Physical, chemical and nutritional characteristics of premature-processed and matured green legumes

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Abstract Premature green legumes are good sources of nutraceuticals and antioxidants and are consumed as snacks as well as vegetables. They are seasonal and have limited shelf-life. Efforts are provided to prepare shelf-stable green legumes to extend their availability throughout the year. Green legumes from chick pea or Bengal gram (*Cicer arietinum*) and field bean (*Dolichos lablab*) have been processed to enhance their shelf-life, and determined their nutritional, physico-chemical and nutraceutical qualities. The shelf stable green legumes (SSGL) show higher water absorption capacity compared to matured dry legumes (MDL). The total colour change in the processed/dried SSGL and MDL samples increased significantly ($p \leq 0.05$) compared to the freshly harvested green samples. The carotenoid content of Bengal gram and field bean SSGLs are 8.0 and 3.2 mg/100 g, and chlorophyll contents are 12.5 and 0.5 mg/100 g, respectively, which are in negligible quantities in matured legumes; the corresponding polyphenol contents are 197.8 and 153.1 mg/100 g. These results indicate that SSGLs possess potential antioxidant activity.

Keywords *Cicer arietinum* · *Dolichos lablab* · Premature green legume · Nutraceuticals

Introduction

Legumes in their premature stage possess green appearance and contain substantial amount of phytochemicals. During

advanced maturity the contents of many of the phytochemicals with nutraceutical qualities decrease considerably due to biotransformation. Due to the presence of active enzymes and high moisture content, the legumes harvested at premature stage are susceptible to quick spoilage, and hence, they are not usually harvested at the early stage. However, a small proportion of some legumes which are harvested at premature stage are used as green vegetables. Most of the legumes are seasonal and they are not available to the consumer throughout the year. Hence, the possibility of processing the green legumes in the dry form by retaining their nutrients and nutraceuticals has been examined.

Generally, drying of most of the mature legume seeds is done by sun drying or mechanical drying but prolonged drying leads to adverse changes in colour, texture and flavour, thereby rendering the product with poor consumer acceptability and shelf-stability (Sharma et al. 1987).

Consumption of premature green legumes is common in several Asian countries. Premature green legumes are available only for short period of the season and have limited shelf-life; preservation of these legumes ensures long availability for use. Ramanathan and Bhatia (1970) prepared dehydrated green Bengal gram with reconstitution time ranging from 8 to 12 min. Yamakawa and Kurihara (2003) described a method for producing frozen blanched green agricultural products including beans and green vegetables. Fresh, frozen and canned immature peas contain glucose, fructose, sucrose and maltose (Gertig and Kulesa 1972). Green peas have highest starch content showing that freezing and canning induce hydrolysis. Fresh peas have highest calorific value, followed by frozen and canned peas (Gertig and Kulesa 1972). Singh et al. (1994) studied the effect of natural tannins on canned green Bengal gram. Jayaraman et al. (1994) dehydrated fresh green field beans

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by employing several drying techniques and evaluated their quality characteristics. Kanawade and Narain (1993) evaluated the quality of peas dehydrated in a fluidized bed dryer. Effects of reprocessing prior to domestic cooking were investigated for frozen green peas and beans (Canet et al. 2004). Reprocessing was found to significantly affect the mechanical behaviour of green bean. Colour changes during heating of green beans and broccoli were monitored by Tijksens et al. (2001). Four varieties of green bean were analyzed for flavonol composition and content (Price et al. 1998). Kaack (1994) showed that inactivation of lipoxygenase in green beans by blanching prevents the development of off-flavours during frozen storage. Changes in peroxidase activity, firmness, chlorophyll, aldehyde and vitamin C contents during blanching were studied. Murcia et al. (2009) evaluated twenty-five vegetables including green bean for their antioxidant activity. Canned vegetables showed a more pronounced loss of antioxidant activity than frozen and fresh vegetables. Sagar and Suresh (2010) reviewed the new dimensions of drying techniques for the production of quality dried products and powders. Drying characteristics and physical quality of green peas were studied by Thakur (2008). However, details of the quality attributes of premature green legumes are still lacking with particular reference to processing and development of convenience foods.

The objectives of the present study are 1) to develop shelf-stable ready-to-eat green legumes, 2) to assess the effect of maturation on their physical and physicochemical properties, 3) to estimate the antioxidant activity, functional properties and nutraceutical contents of the legumes during maturation, and 4) to observe the cellular structure of the seeds before and after processing.

Materials and methods

Whole plants of green Bengal gram (*Cicer arietinum*) and field bean (*Dolichos lablab*) along with their leaves and pods at their green stage were collected from a nearby agricultural farm, Mysore, India and were de-podded manually. Matured yellow seeds of these legumes were also collected simultaneously from the same farm. 1,1-diphenyl-2-picrylhydrazyl (DPPH), and tannic acid were obtained from Sigma Chemical Co. (St, Louis, Mo., USA). All solvents/chemicals used were of analytical grade and were obtained from Merck, Mumbai, India.

Preparation of shelf-stable green legumes: Good quality uniform matured legume seeds were hand peeled followed by drying in a mechanical tray dryer (INDLAB, Chennai, India) at 50 °C for 4 h. The tender fresh seeds were then processed to make shelf-stable ready-to-eat green legumes (SSGL) by blanching of the legume seeds in boiling water (containing 1% sodium chloride, 0.2% magnesium oxide and 0.05% sodium bicarbonate) for 8 min followed by draining out excess water and dried in a freeze dryer (Model # LT5B, Lyodryer, USA). The proximate composition, as determined by following AOAC (2002) methods on triplicate samples, is presented in Table 1.

Physical properties A Vernier caliper was used to measure the size; bulk density by using a Hecto litre tester (OHAUS Scales Corporation, USA) and colour was measured by using a colour meter (Model # LABSCAN XE, Hunter Associate Laboratory, Virginia, USA) for the freshly harvested seed (raw green), shelf stable green legume (SSGL), and matured dry legume (MDL) for

Table 1 Chemical composition of Bengal gram and field bean

Composition (% dry basis)	Bengal gram		Field bean		Standard error of mean (±)
	SSGL	MDL	SSGL	MDL	
Moisture*	2.7±0.1 ^a	9.9±0.7 ^c	5.2±0.4 ^b	11.6±0.8 ^d	0.33
Fat	5.1±0.4 ^b	5.8±0.6 ^b	1.4±0.2 ^a	1.4±0.2 ^a	0.21
Ash	2.1±0.3 ^a	3.3±0.4 ^b	3.3±0.3 ^b	3.6±0.2 ^b	0.18
Protein	16.5±0.6 ^a	21.4±0.9 ^b	23.5±1.4 ^c	28.1±1.2 ^d	0.19
Starch	38.3±2.1 ^a	42.5±2.5 ^b	47.0±2.2 ^c	50.4±2.6 ^d	0.97
Soluble dietary fiber	3.2±0.3 ^a	3.4±0.4 ^a	4.7±0.6 ^b	4.7±0.5 ^b	0.31
Insoluble dietary fiber	29.2±1.3 ^c	25.2±10.9 ^b	24.9±2.1 ^b	22.5±1.8 ^a	1.07
Total sugars	12.2±0.5 ^d	10.2±0.6 ^b	11.0±0.4 ^c	8.45±0.3 ^a	0.22
Energy, kcal/30 g	125.1±1.5 ^b	124.7±2.3 ^b	118.1±0.4 ^a	117.8±1.1 ^a	0.79

SSGL Shelf-stable green legumes, MDL Matured dried legumes

*Expressed as wet basis

Values are mean±SD of 3 observations; degrees of freedom=11

Values in the same row with different subscripts differ significantly at $p \leq 0.05$

Table 2 Physical properties of raw, SSGL and MDL of Bengal gram and field bean

Parameters	Bengal gram			Field bean			Standard error of mean ($\pm$)
	Raw green	SSGL	MDL	Raw green	SSGL	MDL	
Moisture (%)	70.3 $\pm$ 2.8 ^d	2.7 $\pm$ 0.1 ^a	9.9 $\pm$ 0.7 ^c	70.9 $\pm$ 3.5 ^d	5.2 $\pm$ 0.4 ^b	11.6 $\pm$ 0.8 ^c	1.08
Bulk density (kgm ⁻³)	652.3 $\pm$ 9.5 ^d	275.7 $\pm$ 3.2 ^a	749.5 $\pm$ 8.6 ^c	625.3 $\pm$ 5.0 ^c	295.4 $\pm$ 4.8 ^b	870.8 $\pm$ 10.7 ^f	2.05
Length (cm)	1.03 $\pm$ 0.07 ^c	0.85 $\pm$ 0.02 ^a	0.85 $\pm$ 0.02 ^a	1.19 $\pm$ 0.07 ^d	0.95 $\pm$ 0.02 ^b	0.95 $\pm$ 0.02 ^b	0.04
Breadth (cm)	0.85 $\pm$ 0.08 ^b	0.74 $\pm$ 0.01 ^a	0.74 $\pm$ 0.01 ^a	0.89 $\pm$ 0.09 ^b	0.74 $\pm$ 0.01 ^a	0.74 $\pm$ 0.01 ^a	0.02
Thickness (cm)	0.67 $\pm$ 0.07 ^b	0.69 $\pm$ 0.06 ^b	0.73 $\pm$ 0.02 ^b	0.55 $\pm$ 0.08 ^a	0.55 $\pm$ 0.02 ^a	0.51 $\pm$ 0.04 ^a	0.05
Peak force (N)	26.2 $\pm$ 2.5 ^a	45.1 $\pm$ 4.0 ^b	147.3 $\pm$ 7.7 ^c	63.5 $\pm$ 2.6 ^c	83.3 $\pm$ 4.2 ^d	517.6 $\pm$ 17.9 ^f	0.63
L*	36.8 $\pm$ 0.3 ^b	43.0 $\pm$ 0.9 ^c	29.2 $\pm$ 0.7 ^a	49.3 $\pm$ 0.5 ^d	55.5 $\pm$ 0.6 ^f	53.1 $\pm$ 0.8 ^e	0.87
a*	-10.2 $\pm$ 0.2 ^d	-8.4 $\pm$ 0.1 ^b	9.0 $\pm$ 0.2 ^c	-9.0 $\pm$ 0.2 ^c	-5.4 $\pm$ 0.2 ^a	10.4 $\pm$ 0.2 ^f	0.20
b*	18.7 $\pm$ 0.6 ^b	20.0 $\pm$ 0.2 ^c	8.1 $\pm$ 0.4 ^a	21.0 $\pm$ 0.2 ^d	32.6 $\pm$ 0.1 ^f	23.4 $\pm$ 0.5 ^e	0.23
ΔE	57.5 $\pm$ 1.2 ^d	61.0 $\pm$ 0.8 ^e	63.0 $\pm$ 0.3 ^f	46.7 $\pm$ 0.6 ^a	49.1 $\pm$ 0.4 ^b	52.0 $\pm$ 1.6 ^c	0.81
Overall colour	Bright green	Dull green	Bright reddish brown	Bright green	Dull green	Bright reddish brown	

Colour indices: L*: Lightness; a*: red/green component and b*: blue/yellow component.

SSGL Shelf-stable green legumes, MDL Matured dried legumes

Values are mean $\pm$ SD of 3 observations; degrees of freedom=17. Peak force values are based on 10 readings; degrees of freedom=59

Values sharing the same superscript in individual rows differ significantly at $p \leq 0.05$

Bengal gram and field bean samples. The total or overall colour change (ΔE) was measured with L*, a* and b* values using Eq. 1.

$$\Delta E = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2} \quad (1)$$

where L_0^* , a_0^* , and b_0^* denote the initial colour parameters for the sample and L*, a* and b* correspond to the final sample.

Texture of the whole legumes was determined by employing a Universal Testing machine (Model #5R, Lloyd, UK) at a crosshead speed of 100 mm/min subjecting to 50% compression.

Starch content and total free sugars Starch content of the legumes was estimated by enzymatic method after removal of fat from the samples by petroleum ether (Ojimekwe et al. 1998), and total free sugar content by Dubois et al. (1956).

Dietary fiber Dietary fiber was estimated according to Asp et al. (1983).

Chlorophyll content Total chlorophyll was estimated based on the method mentioned by Ranganna (1977) using Eq. 2.

$$\text{Total chlorophyll (mg/l)} = (7.12 \times \text{absorbance at 660 nm}) + (16.8 \times \text{absorbance at 642.5 nm}) \quad (2)$$

Table 3 Carotenoid, chlorophyll, polyphenol content and enzyme activity of Bengal gram and field bean

	Bengal gram		Field bean		Standard error of mean ($\pm$)
	SSGL	MDL	SSGL	MDL	
Total carotenoids (mg/100 g)	8.0 $\pm$ 0.6 ^c	—	3.2 $\pm$ 0.3 ^b	0.67 $\pm$ 0.20 ^a	0.32
Total chlorophyll (mg/100 g)	12.5 $\pm$ 0.7 ^b	—	0.5 $\pm$ 0.2 ^a	—	0.21
Total polyphenols (mg/100 g)	197.8 $\pm$ 0.21 ^d	100.8 $\pm$ 0.22 ^b	153.1 $\pm$ 0.24 ^c	0.77 $\pm$ 0.05 ^a	0.10
Protease activity (unit/g)	113.8 $\pm$ 9.6 ^d	16.3 $\pm$ 1.6 ^b	92.5 $\pm$ 8.4 ^c	10.0 $\pm$ 1.3 ^a	0.63
Protease inhibitor activity (unit/g)	—	230.3 $\pm$ 10.6 ^b	—	225.0 $\pm$ 0.3 ^a	2.22
α -Amylase activity (unit/g)	14.1 $\pm$ 1.5 ^a	—	91.5 $\pm$ 2.2 ^b	—	1.08

(—) Indicates negligible quantity

Values not sharing the same superscript within a row are significantly different at $p \leq 0.05$.

SSGL Shelf-stable green legumes, MDL Matured dried legumes

Values are mean $\pm$ SD of 3 observations. Degrees of freedom are: 11 for total polyphenols and protease activity, 8 for total carotenoids, and 5 for total chlorophyll, protease inhibitor and α -amylase activity.

Fig. 1 Polyphenol content of Bengal gram (Bg) and field bean (Fb) extracted by two different solvents. Values are mean and standard deviation of three observations

Fig. 2 Reducing power and scavenging activity of Bengal gram (Bg) and field bean (Fb). Values are mean and standard deviation of three observations

Carotenoids content Extraction, separation and drying procedures used for the estimation of carotenoids are similar to that of chlorophyll using Eq. 3.

$$\text{Total carotenoids (mg/100 g)} = \frac{3.857 \times \text{absorbance} \times \text{volume} \times 100}{\text{Mass of sample} \times 1000} \quad (3)$$

Preparation of phenolic extract Approximately 500 mg of flour sample was extracted with 5 ml of methanol for 2 h at room temperature (26 ± 2 °C) for the estimation of free phenols and the residue was re-extracted with 5 ml of acidified-methanol for the estimation of bound phenols. These extracts were mixed and stored at -20 °C till analysis to determine total phenolic content, reducing power and scavenging activity.

Total phenolic content Free and bound phenolic content was estimated using the extracts according to the method of Singleton and Rossi (1965) using tannic acid (TA) as the standard and was expressed as tannic acid equivalents (mg of TAE/100 g sample).

Reducing power The reducing power of sample was determined as described by Yen and Duh (1993) using the extract of the legumes against BHA as the standard.

Scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical Free radical scavenging activity of the legume extract was assayed using a stable free radical DPPH, according to the method of Blois (1958) and results were expressed in mg/g BHA.

Alpha-amylase activity Alpha-amylase activity of the SSGL and MDL was determined following the method of Nirmala and Muralikrishna (2003).

Protease activity Protease activity of the SSGL and MDL was determined by the casein digestion method of Kakade et al. (1969).

Protease inhibitory activity Protease inhibitory activity of the legume seed extracts was determined according to the procedure described by Kakade et al. (1974). Protease inhibitory unit (unit/g) is defined as the number of protease units inhibited under the same assay conditions.

Functional property Water and oil absorption capacities of the legume flours were determined according to the procedures of Elhardallou and Walker (1993) and Sosulski et al. (1976), respectively. The values were reported in g of water (or oil) absorbed per g of dry flour. All determinations were replicated thrice.

Table 4 Functional properties of different Bengal gram and field bean samples

Properties	Bengal gram		Field bean		Standard error of mean ($\pm$)
	SSGL	MDL	SSGL	MDL	
Water absorption capacity (g/g)	0.25 ± 0.03^b	0.13 ± 0.02^a	0.30 ± 0.03^c	0.14 ± 0.03^a	0.03
Oil absorption capacity (g/g)	0.13 ± 0.04^a	0.15 ± 0.05^a	0.12 ± 0.03^a	0.15 ± 0.02^a	0.02

Values not sharing the same superscript within a row are significantly different ($p \leq 0.05$)

SSGL Shelf-stable green legumes, MDL Matured dried legumes

Values are mean $\pm$ SD of 3 observations. Degrees of freedom are 11

Fig. 3 Low magnification photographs of SSGL cross-sections of the whole kernel of **a** Bengal gram and **b** field bean

Microstructure Bengal gram and field bean legume seeds of SSGL and MDL were freeze dried, coated with gold in a sputter coating equipment and viewed at different magnifications of 50 or 75 $\times$, and 1 k $\times$. All examinations were made at an accelerating voltage of 20 kV using a scanning electron microscope (Model #435VP, Leo Electron Microscopy, Cambridge, UK).

Statistical analysis One-way and two-way ANOVA (factorial) with replications were used to analyse the significant difference among the results of different samples. Multiple comparisons were made for all experiments employing Duncan's multiple range test (DMRT) at 5% level of significance.

Results and discussion

Physical and chemical properties SSGLs contain lesser starch as well as protein content than that for corresponding MDL samples, but no significant difference exists in their fat content (Table 1). SSGL for both the legumes contains higher total dietary fibre and free sugars compared to the corresponding mature seeds. Presence of more free sugars thus imparts sweetness to the green premature legumes as

evidenced from their sweet taste. Both the processed and dried legumes provide nearly same energy per serving (Table 1), but Bengal gram products give marginally higher energy compared to field bean possibly due to more fat content in Bengal gram.

The average dimensions (length, breadth and thickness), bulk densities, and colour of different legume seeds are shown in Table 2. The dimensions of the freshly harvested seeds are higher compared to the corresponding SSGL and MDL samples for both the legumes due to shrinkage that occurs during drying. The higher bulk densities of the freshly harvested legumes can be attributed to the higher moisture contents compared to processed/dried SSGL and MDL samples. SSGL samples having lower a^* negative values are dull green in colour than freshly harvested (raw green) samples which may be due to blanching and drying of the green legumes. Since greenness is an indication of the presence of chlorophyll in the sample, SSGL samples possibly have more chlorophyll content than MDL samples. The total or overall colour change in the processed/dried SSGL and MDL samples increased significantly ($p \leq 0.05$) compared to the freshly harvested green samples. This may be due to the gradual dullness in colour of the processed SSGL samples, and for MDL samples for becoming brown in appearance due to maturation.

Fig. 4 Low magnification photographs of MDL cross-sections of **a** Bengal gram and **b** field bean

Fig. 5 Photomicrographs of SSGL endosperms of **a** Bengal gram and **b** field bean at a high magnification of 1000X

The MDL samples are significantly harder than SSGLs while freshly harvested seeds (raw green) exhibit the least peak forces (Table 2) due to their high moisture contents. At low moisture content, it is obvious that a grain shrinks and offers more resistance towards compression.

Chlorophyll and total carotenoid content The total chlorophyll contents of both legume MDLs are significantly lower as compared to the SSGL samples (Table 3). The SSGL samples, even in spite of blanching treatment, contain 8.0 and 3.2 mg of carotenoids per 100 g Bengal gram and field bean green seeds, respectively (Table 3). Probably, salts present might have a tissue fixing effect that in turn have prevented leaching loss of chlorophyll to the blanching solution (Ramanathan and Bhatia 1970). Carotenoids have been mainly credited as pro-vitamin and act as a precursor. Recently, their beneficial effects to human health such as enhancement of immune system, reduction of degenerative diseases such as cardiovascular, cataract and muscular degeneration, anticancer, antidiabetic, antiviral, antimalarial, hepatoprotective effects have attracted researchers from different field of studies (Anderson and Polansky 2002; Noonan et al. 2007).

Total phenolic content Total phenolic content is high in both SSGL (Table 3) which reduces during maturation. Acidified methanol extract shows high polyphenol content which indicates that in legumes, polyphenols exist mostly as bound form (Fig. 1). Dry heat treatment reduces the phenolic compounds for MDL samples which may be attributed to the presence of polyphenol oxidase that results in the degradation of phenolic compounds in MDL (Gamel et al. 2006).

Reducing power and scavenging activity SSGLs possess higher reducing power compared to corresponding MDL samples (Fig. 2). The antioxidative properties are concomitant with the development of the reducing power. Polyphenolic substances present in green seed extracts appear to function as good electron and hydrogen atom donors, therefore, able to terminate radical chain reaction by converting free radicals and reactive oxygen species to more stable products. Similar observation has also been reported for several plant extracts including tea (Amarowicz et al. 2004; Zhu et al. 2002). The order of scavenging activity of the legume seeds is Bg SSGL>Fb SSGL>Bg MDL>Fb MDL. Observation of high correlation between the level of phenolics and

Fig. 6 Photomicrographs of MDL endosperms of **a** Bengal gram and **b** field bean at a high magnification of 1000X

antioxidant property (Guo et al. 2008) signifies the potential benefit of SSGL phenolic compounds in the prevention and treatment of different health problems.

Enzyme activity In SSGL, both protease and α -amylase activities are present while in MDL samples, there is hardly any α -amylase activity (Table 3). It is possible that these enzyme activities to some extent depend on the maturity of seeds and the extent of dry heat treatment. Gamel et al. (2006) have reported a reduction in the α -amylase activity under dry heating and autoclaving conditions.

Functional properties The water absorption capacity (WAC) and oil absorption capacity (OAC) of different legume samples are shown in Table 4. SSGLs show a distinctly higher WAC than corresponding MDL samples. This probably indicates that green legumes contain more hydrophilic constituents like polysaccharides. The enhanced ability of flour to absorb and retain water and oil may help to improve binding of the structure, enhance flavour retention, improve mouth feel and reduce moisture loss (Prinyawiwatkul et al. 1997). The SSGL samples have lower OAC values compared to MDLs (Table 4). This may be attributed to higher protein content in MDL samples which in turn, influences the oil absorption capacities. The thermal treatment of SSGLs increases the water absorption which may be attributed to starch gelatinization and protein denaturation (Prinyawiwatkul et al. 1997).

Microstructure Both the SSGL and MDL samples show similar pattern of microstructure (Figs. 3 & 4a). SSGLs for both the seeds at a low magnification (Fig. 3) show large gaps between the cotyledons, and between the seed coat and cotyledon possibly due to immature nature of the seeds. The shrinkage during sample preparation may also be responsible. On the other hand, MDLs possess a compacted structure and the above mentioned gaps are less (Fig. 4). At a high magnification like 1000x, the endosperm of MDL shows that the starch granules are still bound to the protein matrix (Figs. 5 and 6) within the cotyledons (Enwere et al. 1998). The size of starch granules higher for Bengal gram compared to field bean. The starch granules have been partially dislodged from their native positions in the protein matrix in SSGL samples unlike MDLs possibly due to less maturity leading to low structural binding. In addition, the process of blanching gelatinizes starch granules resulting in expansion of cell to damage structure in SSGLs that are separated from protoplasts, leaves some empty spaces and creates intracellular voids (Spaeth and Debouck 1989). Green legumes are richer sources of antioxidants than the matured legumes and may be exploited or incorporated in health food formulations. A high water absorption value of flour is regarded as a desirable property for reconstitution

of the processed ready-to-eat legumes for use it in convenience foods like soup mix, beverage powder, etc.

Conclusions

Green legumes are richer sources of antioxidants than the matured legumes. Higher antioxidant activities of SSGLs are due to the higher carotenoid, chlorophyll and phenolic contents compared to MDLs; the former possess higher WAC compared to latter samples of Bengal gram and field bean; the MDLs have higher protein content compared to corresponding SSGL samples. Electron photomicrographs show that most of the starch granules in both the SSGLs are in a weakly bound form due to processing and are dislodged from their native positions in the protein matrix. A high water absorption value of flour is regarded as a desirable property for reconstitution of the processed ready-to-eat legumes for use it in convenience foods like soup mix, beverage powder, etc. As the SSGLs possess potential antioxidant activity, it may be exploited or incorporated in health food formulations.

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