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Understanding the antioxidant mechanism of Soybean (Glycine max L. Merril)

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Abstract:

In last few decades, the role played by different ROS, in the development of fatal diseases, has compelled the scientists for exploration of new anti-oxidants.

In this review, we have established, a comparatively new food entity in Indian diet, namely Soybean (Glycine max L. Merril) as a prospective anti-oxidant. Soybean, also known as Rajashimbi, is not only a cost effective source of proteins in rural India, but also a good source of antioxidants like vitamin C, and E. But the most important constituents, it possesses, are the high percentage of cysteine, glutamic acid and glycine, from which Glutathione is biosynthesized in the body. Glutathione is an electron donor, responsible for sustaining the ratio of GSSH/GSH present in the cell, and maintaining the reducing environment of the cell. As Soybean is not only rich in glutathione producing amino-acids, but it is most economical in comparison to the chemical anti- oxidants and easily available in rural India. In many states, Soybean is included in the nutrient food supplement for school children. Though, Soybean is described in Ayurvedic texts as in possession of madhura-kashaya, guru, snigdha and ushna, the role played by madhura and kashaya rasa are very important in the purview of extending the life-span of the cells. We have discussed how Soybean is responsible for its anti-aging property in the cell. In the conclusion we have established the role of Soybean, a prospective nutraceutical, as easily available cost-effective anti- oxidant for the control of ROS in young India.

Key words - Soybean, antioxidant, Glutathione, free radicals, DPPH scavenging activity

Introduction:

We are aware of the fact that oxygen is essential for life and without oxygen, human being is unable to survive. On the other hand, oxygen causes some toxic reactions, resulting in a constant danger to the life. Though there are many mechanisms in the body, which give protection from the by- products of our metabolism, these mechanisms are imperfect, as they only minimize the harm caused by oxygen, but are unable to eliminate them completely. Scientist have revealed that slowly, oxygen-induced damage of the tissues accumulate in the body and become a major contributor to aging as well as cardio-vascular disease, cancer, cataracts, and degenerative diseases of the nervous system [1], but we must not forget that in some cases, free radicals are beneficial also, as they can be utilized for useful purpose, as is always used by phagocytes in destruction of harmful microbes. X-ray irradiation is responsible for generating the hydroxyl radical, while ultraviolet light causes the generation of electronically excited states, resulting in formation of radicals. Ultrasound and microwave radiation are also the causes of ROS generation.

The worst effect of oxygen found in the body, is due to the formation of reactive oxygen species. These ROS can be classified in free radicals and non-radicals groups and act as oxidants, means they have a tendency to donate oxygen to other entity. Free radical, being in possession of one or more unpaired electrons, is mostly unstable and highly reactive. Hydroxyl, Superoxide, Nitric oxide and Lipid peroxyl are important free radicals found in the body. On the other hand, Hydrogen peroxide, Singlet oxygen, Hypochlorous acid and Ozone are important non-radicals. ROS are produced constantly as a result of normal metabolic processes and if they are not disabled, they can destruct cellular macromolecules like proteins, carbohydrates, lipids and nucleic acids. If proteins are targeted by free radicals, then it causes increased turnover, decreased enzyme activity and membrane damage all leading to the cell injury. If lipid oxidation is targeted, it also damages cell membrane and LDL damage, causing atherosclerosis. The adverse effects on DNA has a major role in the foundation of cancer. If DNA is targeted, it results in mutation, causing in cell injury. If carbohydrates are targeted by free radicals, they alter cell receptor and reduces viscosity.

To fight back the free radicals, the nature has powered the human being with a category of compounds, labeled as anti-oxidants, some of which can be produced in the body and some can be supplied as food supplement. These anti-oxidants include different enzymes like superoxide dismutase, catalase, and glutathione-peroxidase and glutathione reductase. Some minerals like copper, zinc, selenium and manganese also work as anti-oxidants. While vitamins like vitamin A, C and E are also in possession of good anti-oxidant qualities. It is well established fact that glutathione, flavonoids, bilirubin and uric acid are also capable of destroying the free radicals.

In normal physiological state, the ratio of formation of free radicals and their destruction is well maintained. If there is any deviation in the balance of this ratio, for example if the formation of free radicals is in excess than their destruction, it causes oxidative stress, which can be in between of the states from very mild to severe, depending on the extent of the deviation. Hundreds of research papers have revealed the fact that this oxidative stress is one of the causes of several diseases such as such as cardiovascular diseases, neurological diseases, cancers, renal disorders, diabetes, inflammatory problems, skin diseases, and aging.

Understanding Soybean

In last few decades, the role played by different ROS in the development of fatal diseases, has compelled the scientific community for exploration of new anti-oxidants.

In this review, we have established a comparatively new food entity from Indian diet, namely Soybean (Glycine max L. Merril), belonging to the Leguminosae family, as a prospective anti- oxidant.

Soybean contains mainly 20% of fats and 40% of high-quality proteins, low starch and in addition it also contains several bioactive compounds like lunasin, trypsin, inhibitors, isoflavones and saponins [2]. Riaz (1999), has stated that Soybean has earned its functional food status due to the presence of isoflavones, which varies from 1-3 mg/g in the mature seeds [3]. Seo and Morr (1984) found that the isoflavones content in Soybean comprise about 72% of total phenols [4].

Cultivation – It is generally thought that Soybean cultivated in a hot climate harvest such seeds which possess less highly unsaturated fat than when cultivated in a colder surroundings. Collins and Sedgwick revealed that Soybean cultivated at the northern end of their range produced oil 1- 2 percentage points higher in linolenic acid and 3-6 higher in linoleic acid than when grown at the southern end of their range [5].

राजशिम्बी सुप्तशिम्बी ज्ञेया च गिरीकन्यका ।
राजशिम्बी गुरुः स्निग्धा मधुरोष्णाकषाया ।
दुर्जरा वातशमनी स्तन्यदा बलवर्धनी।

In the Dravyaguna Vigyan of Acharya PriyavatSharma, Soybean is described as Rajashimbi, which is very popular in Indian diet for its high nutritional value. It is highly rich in proteins and easily available throughout the country. 100 grams of raw soybeans supply 446 calories. It contains high percentage of glutamic acids, cysteine and glycine. It also Contain vitamin A, C, E and small percentage of vitamin B1,B2, B9,B12 and K. Minerals like sodium, calcium, magnesium, iron, copper is also present. Synonyms of Rajashimbi are suptashimbi, and girikanyaka [6]. Soybean is an erect branching plant and its height can be more than 6.5 feet. Flowers of Soybean are self-fertilizing and are of a white or a shade of purple. Seeds can be yellow, green, brown, black, though most commercial varieties have brown or tan seeds, with one to four seeds per pod. Soybeans are generally harvested mechanically, after the leaves have dropped off the plant and the moisture content of the seed has decreased up to 13 percent, helpful for safe storage.

Discussion

The antioxidant constituents in soybeans are cysteine, glycine, isoflavones and some phenolic contents. Ferial M. Abu-Salem and others (2013), have described the anti-oxidant properties of Soybean to a number of properties, including their ability to scavenge free radicals, to act as metal- ion chelator, oxygen quencher or hydrogen donor and to the possibility of preventing the penetration of lipid oxidation initiators by forming a membrane around oil droplets [7].

Here we are going to discuss the ability of Soybean as hydrogen donor while working as an anti- oxidant. Ferial M. Abu-Salem and others (2013), have established that Soybean flour, soybean protein isolate and soybean protein isolate hydrolysate contain 21.1%, 1% and 17.9% of Glutamic acid. Soybean flour is made by grinding roasted Soybeans into a powder. Soy protein isolate, a highly refined form of soy protein made from *defatted* soy flour that has most of its fats and carbohydrates removed. Hydrolysate means a substance produced by hydrolysis, and hydrolysis is a step in the degradation of a substance, where cleavage of chemical bonds takes place by addition of water and this addition of water causes both substance and water molecule to split into two parts. In pāṁcabhautika sense of the constitution of these three products of Soybeans, here we can observe that Soybean protein isolate is formed by removing the maximum quantities of Pṛ thvi and Jala mahābhuta, along with the removal of fat from it, results in presenting minimum madhura rasa in it. On the other hand, Soybean flour is just roasted before grinding it, so some portion of the Jala mahābhuta is removed, still it maintains a good part of Madhura rasa in it. Soybean protein isolate hydrolysate is prepared by adding water, so it maintains the Jala mahābhuta and retains a good concentration of Madhura rasa.

Product	pṛ thvi	Jala	Tej	Vayu	Aakash	Glutamic acid	Rasa Probability
Soybean flour	++++	+--	+	+	+	21.1%	Madhura
soybean protein isolate	+ - - -	+--	--	+++	+++	01.00%	Tikta
soybean protei n isolate	++- -	+--	+	+	+++	17.9%.	Kashaya
hydrolysate							

Different procedures, like adding or removing water contents or removing the fat from Soybean, changes its percentage of anti-oxidant activity.

Ferial M. Abu-Salem and others (2013) observed that the anti-oxidant activities give the same trend of peptide content which had high anti-oxidative activities and high peptide content between fractions 15 to 50. With increasing peptide concentration, the scavenging effect on DPPH radical was increased until about 70%. Butylated hydroxyl-anisole (BHA) is well-established antioxidant, which is used as preservative in food. Ferial M. Abu-Salem and others (2013) [7] demonstrated that compare to different concentration of BHA, which exhibited higher activity (90%), Soybean protein hydrolysate also exhibited high anti-oxidant activities (70%). As shown in the above table, we can observed that when Soybean is transformed in Soybean protein isolate hydrolysate, portion of Madhura rasa is omitted and Kashaya rasa is replaced at its site. That is the reason that Soybean protein isolate hydrolysate, now having Kashaya Pradhan-Madhura rasa, is more anti-oxidant in comparison to Soybean protein isolate, which is in possession of madhura and tikta rasa. So the different shades of Madhura rasa in the preparation of different Soy products have different levels of anti-oxidative functionalities. Ferial M. Abu-Salem and others (2013) [7], also found that Soybean peptides contained substances that were proton donors and react with free radicals to convert them to stable diamagnetic molecules. Their previous studies had also shown that Soybean protein isolate hydrolysates are capable of interacting and quenching DPPH radicals.

Dan E., Pratt, Paula M. Birac (1979) [11] found that Soybeans, defatted soy flour, soy protein concentrates, and soy isolates retains substantial antioxidant activity, due chiefly to polyphenolic compounds. Antioxidant action of soy protein isolates are also due to polyphenolic compounds but other compounds also show a marked antioxidant effect. Polyphenolic antioxidants of soy were found to be isoflavones, chlorogenic acid isomers, caffeic acid, and ferulic acid.

One more important finding mentioned by SB Zhang and others (2008), is that in the electrophoresis analysis, low molecular weight hydrolysate fractions appeared to have higher DPPH scavenging activities than high molecular weight fractions [12]. We have already discussed that after conversion from Soybean, Soybean protein isolate hydrolysate is in possession of excess Aakash mahābhuta in its pāṁ cabhautika constitution, resulting in the lower molecular weight of the peptides due to the sūkṣ ma attribute of Aakash mahābhuta. So it is not a miracle that Soybean protein isolate hydrolysate, with its sūkṣ ma quality, is in possession of higher DPPH scavenging activity.

Though, Panthee DR and others (2010) [10] have mentioned that the major limitation of soy proteins is their deficiency in sulfur-containing amino acids, methionine (Met) and cysteine (Cys), the quantity of Cys in Soybean is nearly 2%, we know better that glutathione is biosynthesized in the body from the amino acids L-cysteine, L-glutamic acid, and glycine. We have mentioned above that Glutathione is a better anti-oxidant.

The sulfhydryl group (SH) of cysteine serves as a proton donor and is responsible for its biological activity. (The proton is a hydrogen cation (H+) and it resembles the protium cation. Protium (H-1) has one proton, one electron but no neutron. When it loses the electron, the only thing left is the proton, hence, this cation is sometimes referred to as a "proton".) Glutathione is an important antioxidant, preventing injury to vital cellular components caused by reactive oxygen species (ROS), such as free radicals, peroxides, lipid peroxides and heavy metals [8]. Glutathione decreases disulfide bonds formed within cytoplasmic proteins, to cysteines by serving as an electron donor. In the process, glutathione is converted to its oxidized form, glutathione disulfide (GSSG).

Glutathione (GSH) is a critical molecule in counterattacking oxidative stress and maintaining the reducing environment of the cell. GSH exists in both reduced (GSH) and oxidized (GSSG) states. In the reduced state, the thiol group of cysteine is able to donate a reducing equivalent (H^{+}+ e^{-}) to reactive oxygen species. In donating an electron, glutathione itself becomes reactive, but readily reacts with another reactive glutathione to form glutathione-di-sulphide (GSSG). Glutathione-disulphide reductase (GSR) is an enzyme that catalyses the reduction of glutathione disulphide (GSSG) to the sulfhydryl form glutathione (GSH).

Glutathione can act as a scavenger for hydroxyl radicals, singlet oxygen, and various electrophiles. Reduced glutathione reduces the oxidized form of the enzyme glutathione peroxidase, which in turn reduces hydrogen peroxide (H2O2), a dangerously reactive species within the cell. In addition, it plays a key role in the metabolism and clearance of xenobiotic, acts as a cofactor in certain detoxifying enzymes, participates in transport, and regenerates antioxidants such as Vitamins E and C to their reactive forms. The ratio of GSSH/GSH present in the cell is a key factor in properly maintaining the oxidative balance of the cell, that is, it is critical that the cell maintains high levels of the reduced glutathione and a low level of the oxidized Glutathione disulfide. This narrow

balance is maintained by glutathione reductase, which catalyzes the reduction of GSSG to GSH [9].

Conclusions:

After careful consideration of the above mentioned different facts we are at conclusion that

4. All the three Soy products, namely Soybean flour, soybean protein isolate and soybean protein isolate hydrolysate, are in possession of different permutations and combinations of Kashaya and Madhura rasa, which have different levels of anti-oxidant capacities and are responsible for the control of the aging process in the body.
5. Lower molecular weight hydrolysate fractions of Soybean peptides, which are in possession of sūkṣ ma and vishad qualities, have higher DPPH scavenging activity than high molecular weight Soybean peptide fractions.
6. Soybean peptides are in possession of such substances that work as proton donor and react with free radicals to convert them to stable form.
7. Soybeans are also in possession of Vitamin E and vitamin c, which are

well-established anti-oxidants.

8. Soybean is a rich source for the biosynthesis of a renowned anti-oxidant, glutathione which works as proton donor.

At the end, we conclude that the easily available and cost-effective Soybean is the best anti-oxidant for the betterment of human being.

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