

Development and Analysis of Ragi Based Antioxidant Rich Premix and Formulation of Recipe

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Abstract: Antioxidants are the components that inhibit or delay the oxidation process by blocking the initiation or propagation of oxidizing chain reactions. Various green leafy vegetables, fruits, spices and condiments contain fairly good amount of antioxidants, which when consumed helps in reducing inflammation and ultimately reduces the risk of non communicable diseases. In the present investigation three Ragi based premixes were prepared. One served as control (Mix C) and other two as antioxidant rich: Mix A (enriched with curry leaves) and Mix B (enriched with both curry leaves and amla) were developed. The vitamin C content of mix C was found to be 57.6mg/100gm, mix A has 83.66 mg/100gm vitamin and 86.33 mg/100gm respectively. The β Carotene content of both the mix A and B was found to be 257.33 μ g/100 gm 277.66 μ g/100 gm respectively when compared to the standard premix which only has 182.66 μ g/100 gm of β carotene content. The Vitamin E content of mix B was much higher than mix A, mix B contain 64.5 mg /100 gm of vitamin E followed by mix A which contain 63.66 mg/100 gm and mix C which only contain 52.46 mg/100 gm. The total polyphenol content ranged between 29.3 to 42.3mg /100 gm. All the three samples showed moderate to little free radical scavenging activity. Mix C showed 67.4 μ g/ml, mix A showed 71.86 μ g/ml and mix B showed 76.32 μ g/ml respectively. Comparative analysis indicates that both mix A and B have more antioxidant activity as compared to mix C. The results of the sensory evaluation of the mathari prepared by all the three mixes revealed that mean overall acceptability of the mathari prepared with the mix A scored highest and was liked very much when compared with mix C and mix B. Thus it can be concludes that antioxidant plays an important role in the protecting the human body against damage by reactive oxygen species (ROS). Natural antioxidants were known to exhibit a wide range of biological effects including antibacterial, antiviral, anti-inflammatory properties. Therefore, it is necessary to enrich our diet with antioxidant to fight against various chronic diseases. The developed mixes have good total antioxidant activity. Therefore utilization of prepared mixes help in the overall well being of the society.

Keywords: Antioxidant, total antioxidant activity, Ragi

1. Introduction

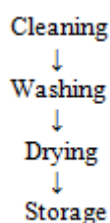
Antioxidants are naturally occurring substances responsible for inhibition of oxidation process by hindering the oxidizing chain reactions thereby preventing the damage caused to cells. The major action of antioxidants in the cells is to prevent damage due to the action of reactive oxygen species (Srinivasan 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 disease like cancer, atherosclerosis, heart disease, stroke, diabetes mellitus, rheumatoid arthritis, osteoporosis, ulcers etc. Studies suggested that Inflammation is one of the major risk factor and one of the potent mechanism which is produced by the immune system. It develops the oxidative stress in the body as oxidative stress and inflammatory processes are linked together for free radical over generation. Another risk factor is excess nourishment, stress in combination with lack of physical inactivity collectively result in overabundance of glucose and fatty acid accumulation within muscle, adipose tissue and pancreatic cells. Exposure to air pollution and behaviour 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 (Shanmugapriya, Saravana, Payal, Mohammed and Williams 2012). 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 fairly good amount of antioxidants which when consumed help in prevention and management of various chronic diseases. Enriching diets with antioxidant compounds helps in ensuring protection against harmful diseases. Hence this results in enhancement of interest in food industry as well as in the field of preventive medicine and development of natural antioxidants from plant materials. 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 possess 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). Green leafy vegetables are rich source of micronutrients and could be used to prepare nutritious mixes to enhance their nutrient content. 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 ragi based antioxidant mix and analyzing the total antioxidant activity.

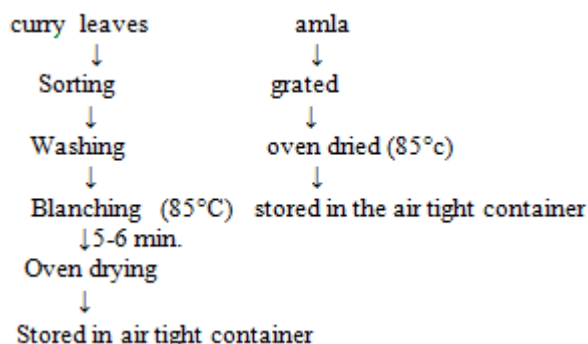
2. Methodology

Ingredients 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 and Ragi was procured from Bengaluru. Some identified varieties of the ingredients include - Bengal gram-RSG-973, ragi- Pr-202, 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 place prior to use. The curry leaves were firstly sorted and then washed. After washing blanching was done at 85 °C boiling water for 5 min, oven dried 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.

3. Preparation of the Seeds



4. Preparation of the leaves and amla-



5. Development of Premix

In the present study one standard mix was prepared. Each 100gms of premix contain ragi 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 ragi flour was supplemented by incorporating 5 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 ragi 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.

6. Determination of Antioxidant Components

6.1 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) Sample was extracted with 2 ml of 80% methanol at room temperature and then reached with 10 fold diluted Folin Ciocalteu reagent. Sodium carbonate at a concentration of 6% (w/v) was added and the final volume was made up in deionized water. After incubation at room temperature in the dark for 2 hour, the premix absorbance was measured against the gallic acid standard at 725 nm. Total phenolic content was expressed as mg gallic acid equivalent (GAE) /100 gm sample. β carotene were determined by using standard method of NIN (2003). 5g of sample is grinded with few crystals of anhydrous sodium sulphate and mixed with 10-15ml acetone. It is then decanted then supernatant is collected in a beaker. The process is repeated twice and transferred the combined supernatant to a separating funnel. 5-10 ml of petroleum ether was added and mixed thoroughly. Two layers were separated out on standing. Discarded the lower layer and collected the upper layer in 100 ml volumetric flask, volume was made up to 100ml with petroleum ether and optical density was recorded at 452nm. Petroleum ether was used as blank. The beta carotene was then calculated using the following expression: β carotene ($\mu\text{g}/100\text{g}$) = $\text{OD} \times 13.9 \times 10^4 \times 100 / \text{wt of sample} \times 560 \times 1000$. Vitamin E was determined by using the standard method of NIN (2003)

6.2 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 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).

6.3 Development of Baked Mathari

Baked mataris were prepared by all the three different premix

Ingredients	Standard premix	Antioxidant premix A	Antioxidant premix B
Ragi 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

6.4 Method of preparation of baked matari

Put the flour in the bowl
 ↓
 Add salt, ajwain and oil and mix well
 ↓
 Prepare dough by adding little amount of water
 ↓
 Prepare mataris out of the dough
 ↓
 Bake it at 160 °C for 25-30 min.

6.5 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.

6.6 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.

7. Results and Discussions

7.1 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 277.66 μ g/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 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 1

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

Statistically significant at(p<0.05) level.

7.2 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 $-\beta$ -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 67.4 μ g/ml, antioxidant rich premix showed 71.86 μ g/ml and antioxidant rich premix showed 76.32 μ g/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 2

Total Antioxidant Activity	Mix C	Mix A	Mix C
	67.4 μ g/ml	71.86 μ g/ml	76.32 μ g/ml

7.3 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 3

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

8. 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 ragi based antioxidant premix has a good total antioxidant activity. Therefore utilization of prepared premixes help in the overall well being of the society. It can be concluded from the present study that green leafy vegetables are the good source of antioxidants. It can be mixed with the regional cereals to enhance its properties.

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