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Phytochemical composition and Total Antioxidant Activity (TAA) of Functional Food Supplements

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Abstract

Functional foods that contain significant amounts of bioactive components may provide desirable health benefits beyond basic nutrition and play important roles in the prevention of chronic diseases. Among the modifiable lifestyle-related factors, the main role is played by diet showing an inverse association between the intake of antioxidant-rich food and the risk of diseases. The present study explored the various phytochemical contents of the developed Functional Food Supplements (FFS) and thereby exhibited their combined effect on the total antioxidant activity. Among the different solvent media used to study the total antioxidant activity of the functional food supplements, aqueous ethanol showed the highest for supplement I (1.09 µg / g) while absolute ethanol extracted the maximum for supplement II (0.83 µg/g). The phytochemical content of the two supplements also showed significant variations due to the differences in their processing techniques.

Keywords- Phytochemicals, Total Antioxidant Activity, Functional Food Supplements, oxidative stress

I. INTRODUCTION

One of the major functions of phytochemicals is their role as antioxidants. Natural antioxidants exhibit a wide range of pharmacological activities, and have been shown to have anticancer, anti-inflammatory and anti-aging properties [1].

Oxidative stress impairment or altered antioxidant status have been suggested as pivotal keys in the onset of certain chronic diseases such as metabolic syndrome (MS), type 2 diabetes, CVD etc. [2, 3].

It has been indicated that an impaired balance between free radical production and an impaired antioxidant defense system resulting in accumulation of oxidative damage, could play important roles in pathological conditions such as insulin resistance, altered energy production and endothelial dysfunction as main risk factors of these diseases [4]. Dietary antioxidants have been reported to protect against oxidative damage and their complications [5].

Phytochemicals have been linked to many other positive health effects in humans and animal studies including coronary heart disease, diabetes, high blood pressure, inflammatory processes, infection, psychotic diseases, ulcers and macular degeneration [6].

Total antioxidant activity (TAA) assessment is an established methodology to measure different elements of antioxidant defense system together [7]. Extracting antioxidants from

plant material most often involves the method of solvent extraction. The choice of solvent has been shown to have a significant influence on the concentration of antioxidants extracted [8].

Studies have shown that phytochemical extracts from food exhibit strong antioxidant and antiproliferative activities and that the major part of total antioxidant activity is from the combination of phytochemicals. The additive and synergistic effects of phytochemicals in foods are responsible for these potent antioxidant and anticancer activities and that the benefit of a diet rich in natural bioactive compounds is attributed to the complex mixture of phytochemicals present in whole foods [9].

Hence, an attempt was taken to develop two functional food supplements (FFS) from naturally available food resources involving two different processing techniques. The developed FFSs were analyzed for their phytochemical properties. The total antioxidant activity (TAA) of the supplements which is the combined effect of various phytochemical constituents was analyzed using different solvent extracting medium.

II. MATERIALS AND METHODS

2.1. Selection of ingredients.

Food substances containing bioactive compounds, which has got greater health benefits and therapeutic effects, but are less utilized or consumed in the daily diet, were selected for the formulation of FFS. Thus based on the support from the previous scientific investigations, the constituents for the FFS was chosen to contain Barley, Ragi, Banana (Rasakadhali / Njalipoovan - mature, unripe), Defatted Soy Flour, Drumstick leaves powder and Mushroom powder in different proportions. The materials for the study was procured locally and processed in the laboratory.

2.2. Standardization of FFS.

In the first processing technique (I), the constituents were dried individually in a cabinet drier, powdered mechanically, sieved thrice to obtain a fine powder and then blended into different proportions to formulate the FFS (I). In the second processing technique (II), fermentation followed by dehydration was the processing techniques. Ragi was cleaned, washed, allowed to soak in triple the amount of water for 12 hrs, drained excess water, kept covered in a muslin cloth, allowed to germinate for 24hrs, cabinet dried for four hours, then powdered, sieved and packed. The other constituents were dried individually, powdered mechanically and then packed separately to formulate the FFS II. Approximate proportion of ingredients for formulating the supplement is as follows: Barley – 20 to 40 per cent, Ragi – 20 to 30 per cent, Banana powder – 20 to 40 per cent, Defatted Soy Flour - 15 to 20 per cent, Drumstick leaves powder – 0 to 10 per cent, Mushroom powder - 0 to10 per cent. The best identified FFSs were further investigated in depth for their phytochemical compositionss and total antioxidant activity (TAA).

2.3. Estimation of phytochemical composition.

The phytochemical contents of the identified FFSs were estimated using standard procedure.

Table 01. Analytical procedures for phytochemicals

Phytochemicals	Method
Flavonoid	Bohm and Kocipai- Abyazan (1974) [10]
Poly phenols	Sadasivam and Manickam (1992) [11]
Tannin	Sadasivam and Manickam (1992) [11]
Saponin	Obdoni and Ochuko (2001) [12]
Alkaloid	Harborne method (1973) [13]
Oxalates	Colorimetric method (AOAC, 2005) [14]
Vitamin C	Sadasivam and Manickam (1992) [11]
Vitamin E	AOAC (2005) -HPLC [14]
ß- carotene	Colorimetric method -Sadasivam and Manickam (1992) [11]

2.4. Determination of Total Antioxidant Activity (TAA).

The antioxidant activity of the extracts was evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al.* (1999) [15]. The assay is based on the reduction of Mo (VI)–Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH. The sample was extracted using various solvents like petroleum ether, Chloroform – water mixture, Acetic acid, Acetone, Ethanol, Aqueous Ethanol, n Butanol, Benzene, Ethyl acetate, Diethyl ether and Hot water. 0.3 ml of extract was combined with 3ml of reagent solution (0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95^0 C for 90 min. Then the absorbance of the solution was measured at 695 nm using a spectrophotometer against blank after cooling to room temperature. The respective solvent (0.3 ml) in the place of extract is used as the blank. The antioxidant activity is expressed as the number of equivalents of ascorbic acid.

2.5. Statistical analysis:

The statistical processing of the data obtained from all studies is expressed as Mean ± standard deviation (SD) of three separate experiments using the computer programme Excel. Student's 't' test were achieved to calculate significant differences in the treatment means.

III. RESULTS & DISCUSSION

The best combination of the constituents for FFS I and II was identified based on their computed nutritional qualities, amino acid scores, phytonutrient contents and sensory

attributes. The identified combination from the dehydration technique was denoted as FFS I and that from the fermentation technique was denoted as FFS II.

3.1. Phytochemical composition of Functional Food Supplements I & II

The phytochemicals are found to possess therapeutic functions like antioxidant and reducing properties. Flavonoids and sulfur-containing compounds are classes of compounds that may be important in reducing the risk of atherosclerosis and other lifestyle diseases. Within these categories are multiple possible compounds, most of which are not well characterized and whose modes of action has to be established [16].

Table 02. Phytochemical composition of FFS I & II

Constituents (per 100 g)	FFS I	FFS II	t values
Flavonoids (%)	4.6	1.23	142.79**
Poly phenols (mg)	73.25	48.25	19.61**
Tannin (Tannic Acid Equivallence, mg)	10.09	5.77	23.15**
Saponin (%)	3.64	1.28	14.55**
Alkaloids (%)	0.8	0.2	47.38**
Oxalates (mg)	5.32	1.92	42.79**
Phytates (mg)	155	93.5	26.73**

Polyphenols which are derived mostly from plant foods are widely distributed in the human diet. Dietary total polyphenol intake could be as high as 1 g/d, which is much higher than all other classes of phytochemicals and known dietary antioxidants e.g. antioxidant vitamins [17].

The major classes of polyphenols are flavonoids. Several in vitro and in vivo studies have suggested that dietary flavonoids, may induct an antioxidant defense system and exert beneficial effects on the vascular system via an improving endothelial function and inhibiting low density lipoprotein oxidation [18]. Moreover, higher intake of flavonoids is associated with good health and wellbeing. The flavonoid contents of FFS I was 4.6 per cent while FFS II was found to contain 1.23 per cent which was highly significant at 5 per cent level. Fermentation technique involved in the processing of FFSs might have contributed to the reduction of flavonoids in FFS II.

From the above table, it was noted that the tannin contents of FFS I and II were 10.09 mg and 5.77 mg respectively. Tannins (tannic acids) and saponins are responsible for the antibacterial activity of the plant seed extracts [19].

Fig: 1 Effect of processing on flavanoids, saponins & alkaloids of FFS I & II

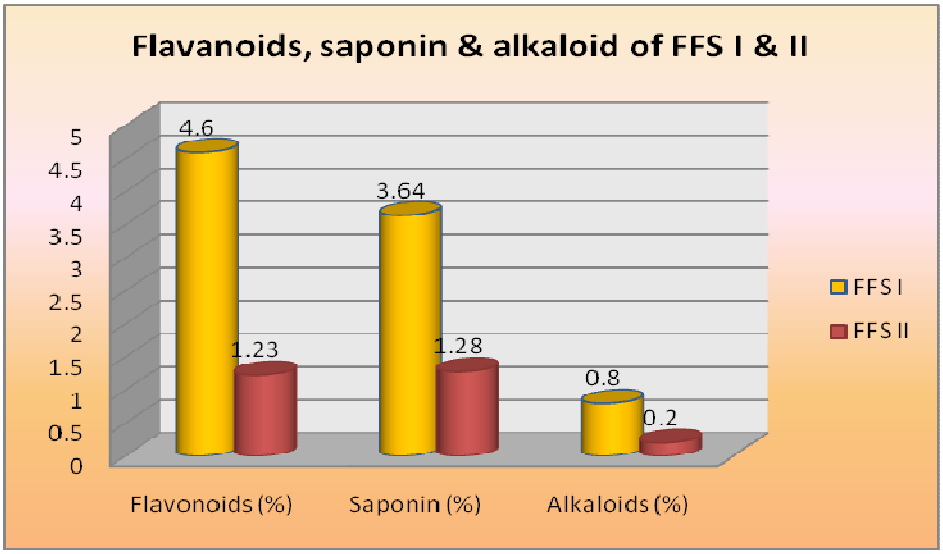
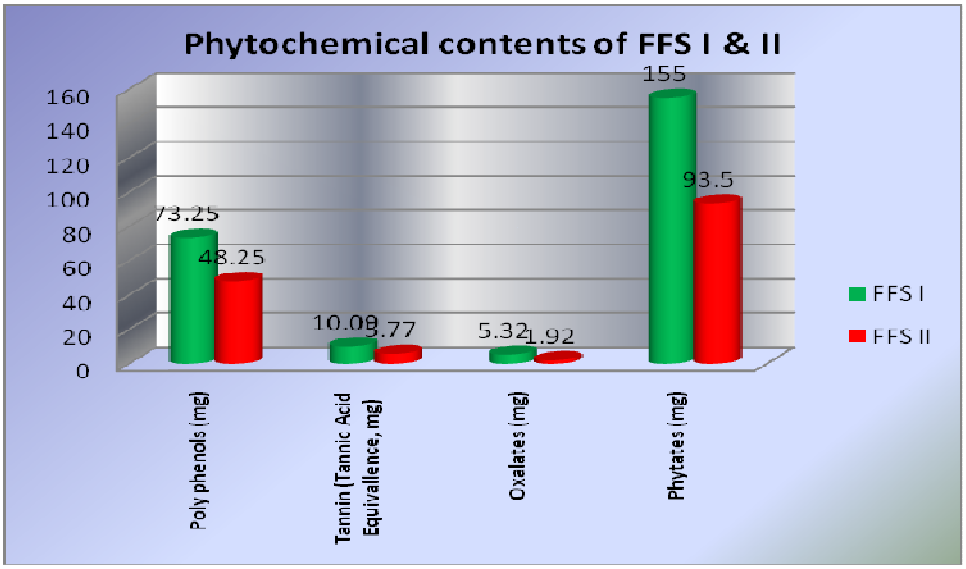


Fig: 2 Effect of processing on phytochemicals of FFS I & II



The results indicated that the phytochemicals viz. polyphenols, alkaloids, saponins, oxalates, tannins and phytates analyzed in FFS I & II follows a similar pattern of reduction on fermentation as that of flavonoids.

3.2. Total Antioxidant Activity (TAA) of Functional Food Supplements I & II.

The consumption of dietary antioxidants will help to prevent free radical damage. Antioxidants have the ability to scavenge free radicals by inhibiting the initiation step or interrupting the propagation step of oxidation of lipid and as preventive antioxidants which slow the rate of oxidation by several actions [20].

Table 03. Total Antioxidant Activity (TAA) (µg / g) of FFS I & II

SOLVENTS	FFS I	FFS II	Mean
Petroleum ether	0.84	0.62	0.728
$CHCl_3$:H_20	0.95	0.66	0.803
Acetic acid	0.68	0.51	0.594
Acetone	0.80	0.60	0.698
Ethanol	0.98	0.83	0.901
Aqueous Ethanol	1.09	0.82	0.953
n butanol	0.74	0.53	0.633
Benzene	0.75	0.55	0.648
Ethyl acetate	0.95	0.66	0.803
Diethyl ether	0.61	0.49	0.55
Hot water	0.57	0.41	0.488
Mean	0.811	0.607	

$F_{(10,66)}$ Solvent	345.21 **	
Product	1869.89 **	
Solvent * product	13.33 **	
CD - solvent	0.022	
CD – product	0.009	
CD – solvent * product	0.023	

Different solvent media were used to extract the total antioxidant activity of the developed FFS I and II. Results indicated that the total antioxidant activity of FFS I was 0.811 µg / g and that of FFS II was 0.607 µg / g. The total antioxidant activity (1.09 µg / g) was highest when aqueous ethanol was used. But on the other hand, for FFS II (0.83 µg / g) the maximum antioxidant activity was derived while using absolute ethanol as solvent extraction medium. However it could be noted that there was not much variation in the activity levels of aqueous ethanol (0.82 µg / g) and absolute ethanol in the case of FFS II. In both FFS I (0.57 µg / g) & II (0.41 µg / g), hot water extraction produced the least antioxidant activity.

For FFS I the total antioxidant activity of different solvents followed the pattern of aqueous ethanol> ethanol> $CHCl_3$:H20> ethyl acetate > petroleum ether > acetone > benzene > n butanol > acetic acid > diethyl ether > hot water. On the other hand FFS II followed the pattern of ethanol> aqueous ethanol> CHCl3:H20> ethyl acetate > petroleum ether > acetone > benzene > n butanol > acetic acid > diethyl ether > hot water. This shows that both FFS I

& II follow a similar trend in the antioxidant activity levels on using different solvent extraction media.

The amount of the antioxidant components that can be extracted from a plant material is mainly affected by the vigor of the extraction procedure, which may probably vary from sample to sample. Amongst other contributing factors, efficiency of the extracting solvent to dissolve endogenous compounds might also be very important [21]. The polarity and solubility of the solvent and the compound also has a role in this activity.

Nout and Ngoddy [22] observed that during fermentation, the reduction of phytic acid, tannin and polyphenol is due to the enzymes like phytase, polyphenol oxidase etc. present in the food grain or micro flora content. Most of these compounds in plants could be removed by several processing methods such as soaking, germination, boiling, autoclaving, fermentation, genetic manipulation and other processing methods [23]. Thus fermentation technique involved in FFS II can be considered as the reason for its reduction in antioxidant activity.

IV. CONCLUSION

Earlier, most of the phytochemicals were considered as anti nutrients and various processing techniques were applied to reduce their content. Later studies were carried out to justify the pharmacological effects of the phytochemicals in various diseases. Though there was considerable variation brought about in the phytochemical contents by the different processing techniques involved in the development of the FFSs, their high bio availability and increased therapeutic action is found to be much favourable.

However, it must also be taken into consideration that, though there was decrease in the phytochemicals in FFS II, fermentation has brought about favourable changes in the other nutrients and also it has increased the activities of the available phytochemicals.

It could be concluded that various solvent extracts exhibited varying levels of TAA in FFS I & II, the antioxidant potency of both the supplements were highly significant. FFS I & II exhibit higher activities in preventing the reactive oxygen species (ROS) and free radicals from oxidizing. This in turn prevents lipid peroxidation and various chronic illnesses. This proves both FFS I & II to be equally good at scavenging properties. This might be brought about by the high levels of vitamins like beta carotene, Vitamin C & E and also selenium. Some of the phytochemicals like flavanoids, polyphenols etc are also found to exhibit greater antioxidant properties.

Functional food supplements from natural resources opens a wide market in the current situation. Thus FFS I with its higher phytochemical contents and FFS II with its higher potency and activity are on the same scales in exhibiting the disease preventing properties.

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