

## Use of Sprouted Legumes as Sources of Antioxidants in Functional Dairy Product Development

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### ABSTRACT

The potential health benefits of phytochemicals and their ability to be incorporated into dairy foods as nutraceuticals has received considerable interest in food industry. Phenolic compounds are added to dairy products to improve both their technological functionality and nutritional value. Sprouted legumes are considered to be power houses of nutrients and hold various bioactivities, which could have many health benefits, improve its nutritional value and prevent oxidation in number of food matrices. Extraction of phytochemicals from these sprouts and incorporation into food based products can be suitable method to improve the functionality of the food and increase the shelf life of fat rich foods due to the potent free radical quenching ability of these phytochemicals.

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This review provides a summary of the antioxidant potential of sprouted legumes as assessed by various methods and thereby providing their suitability as potential candidates in the development of functional foods of livestock origin.

**Keywords** Sprouted legumes, Antioxidants, Dairy products, Functional food, Phytochemicals.

### INTRODUCTION

Free radicals are generated through normal reactions in human body. These physiologically produced radicals can exert diverse functions like signaling roles and providing defense against infections. However, an excessive amount of reactive radicals can result in cellular damage, which in turn, initiates several diseases including atherosclerosis, arthritis, diabetes and cancer (Phaniendra *et al.* 2015). Under normal conditions, antioxidant defense systems can remove reactive species through enzymatic (like superoxide dismutase and glutathione peroxidase) and non-enzymatic antioxidants (like antioxidant vitamins, trace elements, coenzymes and cofactors). However, in certain circumstances, the endogenous defense system fails to protect the body against reactive radicals on its own resulting in oxidative stress, a condition in which the generation of highly reactive molecules

such as reactive oxygen species and reactive nitrogen species exceed their elimination and/or when their elimination is inadequate (Liu *et al.* 2008). In humans, oxidative stress usually plays the role of a promoter rather than an initiator of chronic diseases, which has fostered research on plant sources and the screening of raw materials for identifying new antioxidants. The antioxidant property is mainly imparted to their phenol-derived structure. Antioxidants “sacrifice themselves” by giving up a hydrogen atom, then rearrange to a stable conformation. Plant-based foods, such as fruits, vegetables, whole grains and legumes, which contain significant amounts of bioactive phytochemicals, may provide desirable health benefits beyond basic nutrition to reduce the risk of developing chronic diseases (Liu 2004).

## MATERIALS AND METHODS

### Legume - power house of bioactive compounds

Legumes believed to be rich sources of bioactive compounds are recently been studied for their antioxidant properties. Consumption of legumes is also correlated to number of positive health benefits such as hypocholesterolemic, antiatherogenic, anticarcinogenic and hypoglycemic properties, reduction in obesity, reduction of coronary heart diseases, which has been noted and confirmed (Angeles *et al.* 2021). These health benefits are known to be associated with dietary fiber and phytochemicals like polyphenols which are considered to be natural antioxidants, representing an important group of bioactive compounds to be used in functional foods and other applications. Wu *et al.* (2004) categorized a wide array of foods in terms of antioxidant capacity and concluded that most legume seeds ranked in the group with the highest activity (>2000  $\mu\text{mol Trolox equiv. /serving}$ ). Legumes, being excellent sources of bioactive compounds can be important sources of ingredients for uses in functional foods and other applications. Legumes are also rich in phenolic compounds ranging from 1.1 to 68 mg/g of dry weight (Vaz Patta *et al.* 2015). Phenolic acids and flavonoids are the most abundant phenolics in legumes and they generally occur as free and bound forms, the latter representing up to 50% of the total phenolic content (Yeo and Shahidi 2015). The content of bioactive compounds of legumes is generally af-

ected by conditions such as planting, environmental and genetic factors such as cultivar, cultivation year, cultivation location and temperature with storage time and germination period having marked effects on the phenolic compounds and isoflavones (Randhir *et al.* 2004). Selection of the right legume varieties combined with a suitable germination process could provide good sources of bioactive compounds and significantly enhance the antioxidant properties of legumes is critical if legumes are to become a competitive source of phytonutrients and incorporation in products as desirable functional ingredients or natural antioxidants in new food formulations.

### Sprouted legumes

Germination causes important changes in the biochemical, nutritional and sensory characteristics of seeds (Smith *et al.* 2018). The reserve materials of the seeds are degraded and used partly for respiration and partly for synthesis of new cell constituents of the developing embryo during germination. In addition of being a good source of basic nutrients, they also contain important health promoting phytochemicals compared with their mature counterparts with disease preventive and health promoting properties (Fernandez-Orozco *et al.* 2006). Sprouting mobilizes polymerized forms, such as concentrated starch and protein, into carbohydrates and free amino acids, respectively (Randhir *et al.* 2004) and have substantial nutritional benefits for the human body because of their high concentration of nutrients which can be used readily by the body. As a consequence of germination, some anti-nutritional factors such as trypsin inhibitors, phytic acid and saponins, decrease or even disappear during germination while some compounds with antioxidant effects such as phenolics, vitamins and a variety of other bioactive compounds that act as reducing agents, free radical terminators, metal chelators and singlet oxygen quenchers increase (Doblado *et al.* 2007) increase during germination process. They are confirmed to possess diverse bioactivities, which could have many health benefits such as reduce the risks of cancer, heart disease and diabetes, inhibition of plasma platelet aggregation, cyclooxygenase (COX) activity and histamine release, *in vitro* antibacterial, antiviral, anti-inflammatory and anti-allergenic activities (Oak *et al.* 2005) and

prevent oxidation in foods and also act as protective factors against oxidative damage in the human body (Kikuzaki *et al.* 2002).

### **Effect of germination on phytochemical profile and antioxidant activity of legumes**

Legume germination has also been suggested as a powerful strategy to increase total antioxidant activity (Fernandez-Orozco *et al.* 2006). During germination the hydrolytic enzymes modify the endosperm and may liberate some of the bound components that play a role in antioxidant activity (Doblado *et al.* 2007). Xue *et al.* (2016) reported that optimum germination time for sprouts was 3-5 days when total bioactive compound content and antioxidant activities both reached their peak values. Phenolics and flavanoid contributed the highest towards the antioxidant activity of mung, soybean and black gram sprouts. Xue *et al.* (2016) observed that vitamin C content increased in a time-dependent pattern and reached a peak on Day 6, amounting to 1.97, 1.94 and 1.93 mg/g FW for mungbean, soybean and black bean, respectively. The vitamin C contents increased in a time-dependent manner and reached peak on day 8 at the concentration of  $285 \pm 25.7$  mg/100 g DW, almost 24 times higher than the initial concentration in mungbean seeds (Guo *et al.* 2012). Doblado *et al.* (2007) reported an increase in vitamin C content in sprouted cowpeas and the content reached upto 23.3 and 25.2 mg/100 g DM after 4 and 6 days of germination, respectively while no vitamin C was detected in raw seeds. Sood and Malhotra (2002) observed that vitamin C content of chickpea increased between 4-7 times during germination while Plaza *et al.* (2003) observed 54%, 218% and 919% increase respectively in wheat, soybean and alfalfa after 96 h at 28°C in the dark. Pajak *et al.* (2014) observed more than 10-fold increase of antioxidant activity against ABTS and DPPH radicals in mungbean after 5-day germination and reported that this increment to be related with changes in the content of antioxidants, such as vitamins and polyphenols while Guo *et al.* (2012) reported that the antioxidant activity of mungbean sprouts were six times higher than in the seeds as measured by the hydrophilic peroxy radical scavenging capacity. López-Amorós *et al.* (2006) studied the antiradical efficacy of pea, bean and

lentil seeds and their sprouts against DPPH radical and observed that germination process modified the antiradical efficacy against DPPH of the legume seeds but differently for each. Four to six day old germs showed higher antiradical capacity than the raw seeds and it was most remarkable after 4 days.

### **Effect of different extraction solvents on antioxidant capacity of different legume extracts**

Solvent extraction is most frequently used technique for isolation of plant antioxidant compounds. The selection of solvent is supported by their different chemical characteristics. Recovery of antioxidant compounds from plant materials is typically accomplished through different extraction techniques taking into account their chemistry and even distribution in the plant matrix. Soluble phenolics are present in higher concentrations in the outer tissues of grains than the inner tissues (Antolovich *et al.* 2000). Antioxidant capacity of different plant extracts is dependent on the solvent nature, temperature, time, pH, presence of oxygen, oxidative agents and enzymes. Polar solvents have been frequently employed for the recovery of polyphenol from a plant matrix. The most suitable of these solvents are (hot or cold) aqueous mixtures containing ethanol, methanol, acetone and ethyl acetate (Peschel *et al.* 2006). Methanol and ethanol have been extensively used to extract antioxidant compounds from legumes. Studies have also demonstrated the efficacy of ethyl acetate to extract polyphenolic compounds from *Vigna sinensis* (Eymur Shafekh *et al.* 2012). Polarity of the solvent is an important factor in optimizing the antioxidant activity of extracts. In general, the amount of total extractable compounds decreased with decreasing polarity of the solvent in the order of water, ethyl acetate, methanol and hexane (Mariod *et al.* 2009).

Hydrogen bonding may induce dramatic changes in the H atom donor activities of phenolic antioxidants. Xu and Chang (2007) suggested 50% acetone was the best solvent for extraction of phenolics from peas, chickpea and yellow soybean, whereas acidic 70% acetone was the best for lentil, black soybean and common beans while Nithiyantham *et al.* (2012) found that 80% methanol served to be a better solvent for extraction of phenolics and tannins in chickpea while 70% acetone, for green peas.

## RESULTS AND DISCUSSION

### Effect of germination on total phenolic content

Phenolic compounds occur as secondary metabolites in all plants (Lin *et al.* 2016). They embrace a considerable range of substances possessing an aromatic ring bearing one or more hydroxysubstituents, although a more precise definition is based on metabolic origin as those substances derived from the shikimate pathway and phenyl propanoid metabolism (Ryan and Robards 1998). Antioxidant properties of polyphenols arise from their high reactivity as hydrogen or electron donors which can stabilize and delocalize the unpaired electron and from their potential to chelate metal ions (termination of Fenton reaction) (Rice-Evans *et al.* 1996).

Sreeramulu *et al.* (2009) reported that pulses and legumes had a TPC that ranged from 62.35 to 418.34 mg/100 g while Marathe *et al.* (2011) reported a TPC in the range of 0.325- 6.378 mg/g GAE in various commonly consumed legumes with the highest concentration being recorded in cowpea (red and brown). Karthiga and Jaganathan (2013) investigated commonly used pulses namely, bengal gram, black gram, green gram, red gram and rajmah to conclude that TPC ranged from 345.2 to 54.43 mg GAE/100 g. Fouad and Rehab (2015) observed a significant increase in the TPC (mg/100 g DW) of lentil seeds during germination which increased from 1341.13 in raw lentil seeds to 1630.20 during germination. Pajak *et al.* (2014) reported that TPC (mg/100 g DM) ranged from 0.72 in mung seeds to 9.95 in mung sprouts where gallic and ferulic acids were the predominant compounds. Huang *et al.* (2014) reported 330% increase in TPC in 4 day germinated soyabean as compared to their seeds. Lin and Lai (2006) reported that germination enhanced phenolics content in soybean with the highest content in 4-day germinated bean. Gharachorloo *et al.* (2013) observed that germinated seeds of lentil, chickpea, white bean and common vetch had higher TPC than their seed counterparts irrespective of the solvent used. Pasko *et al.* (2009) also reported higher TPC in sprouts compared to seeds, suggesting that synthesis of phenolic antioxidants may occur during germination.

### Effect of germination on various antioxidant assays

#### DPPH radical scavenging activity

Model of scavenging free radicals is widely used to evaluate the antioxidant properties in relatively short period, as compared to other methods. DPPH assay is widely used for assessment of free radical scavenging potential due to its stability and simplicity, involving only the direct reaction between the radical and an antioxidant (Kedare and Singh 2011). DPPH free radical scavenging activity by antioxidants is due to their hydrogen donating ability; the more the number of hydroxyl groups, the higher the possibility of free radical scavenging ability. Marathe *et al.* (2011) classified cowpea (brown, red), common beans, peanut, horse gram and fenugreek as legumes with high antioxidant activity while pigeonpea, green gram, chickpea (small brown), cowpea (white), common bean (maroon), black gram, lentils and soyabean exhibited moderate DPPH activities. Chickpea (cream, green and big brown), moth bean, butter bean, pea and common bean (white) had lowest antioxidant activities. DPPH radical scavenging activity ranged from 0.26–1.07 trolox equ mg/g among different pulses as observed by Sreeramulu *et al.* (2009). They reported highest activity in *P. vulgaris* followed by green pea, green gram, red gram, bengal gram and black gram. Dark seed colored cowpea extracts had higher antioxidant activities as compared to light colored seeds however the activity was significantly lower than that of standard antioxidants (Siddhuraja and Becker 2007). Xu and Chang (2007) suggested 70% methanol to be the best solvent for study of DPPH activity from yellow pea and chickpea while 80% acetone to be a better solvent for lentil, black soyabean, yellow soyabean and red kidney beans. Nithiyantham *et al.* (2012) observed significant differences between antioxidant activities of methanolic (IC<sub>50</sub> of 12.17g extract/g DPPH) and acetonic (3.72 g) raw chickpea extracts thereby reflecting the fact that acetone extracts had a greater antioxidant potential while in case of raw peas higher DPPH (IC<sub>50</sub> 13.67 g extract/g DPPH) scavenging activity was observed in methanolic extracts. Higher DPPH inhibition activities were registered in ethyl acetate extracts of raw and sprouted green beans as compared to methanol,

n-hexane and n-butanol extracts (Kim *et al.* 2012). Gharchaloo *et al.* (2013) reported that acetone extracts of chickpea sprouts functioned more efficiently at delaying oxidation in tallow as compared with hexane and methanolic extracts, which might be due to the differences in solvents polarity and consequently the type of the extracted compounds. Ethanolic extracts of raw and sprouted lentil seed exhibited 40.76 to 62.19% inhibition of DPPH free radical (Fouad and Rehab 2015). The antioxidant capacity of raw cowpeas (27.4  $\mu\text{mol TEAC/g DM}$ ) increased by 58% and 67% after 4 and 6 days of germination respectively (Doblado *et al.* 2007).

#### **ABTS radical scavenging activity**

ABTS, generated from oxidation of 2, 2-azino-bis (3 ethylbenzothiazoline-6-sulphonic acid) by potassium persulfate, is presented as an excellent tool for determining the antioxidant activity of hydrogen-donating antioxidants (scavengers of lipid peroxy radicals). Marathe *et al.* (2011) grouped cowpea (red and brown), soyabean, common beans (red, brown), horse gram and fenugreek under legumes showing high ABTS activity ( $>12 \mu\text{mol TEAC/g}$ ) and high phenolic content while common bean (white), lablab bean, pea (black), pigeonpea, green gram, chickpea, cowpea (white), common bean, lentil and black gram showed moderate activity (6-12  $\mu\text{mol TEAC/g}$ ) against ABTS free radical. Legumes with low phenolic content such as lablab bean (white, cream), chickpea (cream, green and big brown), butter bean and pea (white and green) showed lowest antioxidant activity ( $<6 \mu\text{mol TEAC/g}$ ). Acetone extracts of raw chickpea exhibited better ability to quench free radicals by the ABTS assay as compared to their methanolic counterparts (Nithiyantham *et al.* 2012) while the same authors reported that methanolic extracts of raw pea exhibited higher ABTS (6155 Troeque (mmol/g extract) scavenging activity. Siddhuraju and Becker (2007) reported that raw cowpea seed samples with the highest TPC have more ability to quench free radicals (ABTS<sup>+</sup>). Villalobos *et al.* (2016) reported 60 % inhibition of the ABTS radical in defatted soyabean flour.

#### **Reducing power assay**

The reducing properties of antioxidants are generally

associated with the presence of reductones and the antioxidant action of reductones is based on the breaking of free-radical chain by donating a hydrogen atom. The reducing power of a compound serves as a significant indicator of its potential antioxidant activity (Das *et al.* 2012). Reducing power explains some aspect of antioxidant activity and can be directly related to the amount of phenolic compounds. Djordjevic *et al.* (2011) reported significantly greater FRAP for mungbeanethanolic extract (24.98  $\text{nmol mg}^{-1}$  extract) when compared with soybean ethanolic extract (8.34  $\text{nmolmg}^{-1}$  extract). Kumar *et al.* (2010) observed that FRAP value of yellow soybeans ranged from 11 to 28 FE  $\mu\text{mol g}^{-1}$ , while of black soybeans and red kidney beans ranged from 12.7-99.3 and 28.5-92.2 FE  $\mu\text{mol g}^{-1}$  respectively. Anwar *et al.* (2007) observed a linear correlation between TPC and reducing power. Siddhuraju and Becker (2007) observed that raw seed extracts of cowpea showed higher FRAP antioxidant activity, DPPH and ABTS activity besides high correlation between total phenolics and antioxidant activity as compared to processed cowpea extracts.

#### **Application in dairy products**

Currently, the potential health benefits of the phytochemicals and their ability to be incorporated into dairy foods as nutraceuticals has received considerable interest in food industry. There has been an increase in the addition of phenolic compounds to dairy products, thus improving their technological functionality and nutritional value (O'Connell and Fox 2001). Attempts have been made to produce fermented dairy foods supplemented with polyphenolic compounds from grape wine (Howard *et al.* 2000), grape pomace (Tseng and Zhao 2013), aronia juice (Nguyen and Hwang 2016), Hibiscus extract (Iwalokun and Shittu 2007), tea infusions (Najgebauer- Lejko *et al.* 2011), apple polyphenols (Sun-Waterhouse *et al.* 2012), polyphenolic extract of strawberry (Singh *et al.* 2013), sour cherry pulp (Sengul *et al.* 2012), strawberry extract (Singh *et al.* 2013) and ethanolic extract of pomegranate peel (Sandhya *et al.* 2018). Various legume extracts have been suitably used to prepare milk shakes containing legume extract-bovine milk blends in the ratio 50:50. These milk blends were found to be sensorially acceptable to consumers (Preeti and Sheel 2015). These



products are considered functional dairy products (Mattila-Sandholm and Saarela 2005).

## CONCLUSION

Enrichment of milk and meat products with germinated legume polyphenolic extracts would offer a new way for delivering biologically active phytochemicals to the human diet. Moreover, natural compounds enjoy positive consumer image and have application in development of novel healthy products. Taking into consideration the potential significance of phenol antioxidants in prevention of wide range of degenerative physiological processes and food deterioration milk and meat products can be enriched with phytochemical/ bioactive compounds such as sprouted legume extract/powders as source of natural antioxidants to increase the functionality and antioxidant activity of these products and in this way to improve the consumer's protection against pathologies related with free radicals.

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