Research Paper

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Development and Analysis of food based Antioxidant Premix and Formulation of Recipe

Food Science

KEYWORDS : Antioxidant , DPPH, Food based Premix

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ABSTRACT

Antioxidant protect human body from oxidative stress caused by various agents because of their antibacterial, antiviral, anti- inflammatory and vasodilatory properties. Natural antioxidants sources include green leafy vegetables, fruits ,spices and condiments which helps in the prevention and management of chronic degenerative diseases. In the present investigation cereal and pulse based mixes were developed named as Mix C, Mix A and Mix B The analysis of nutrients and antioxidants was done by using the standardized techniques(AOAC,2005). The total antioxidant activity was determined by using DPPH method. Results of the nutritional and antioxidant components highlighted that Mix C (Moisture 10.3%; Ash 4.40g; Protein 14.57gm; Fiber 2.32gm; Carbohydrate 62.19 k cal and Fat 6.47gm, vitamin C 57.6mg, vitamin E 52.46mg,β-carotene 182.66µg, total polyphenols29.43Mg); Mix A (Moisture 9.93%; Ash 3.39 gm; Protein 13.2gm; Fiber 6.03gm; Carbohydrate 61.64 k cal and Fat 5.77 gm, Vitamin C 83.66mg, Vitamin E 63.66 mg, β carotene 257.33µg and total polyphenols 31.66mg); Mix B (Moisture 10.33%; Ash 3.2 gm; Protein 14.32gm; Fiber 5.61 gm; Carbohydrate 59.95 Kcal and fat 5.57 gm, Vitamin C 86.33, Vitamin E 64.5 mg, β carotene 277.66µg and total polyphenols 42.3mg) per 100 gram basis. All the three samples showed moderate to little free radical scavenging activity. Mix C showed 67.4µg/ml, mix A showed 71.86µg/ml and mix B showed 76.32µg/ml respectively. Mathari prepared by mix were evaluated for organoleptic characters like appearance, colour, texture, after taste and overall acceptability by using five point hedonic rating scale. Therefore utilization of prepared mixes help in the overall well being of the society.

Introduction

Antioxidants are naturally occurring substances which are responsible for inhibition of oxidation process by hindering the oxidizing chain reactions and prevent cells to get damage. (Srinivasan and Gayatri 2012). The highly reactive free radicals generated by oxidative stress start chain reactions which are capable of attacking the healthy cells of the body resulting into the damage of their structure and function resulting into the development of various chronic degenerative disease like cancer, atherosclerosis, heart disease, stroke, diabetes mellitus, rheumatoid arthritis, osteoporosis, ulcers etc. Studies suggested that Inflammation is the major risk factor and one of the potent mechanism which is produced by the immune system which leads to the development of the oxidative stress in the body.. Another risk factor is excess nourishment, stress in combination with lack of physical inactivity collectively result in increased blood glucose level and fatty acid accumulation within muscle, adipose tissue and pancreatic cells. Exposure to air pollution and behavoiur such as smoking, tobacco chewing unhealthy diet and physical inactivity can lead to obesity. Antioxidant and antimicrobial properties are responsible for the well being of human body. Naturally occurring antioxidants reflect number of biological effects which include antibacterial, antiviral, anti-inflammatory and vasodilatory activities. The consumption of natural antioxidants can improve the quality of life by reducing oxidative stress caused by various agents .Various green leafy vegetables and fruits ,spices and condiments contain good amount of antioxidants which when consumed help in prevention and management of various chronic diseases. The possible toxicity of synthetic antioxidants has resulted in decreased use of these compounds in foods for human consumption. As a result of this and due to the appeal of natural products to consumers, numerous studies have been carried out in order to identify naturally occurring compounds which posses antioxidant activities such as phenolic phytochemical (Jacob and Shenbagaraman 2011).Human diet works as a tool to protect against cellular damage caused due to exposure to stress resulting into production of free radicals. Hence the diet containing good amount of fruits and vegetables contains optimal mix of antioxidants like Vitamin C, Vitamin E, Polyphenols , Carotenoids and complex carbohydrate (Mahattanatawee et al 2006).There are many studies available on the development, evaluation and supplementation of various health mixes utilizing whole and sprouted cereals , millets and oil seeds. Studies related to incorporation of green leafy vegetables and amla are limited. (Kawsalya and Indira, 2010). Keeping all the above mentioned points in mind the research was planned with the objective of developing cereal based antioxidant mix and analyzing the total antioxidant activity.

METHODOLOGY

Ingredients used for the development of premix were selected on the basis of nutrient content, cost and antioxidant and anti inflammatory properties . Commercially released varieties of the ingredients were procured from Agriculture Research Station, Durgapura, Jaipur. Some identified varieties of the ingredients include – Wheat Raj-1482 Bengal gram-RSG-973, Soybean-Pratap soya, Amla-Banarsi and Curry leaves The grains were cleaned , washed dried and then stored in a polythene bag in a cool and a dry palace prior to use. The curry leaves was firstly sorted and then washed. After washing blanching was done at 85 °C boiling water for 5 min , oven died for 85°C for 1 hour and then grinded to convert in fine powder and stored in a air tight container in dry and cool place. The same procedure was followed for the amla instead of blanching it was grated. Flow chart for the different premixes Fig 1.

Preparation of the seeds.

Cleaning
↓
Washing
↓
Drying
↓
Storage

Preparation of the leaves and amla-

curry leaves	amla
↓	↓
Sorting	grated
↓	↓
Washing	oven dried (85°c)
↓	↓
Blanching (85°C)	stored in the air tight container

↓ 5-6 min.
Oven drying
↓
Stored in air tight container

Development of premix.

In the present study one standard mix was prepared named as Mix C .Each 100gms of premix contain wheat 75 gms, Bengal gram 15 gms, soybean 10 gms. Another premix named Antioxidant rich premix A was prepared by using the above mentioned ingredients in which 5 gms of wheat flour was supplemented by incorporating5 gms of Antioxidant rich curry leaves. The third type of premix was Antioxidant rich premix B which was also prepared by above mentioned ingredients in which the content of wheat flour was supplemented by incorporating the antioxidant content like 2.5 gms amla and 2.5 gms curry leaf powder. Chemical analysis of these mixes was done using below mentioned parameters.

Nutrient Analysis

Proximate analysis was carried out by using the standardized method of AOAC (2005). The moisture content of all the three prepared premixes were determined by drying at 105°C until a constant weight is attained The nitrogen content thus determined was multiplied by factor 6.25 to get crude protein content. Fat was determined by extraction with petroleum ether (80°C) with the help of Soxlet apparatus. Ash content was estimated by using Muffle furnace at a temperature of 550°C . The estimation of crude fiber content was carried out by acid alkali method using the apparatus fibra plus. Carbohydrate was calculated by difference by subtracting the values of moisture, ash, protein ,fat and fiber.

Determination of antioxidant components

EXTRACTION- All the three samples of premixes were extracted with 80% aqueous methanol. At 200 rpm for 2 hour at ambient temperature with continuous stirring in a dark bottle. Filtration was done by using filter paper(whatman No. 4). The obtained solution were then used for β- carotene, total polyphenols and total antioxidant activity.

The non enzymatic antioxidants like vitamin C were estimated by using dye method of Sheel Sharma (2007). Total polyphenols were determined by using the Folin Ciocalteu (FC) reagent method of AOAC(2005) . Total phenolic content was expressed as mg gallic acid equivalent (GAE) /100 gm sample. Bcarotene were determined by using standard method of NIN (2003). The beta carotene was then calculated using the following expression: β carotene (µg/100g) = ODx13.9x 10^4x100/wt of samplex560x1000. Vitamin E was determined by using the standard method of NIN (2003)

Determination of Total Antioxidant Activity

Free radical scavenging activity of the mixes was measured using 1,1-diphenyl-2- picryl-hydrazil (DPPH) by the method of Gupta and Prakash (2009) .DPPH is a commercial oxidizing radical which is reduced by antioxidants. The disappearance of the DPPH radical absorption at a characteristic wavelength is monitored by decrease in optical density.(Singh et al 2002).

Development of Baked Mathari

Baked mataris were prepared by all the three different premix

Ingredients	Standard premix	Antioxidant premix A	Antioxidant premix B
Wheat flour	75 gms	70 gms	70 gms
Soybean flour	15 gms	15 gms	15 gms
Roasted Bengal gram flour	10 gms	10 gms	10 gms
Curry leaves powder	-	5 gms	2.5 gms
Amla powder	-	-	2.5 gms
Salt	Acc to taste	Acc to taste	Acc to taste
Ajwain	½ tsp	½ tsp	½ tsp
Baking powder	½ tsp	½ tsp	½ tsp
Oil	20 gms	20 gms	20 gms

Sensory evaluation

Sensory evaluation of recipe prepared (mathries) was based on five point hedonic rating scale. All the sensory attributes in terms of its appearance, color,texture, flavor and overall acceptability were evaluated by a semi trained panel of ten members.

Statistical Evaluation

The readings of estimations were taken in triplicates which were then tabulated and subjected to statically analysis. The results were finally expressed as means, sds and t test .

Results and discussions

The result of the proximate composition of the three prepared premixes was shown in table 1. Moisture content of the premixes ranged from 10 to 11%. Similar trends were noticed by several researchesrs. Banu et al (2012) and Zaltica& Jolana(2010) reported moisture content of 11.3% to 11% for different composite mixes. The Ash content was highest in standard premix and was found least in antioxidant rich premix B which may be due to the seed coats as majority of grains were used with the seed coat. Similar result were noticed by Banu et al (2012). There is a slight difference in the protein content of all the three samples of premixes because the amount of soybean remains the same. Ziaulhaq et al (2004) reported the similar results since it contain the micronutrient of wheat bran and germ. Crude fiber includes mostly lignin, cellulose and hemicelluloses (islam et al 2007). The fiber content varies from 2 to 6%. Mix A had the highest fiber content and the standard premix had least fiber content, this is due to the presence of curry leaf powder and amla powder in the antioxidant premix respectively. The significant rise in the fiber content of the antioxidant rich premix has several health benefits (Jideani & onwubali2009). According to a well documented studies it is now obvious that dietary fiber plays a significant role in the prevention of several chronic diseases like cancer diabetes etc. Carbohydrate determined by composite method varied from 59 to 62% among which standard premix contain highest carbohydrate as this contain high sources of energy as compared to other antioxidant rich premixes. Fat content of all the premixes varied from 5 to 6 gm per 100g respectively.

Table 1 Proximate composition of standard and antioxidant rich premixes.

Proximate principles	Standard premix	Antioxidant rich premix A	Antioxidant rich premix B
Moisture %	10.3±0.04	9.93±0.04	11.33±0.30
Ash (g/100g)	4.40±0.15	3.39±0.12	3.2±0.29
Protein (g/100g)	14.57±0.33	14.20±0.05	14.32±0.37
Crude fiber (g/100g)	2.32±0.01	6.03±0.02*	5.61±0.27
Carbohydrate(k cal)	62.19±0.19	61.64±0.04	59.95±0.77
Fat (g/100g)	6.47±0.04	5.77±0.04	5.57±0.32

Statistically significant at($p<0.05$) level.

Antioxidant components

Ascorbic acid is a water soluble antioxidant which readily scavenges reactive oxygen there by prevent oxidative damage. (Srinivasan and Gayatri 2012). It involves in the activities and function of cells. It promotes the absorption of iron, boost immune system neutralizes blood toxin, helps in maintaining the epithelial tissue of the skin. The Vitamin C content of antioxidant premix B was found to be highest (table 1). The β carotene level of antioxidant rich premix A was found to be 277.66µg/100 gm when compared with the other two premixes. Similar study were reported by Reddy (1999) assured that green leaf contain high amount of β carotene. Vitamin E (Tocopherol) is found in number of animal and plant product. Tocopherol are important antioxidant in foods specially vegetable oils. The mainly helps in inhibition of unsaturated fatty acids in the tissues, also helps

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to maintain healthy cells, protects unsaturated fatty acids and Vitamin A against oxidation.(Philippo et al 2005). Antioxidant premix B contain 64.5 mg/100gm of vitamin E followed by Antioxidant premix A which contain 63.66 mg/100gm and standard premix which only contain 52.46mg/100gm. Phenolics are the secondary aromatic plant metabolites which is widely present throughout plant kingdom and are responsible for the colour, sensory attributes , nutritional and antioxidant properties. The green leafy vegetables and fruits like amla have varying level of polyphenols. The total polyphenol content of content of Antioxidant rich premix B was highest when compared to both the premixes. The total polyphenol content ranged between 29.3 to 42.3mg /100 gm, the variation in polyphenol content is widely depending on the variety of vegetables and fruits therefore the comparison is very difficult. (Gupta and Prakash 2008).

Table 2- Antioxidants components of standard and Antioxidant Rich Mixes.

Components	Mix C	Mix A	Mix B
Vitamin C (mg/100gm)	56.6±0.16	83.4±1.52	86.23±0.28*
Vitamin E (mg/100gm)	52.46±0.33	63.66±1.52	64.5±0.5
B-carotene (µg/100mg)	182.26±0.12	277.66±1.52	257.33±1.52
Total polyphenols(mg/100gm).	29.3±0.12	31.66±0.57	42.3±0.21

Statistically significant at($p<0.05$) level.

Total antioxidant activity

Auto oxidation of unsaturated lipid in the food is caused by free radical but presence of antioxidants reduce these radical chain of oxidation and donate electron and form stable form end product (Kaur and Perkins 1991, Sherwin 1978). The three samples of premixes prepared were tested by DPPH method and the result is shown in table 2.Antioxidants react with DPPH which convert into α,α-diphenyl –β-picryl hydrazine. The degree of discoloration indicates the scavenging potential of antioxidant present in flour (Singh et al 2002). All the three samples showed moderate to little free radical scavenging activity. Standard premix showed 67.4µg/ml, antioxidant rich premix showed 71.86µg/ml and antioxidant rich premix showed 76.32µg/ml respectively. Comparative analysis indicate that both antioxidant rich premix A and B showed a significant ($p<0.05$) increase in the antioxidant activity as compared to standard premix. Earlier Anita and Jayshree(1999) indicated that curry leaves are the potent source of antioxidants.

Table 3

Total Antioxidant Activity	Mix C	Mix A	Mix C
	67.4µg/ml	71.86µg/ml	76.32µg/ml

Sensory Evaluation:

The mean scores of sensory evaluation were calculated and compared with standard recipe mean scores. The mean score for appearance of mathari prepared by using Antioxidant premix A were similar to the mathari prepared by standard flour i.e 3.8, however the score of mathari prepared prepared from antioxidant rich flour was slightly low i.e 3.6. colour attribute of both standard and Antioxidant A was also similar i.e. 3.4 and it was liked however in case of antioxidant B it was not liked and scored just 2.8. The taste of mathari prepared by Antioxidant rich A was best appreciated and liked as it scored 4.2 which was better than mathari prepared by standard mix which proves that adding curry leaves enhance the taste of the product. In case of mathari prepared by using Antioxidant premix B a peculiar after taste was felt which may be because of the presence of amla powder. However the overall acceptability of all the three matharis was almost similar and liked by the panelist.

Table 4

Sensory attributes	Standard premix	Antioxidant premix A	Antioxidant premix B
Appearance	3.8±0.44	3.8±0.44	3.6±0.54
Colour	3.4±0.54	3.4±0.54	2.8±0.44
Taste	3.3±0.44	4.2±0.44	3.4±0.54
After taste	3±0.70	3.4±0.54	2.4±0.54
Overall acceptability	4±00	4±00	3.6±0.48

Conclusions

Antioxidant has a very specific role to protect the human body from the damage caused by reactive oxygen species. Natural plant based antioxidant has a wide range of biological effects including antibacterial antiviral and anti inflammatory properties. Therefore, it is necessary to enrich our diet with antioxidant to fight against various chronic diseases. To bring about the healthy life, it is necessary to have a sufficient and nutritive diet. In conclusion the developed Wheat based antioxidant premix has a good total antioxidant activity. Therefore utilization of prepared premixes help in the overall well being of the society.

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