Bio Availability Studies on Ragina & Energy Protein Rich Food

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Abstract

The Horse gram (Dolichos Biflorus) which is commonly used for cattle feed can be diversified for human consumption with less investment. Processed Horse gram flour was prepared using puffing and roasting, processed Soya bean (Glycine max) flour was prepared by dehulling and roasting. The low cost energy protein rich products namely RAGINA and EPRF were prepared using the simple home scale processing methods like germination, roasting and puffing, to improve the nutritional status. Horse gram has been identified as potential food resource for the tropics and also occupies an important place among pulses because of its ability to resist severe drought conditions. Soya bean is one of the best vegetable proteins and has tremendous potential to meet the protein deficiency in the cereal based Indian diets at a low cost. Product development can be taken as income generating activity in the rural areas by the illiterate women. Products can be included in supplementary feeding programmes in order to improve the nutritional status of the vulnerable groups of the population.

Keywords
dolichos Biflorus, soya bean, protein

1. Introduction

India is the second most populous country in the world, 120 million women live in poverty. As per the National and Regional Survey Prevalence of anemia 74% in children below 3 years of age, 85% in pregnant mothers and 90% among adolescent girls. In a developing country like India, there is a genuine need for nutritious food supplements which can be prepared from readily available raw materials [1]. Since horse gram occupies an important place among the pulse because of its ability to resist severe drought conditions. Horse gram was selected as one of the ingredient in developing mixes. Vitamin A deficiency is prevalent among large segments of the society in many countries [2].

Two different products Ragina and Energy Protein Rich Food (EPRF) were developed incorporating Red palm oil (obtained from the fruits of tree Elaeis guineensis Jacquat) 5% level which is a rich natural source of β carotene. The composition given below.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Amount</th>
<th>Ingredients</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAGINA</td>
<td></td>
<td>EPRF</td>
<td></td>
</tr>
<tr>
<td>Germinated Ragi Flour</td>
<td>40g</td>
<td>Dehusked roasted Horse gram flour</td>
<td>40g</td>
</tr>
<tr>
<td>Puffed Horse gram</td>
<td>20g</td>
<td>Roasted Soya bean flour</td>
<td>20g</td>
</tr>
<tr>
<td>Jaggery Powder</td>
<td>35g</td>
<td>Jaggery Powder</td>
<td>35g</td>
</tr>
<tr>
<td>Red palm Oil</td>
<td>05g</td>
<td>Red palm Oil</td>
<td>05g</td>
</tr>
<tr>
<td>Total</td>
<td>100g</td>
<td>Total</td>
<td>100g</td>
</tr>
</tbody>
</table>

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2. Materials and Methods
The raw materials used in the preparations of the products such as Ragi and Soyabean were obtained from Regional Agricultural Research Station, Palem, Mahaboobnagar, Horsegram from local market, jaggery powder from Regional Agricultural Research Station, Anakapalli and Red Palm Oil from AP. Co-operative at Seeds Grower’s Federation, Pedevagi were obtained.

2.1. Estimation of minerals, energy and β carotene content of developed foods
The estimates were carried out as per the standard procedures (AO AC, 1983; JH and Tan, 1988)

2.2. Bio-Availability Studies
The growth and Bio - Availability Studies (Protein efficiency ratio, overall Digestibility, Nitrogen Growth Index) using wistar strain male albino rats were conducted.

2.3. Growth & Protein Efficiency Ratio (PER)
Weanling male rats 8 per group (wistar strain) were housed individually in cages with wire mesh bottom and fed with diets containing Ragi, casein (control) and ERPF at 10% of protein. The diets contained following other ingredients i.e. refined groundnut oil (9%), vitaminised starch 1% (Chapmen et al 1959), and vitaminised oil 1% (Hubbell et al, (1937), fibre 4% and com starch was added to make up to 100 per cent

The diet was stored in closed containers for the duration of the experiment. The diet and water was supplied at ad libitum. The feed was given in especially designed porcelain cups to prevent spillage. The diet was moistened and made into a semi-solid form with the help of warm water. Records of daily food intake were observed and the left over food was collected, dried and weighed for calculating the daily food consumption. Weekly gain in weights of rats were also observed and recorded.

2.3.1. Protein Efficiency Ratio
PER of Ragi, EPRF and casein (control) were determined according to the procedure given by Chapman et al (1959)

After PER experimental period, the faeces was collected for 3 days dried to constant weight, powdered and used for nitrogen analysis. Diet intake was also recorded for three days and analysed for nitrogen.

2.3.2. Overall Digestibility (O.D.)
Overall digestibility of the developed products & control was measured using the following formula:

\[
\frac{\text{Food intake(g)} - \text{Faeces excreted(g)}}{\text{Food intake (g)}}
\]

2.3.3. Nitrogen Growth Index
The mean gain in body weight and mean nitrogen intake of the two experimental and control group were plotted on a graph.

2.3.4. Serum analysis
The rats were anaesthetized with petroleum either at the end of experiment and blood was collected by decapitation technique. Ventral lateral neck incision was made and jugular vain was located to collect the blood. Serum was separated by centrifuging the blood samples at 3000 rpm, for \textit{in vitro} quantities’ determination of protein, albumin and globulin. The albumin and globulin were calculated using the formula.

\[
\text{Total Protein (g %)} = \frac{A_{o(T)}}{A_{o(S)}} \times \text{Cone. of Total Protein}
\]

\[
\text{Albumin (g %)} = \frac{A_{o(T)}}{A_{o(S)}} \times \text{Cone. of Albumin}
\]

\[
\text{Globulin (g %)} = \text{Total Proteins in g %} - \text{Albumin in g %}
\]
2.4. ORGAN-WEIGHTS

The animals were dissected and the organs such as liver, kidney and brain were collected and weighed.

3. Results and Discussions

3.1. Mineral, Energy and β-carotene content of Developed Foods

The mineral composition of the developed products (Table 1) showed that the calcium of Ragina (243.66 mg) was considerably higher compared to EPRF (186.31 mg). This may be due to the high calcium content of the ragi which is one of the major ingredients in the preparation of Ragina [3].

Table 1. Mineral composition of developed products (g/100g)

<table>
<thead>
<tr>
<th>Product</th>
<th>Calcium (g)</th>
<th>Magnesium (g)</th>
<th>Copper (mg)</th>
<th>Iron (mg)</th>
<th>Zinc (mg)</th>
<th>Manganese (mg)</th>
<th>Energy (Kcal)</th>
<th>β-carotene (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ragina</td>
<td>243.66*</td>
<td>116.9</td>
<td>0.742</td>
<td>4.6</td>
<td>1.59</td>
<td>4.33</td>
<td>397.5*</td>
<td>1070.2*</td>
</tr>
<tr>
<td>EPRF</td>
<td>186.33*</td>
<td>116.45</td>
<td>0.93</td>
<td>4.6</td>
<td>2.08</td>
<td>--</td>
<td>652.87*</td>
<td>1377.3*</td>
</tr>
</tbody>
</table>

Statistical analysis by students’ t-test; *Significantly different at 5% level

The magnesium, copper and iron contents of both the products developed viz., Ragina and EPRF were similar indicating the values as 116.9 mg, 0.742 mg, 4.6 mg for Ragina and 116.4 mg, 0.93 mg, 4.6 mg for EPRF respectively. The zinc content of EPRF 2.08 mg was found to be higher when compared to Ragina 1.59 mg. The manganese content of Ragina was 4.33 mg while EPRF did not contain manganese. The energy content of EPRF is markedly higher 652.87 Kcal when compared to Ragina 397.5 Kcal. This may be due to the use of Soya bean which is rich in fat content. The β-carotene content of EPRF is 1377.3 mg while in Ragina it is 1070.2 mg [4].

Statistical analysis showed that calcium, Energy and β-carotene values of Ragina are different from EPRF. No significant difference was observed in other minerals. Red Palm oil provides a solution to prevent vitamin A deficiency which is an alarming problem, as it is a concentrated source (400 µg/g) of β-carotene, which is a precursor of vitamin A. The present study was undertaken to develop the low cost protein rich products which will be beneficial to improve the nutritional status of the vulnerable group of the population. Study conducted by Vijaya Khader and Aruna (2008) to screen the effect of supplementation of Red Palm Oil revealed increase in height & weight of children. In most of the centers, normal nutrition and grade-1 increased with simultaneous decrease in grade 11 and grade 111 malnutrition [5].

3.2. Growth and Protein efficiency ratio (PER)

The gain in weights of rats and PER of the two developed products Ragina and EPRF are given in Table 2, the values are significantly different with each at 5% level. The weight gain and PER of the rats fed on Ragina was markedly higher 62.1g 2.2 than that observed with EPRF 37.8g and 1.0 respectively. The higher weight gain of the rats fed on Ragina might be due to the puffing of horse gram which included sudden heat treatment for short period. The processing increased the PER of protein as it destroys the anti-nutritional factors (Borchers et al, 1947) and results in better utilization of protein. Vijaya Khader and Venkat Rao (1986) reported that cooked dehusked horse gram gave a slightly higher PER than autoclaved whole horsegram. The increase in gain in weight may also be attributed to the germinated ragi used in Ragina. During germination the starch breaks down, which increases amylase and phosphorylase activity in respiratory metabolism and promote digestibility of the sprouted millet. Chandra Shekhar and Chithra (1978) reported the protein quality of germinated horse gram to be higher than that of raw seeds. Similar results were reported by Sudha et al., (1994).
Table 2. Protein efficiency ratio of Ragina, EPRF (experimental period -4 weeks 8 male rats group)

<table>
<thead>
<tr>
<th>Product</th>
<th>Weight Gain(g)</th>
<th>Food Given (g)</th>
<th>Left Over (g)</th>
<th>Amount food intake(g)</th>
<th>Protein content of diet (g%)</th>
<th>Protein intake (g)</th>
<th>PER (Actual)</th>
<th>PER* (Corrected)</th>
<th>‘t’ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ragina</td>
<td>62.1</td>
<td>348</td>
<td>102.83</td>
<td>265.16</td>
<td>6.36</td>
<td>16.8</td>
<td>3.6</td>
<td>2.1</td>
<td>7.85**</td>
</tr>
<tr>
<td>EPRF</td>
<td>37.1</td>
<td>368</td>
<td>151.41</td>
<td>216.58</td>
<td>10.0-</td>
<td>21.0</td>
<td>1.7</td>
<td>1.1</td>
<td></td>
</tr>
</tbody>
</table>

*The PER of casein was considered as 2.5 for correction. ** Significant at 5% level

3.3. Overall Digestibility

The food intake of rats fed on Ragina and EPRF are 26.77 g and 28.88 g and the overall digestibility is 85.69% and 86.38. No significant difference was observed between these products but they are significantly different from the control diet (Table 3).

Table 3. Overall digestibility of rats fed on developed foods and control

<table>
<thead>
<tr>
<th>Group</th>
<th>Diet</th>
<th>Food intake (g)</th>
<th>Faecal weight (g)</th>
<th>Overall Digestibility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ragina</td>
<td>26.77</td>
<td>3.7</td>
<td>85.69a</td>
</tr>
<tr>
<td>2</td>
<td>EPRF</td>
<td>28.88</td>
<td>3.7</td>
<td>86.38a</td>
</tr>
<tr>
<td>3</td>
<td>Control (Casein)</td>
<td>37.03</td>
<td>2.3</td>
<td>93.66b</td>
</tr>
</tbody>
</table>

Statistical analysis of variability in the data by ‘F’ ration at 5% level Values not sharing common superscript letters are significantly different

3.4. Nitrogen - Growth Index

This index is used to obtain a curve which indicate the gain in body weight for the nitrogen intake. Similar studies were conducted by Allison et al., (1955). The values are represented in Table 4.

Table 4. Nitrogen growth index

<table>
<thead>
<tr>
<th>Product</th>
<th>Gain in weight (g)</th>
<th>Nitrogen intake (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ragina</td>
<td>61.2</td>
<td>2.68</td>
</tr>
<tr>
<td>EPRF</td>
<td>42.9</td>
<td>3.45</td>
</tr>
<tr>
<td>Control (Casein)</td>
<td>117.1</td>
<td>4.68</td>
</tr>
</tbody>
</table>

Although the nitrogen intake of the rats fed on the diet Ragina (2.68 g) was comparatively lower than that of EPRF (3.45g) the weight gain was higher in case of Ragina a (61.2 g). Reduced gain in weight of the rats fed on EPRF (42.9 g) may be due to the anti nutritional factors present in the soybean which might have interfered with the protein utilization. The better gain in weight of Ragina was due to processing like germination and puffing of ragi and horse gram incorporated in the preparation of the product which might have increased the protein utilization [6].

3.5. Organ weights

The weights of different organs collected from the rats fed on Ragina, EPRF and control diet for a period of 5 weeks are given in Table 5. The group of rats fed on Ragina gained maximum weight (61.2 g) as compared to rats fed on
EPRF (42.9 g). However, the body weights of rats fed on Ragina comparatively less as compared to rats fed on casein diet at 10% level. This may be due to the less protein content of Ragina.

**Table 5. Mean weight of various organs of rats fed on ragina, EPRF and control (duration of experiment -5weeks; 8 male rats per group)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean body weight</th>
<th>Gain weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>Ragina</td>
<td>32.4</td>
<td>93.6</td>
</tr>
<tr>
<td>EPRF</td>
<td>32.6</td>
<td>75.5</td>
</tr>
<tr>
<td>Control</td>
<td>32.5</td>
<td>149.7</td>
</tr>
</tbody>
</table>

The rats fed on EPRF showed markedly less gain in body weight as compared with Ragina and casein diets in spite of 10% protein. This may be due to the variety of Soyabean used and also the anti-nutritional factors present in that particular variety. Similar type of results are observed in the case of liver weights.

The weights of the kidney and brain of rats fed on EPRF are comparable to that of rats fed on casein diet. Whereas, the kidney as well as brain weights of rats fed on Ragina are much lower as compared to rats fed on EPRF and casein diets. This may be due to the less protein contents of the Ragina diet.

### 3.6. Serum Analysis

There is no significant difference between the developed products (**Table 6**) with regard to total protein, albumin, globulin and A/G ration; but markedly different from control group.

**Table 6. The mean protein, albumin, globulin and A/G ratio**

<table>
<thead>
<tr>
<th></th>
<th>Total protein (g)</th>
<th>Albumin (g)</th>
<th>Globulin (g)</th>
<th>A/G ration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ragina</td>
<td>4.5</td>
<td>2.6</td>
<td>1.8</td>
<td>1.6</td>
</tr>
<tr>
<td>EPRF</td>
<td>4.4</td>
<td>2.5</td>
<td>1.8</td>
<td>1.6</td>
</tr>
<tr>
<td>Control</td>
<td>5.0</td>
<td>3.2</td>
<td>1</td>
<td>2.2</td>
</tr>
</tbody>
</table>

### 3.7. Summary

PER of Ragina higher than EPRF. This might be due to the puffed horse gram used in the preparation of Ragina. Puffing makes the pulse very light and easy to digest due to the breakage of protein into simpler amino acids indicating the beneficial effect of puffing. No difference was observed in the overall digestibility of developed products. Though the nitrogen intake of the rats fed on the diets Ragina was comparatively lower than that of EPRF the weight gain was higher in case of Ragina. Reduced gain in weight of the rats fed on EPRF may be due to the anti-nutritional factors present in the soya bean which might have interfered with the protein utilization. The better gain in weight of Ragina was due to processing like germination and puffing of ragi and horse gram incorporated in the preparation of this product which might have increased the protein utilization [7].

The weights of the kidney and brain of the rats fed on EPRF are comparable to that of rats fed on casein diet. Whereas, the kidney as well as brain weights of rats fed on Ragina are much lower as compared to rats fed on EPRF and casein diets. This may be due to the less protein content of the Ragina diet [8].

The purpose of the present study was to investigate the bioavailability of the Red Palm oil incorporated mixes developed using horse gram, ragi and soya bean. Crude Palm Oil is Nature’s richest source of the carotenoid with concentrations in
the order of 700-1000 ppm. This is about 30 times more than what is present in carrots. Product development is a new approach to overcome Vitamin A deficiency in many parts of the developing world [9-10].

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**REFERENCES**


