 1960, pp. 1-22.
69. T. N. Barry, *Journal of agricultural science*, 1984, **102**, 479.
70. T. N. Barry, *Journal of agricultural science*, 1984, **102**, 635.
71. G. L. Larsen, *Xenobiotica*, 1985, **15**, 199-209.
72. G. L. Larsen and J. L. Stevens, *Molecular Pharmacology*, 1986, **29**, 97-103.
73. F. Murakami, Vitamins (Kyoto), Editon edn., 1960, vol. 20, pp. 126-131.
74. J. Nomura, *Journal of biological chemistry*, 1963, **238**, 1441.
75. R. Smith, Ann. Rep. Rowett Res. Inst., Editon edn., 1974, vol. 30, pp. 112-131.
76. H. Tomisawa, *Journal of biological chemistry*, 1984, **259**, 2588.
77. M. Tateishi and H. Shimizu, Academic Press, New York, Enzymatic Basis of Detoxication, Editon edn., 1980, vol. 2, pp. 121-130.
78. H. Tomisawa, *Biochemical journal*, 1986, **235**, 569.
79. F. Binkley, *Journal of Biological Chemistry*, 1950, **186**, 287-296.
80. H. Tomisawa, N. Ichimoto, S. Ichihara, H. Fukazawa and M. Tateishi, *Xenobiotica*, 1988, **18**, 1029-1037.
81. G. Rosenberger, Deutsche-österreichische tierärztliche Wochenschrift, Deut. Tierärztl. Wochenschr., Editon edn., 1939, vol. 49, pp. 244-246.

82. G. Rosenberger, Deut. Tierärztl. Wochenschr., Editon edn., 1943, vol. 51, pp. 63-67.
83. G. JFD, Occ. Publ. Rowett Res. Inst., The Future of Brassica Fodder Crops, Editon edn., 1971.
84. F. S. Steven, *The FEBS journal*, 1981, **119**, 75.
85. R. Smith, *Veterinary Research Communications*, 1978, **2**, 47-61.
86. E. M. Tucker, *British journal of haematology*, 1981, **48**, 403.
87. H. S. Jacob, *The Journal of clinical investigation*, 1968, **47**, 2664.
88. H. Steger, *Archives of animal nutrition*, 1964, **14**, 113.
89. B. Piatkowski, *Archiv für Tierzucht*, 1965, **8**, 167.
90. J. R. Carlson, *Journal of Animal Science*, 1984, **58**, 1040.
91. R. H. Smith, *Veterinary record*, 1980, **107**, 12-15.
92. G. S. Stoewsand, *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association*, 1995, **33**, 537-543.
93. T. Kawamori, T. Tanaka, M. Ohnishi, Y. Hirose, Y. Nakamura, K. Satoh, A. Hara and H. Mori, *Cancer Res*, 1995, **55**, 4053-4058.
94. B. TN, Proc. Agron. Soc. N.Z. , Editon edn., 1978, vol. 8, pp. 143-148.
95. T. N. Barry, T. R. Manley and K. R. Millar, *The Journal of Agricultural Science*, 1982, **99**, 1-12.
96. T. Mae, *Plant & Cell Physiology*, 1971, **12**, 1.
97. G. A. Maw, *Biochemistry of S-Methyl-L-Cysteine and Its Principal Derivatives*, Taylor and Francis, 1982.
98. S. L. Fales, *Journal of dairy science*, 1987, **70**, 2402.
99. C. A. Grant, P. Holtenius, G. Jnsson, J. Luthman and C. B. Thorell, *Acta Veterinaria Scandinavica*, 1968, **9**, 141-150.
100. J. F. Greenhalgh, G. A. Sharman and J. N. Aitken, *Research in Veterinary Science*, 1969, **10**, 64-72.
101. J. F. D. Greenhalgh, *The Proceedings of the Nutrition Society*, 1969, **28**, 178.
102. J. F. Greenhalgh, G. A. Sharman and J. N. Aitken, *Research in Veterinary Science*, 1970, **11**, 232-238.
103. R. Penny, J. David and A. Wright, Vet. Rec., Editon edn., 1964, vol. 76, pp. 1053-1059.
104. K. Dawson, M. Rasmussen and M. Allison, Chapman and Hall, London, The Rumen Microbial Ecosystem, Editon edn., 1997, pp. 633-660.
105. L. Nugon Baudon, *Xenobiotica*, 1990, **20**, 223.
106. S. Rabot, *British journal of nutrition*, 1993, **70**, 323.
107. M. H. Maxwell, *Research in Veterinary Science*, 1981, **30**, 233-238.
108. C. R. A. Earl, *Journal of the Science of Food and Agriculture*, 1983, **34**, 23.
109. S. R. Case GL, CRC, Boca Raton, Fl., USA,, Toxicants of plant origin vol III. (Proteins and amino acids). Editon edn., 1989, vol. vol III, pp. pp 203-228.
110. D. B. McPhail, *Free radical research*, 1992, **16**, 277.
111. S. Mitchell, P. Carmichael and R. Waring, Biologist, Editon edn., 2004, vol. 51, pp. 212-215.
112. S. Prache, *Veterinary research*, 1994, **25**, 497-520.
113. M. J. Hill, *Gut*, 1975, **16**, 318.
114. G. S. Stoewsand, *Food and Chemical Toxicology*, 1995, **33**, 537-543.
115. M. A. Shamat, *International journal of pharmaceutics*, 1993, **97**, 1.

116. F. S. Steven, M. M. Griffin and R. H. Smith, *European Journal of Biochemistry*, 1981, **119**, 75-78.
117. M. Masters, *Biochemical journal*, 1939, **33**, 1304.
118. F. Ostermayer, *Journal of the American Chemical Society*, 1960, **82**, 3752.
119. A. Stoll, *Helvetica chimica acta*, 1949, **32**, 197.
120. H. S. Marks, J. A. Anderson and G. S. Stoewsand, *Food Chem Toxicol*, 1993, **31**, 491-495.
121. S. Y. Kim, *International journal of oncology*, 2008, **32**, 869.
122. S. Y. Kim, *Bioorganic and medicinal chemistry letters*, 2008, **18**, 199.
123. J. H. Lee, *Toxicology Letters*, 2009, **188**, 142.
124. K. W. Park, *Journal of agricultural and food chemistry*, 2007, **55**, 7957.
125. Y. K. Nakamura, T. Matsuo, K. Shimoi, Y. Nakamura and I. Tomita, *Biosci Biotechnol Biochem*, 1996, **60**, 1439-1443.
126. Y. Ito, Y. Nakamura and Y. Nakamura, *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 1997, **393**, 307-316.
127. Y. Morishita, *Cancer science*, 1997, **88**, 815.
128. B. S. Reddy, T. Kawamori, R. Lubet, V. Steele, G. Kelloff and C. V. Rao, *Carcinogenesis*, 1999, **20**, 1645-1648.
129. Y. Itokawa, K. Inoue, S. Sasagawa and M. Fujiwara, *J Nutr*, 1973, **103**, 88-92.
130. M. Fujiwara, *Cellular and molecular life sciences*, 1972, **28**, 254.
131. H. Nakamura and M. Ishikawa, Kanzō (Acta Hepatologica Japonica), Editon edn., 1971, vol. 12, pp. 673-677.
132. K. Kumari and K. T. Augusti, *Journal of Ethnopharmacology*, 2007, **109**, 367-371.
133. K. Kumari, B. C. Mathew and K. T. Augusti, *Indian J Biochem Biophys*, 1995, **32**, 49-54.
134. C. G. Sheela, K. Kumud and K. T. Augusti, *Planta Med*, 1995, **61**, 356,357.
135. K. Kumari, *Indian journal of experimental biology*, 2002, **40**, 1005.
136. W. Komatsu, Y. Miura and K. Yagasaki, *Lipids*, 1998, **33**, 499-503.
137. S. Black, in *Methods in Enzymology*, ed. P. C. a. N. O. K. Sidney, Academic Press, Editon edn., 1962, vol. Volume 5, pp. 992-998.
138. L. F. Stancato, *Biochemistry*, 1993, **32**, 3729.
139. A. R. Karala, *Antioxidants & redox signalling*, 2007, **9**, 527.
140. *Japan Pat.*, 2002.