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Macronutrient and Amino Acid Evaluation of Formulated Edible Seed Protein Mix

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Abstract:- The study was done to formulate a protein mix powder (pumpayarind powder), from the edible seeds and evaluate its macronutrient and amino acid profile. Standard procedures from Association of Analytical Communities (AOAC) were used for the estimation of protein and amino acid. The results were subjected to t test to check the significance. The nutrients were compared with the reference protein (egg) and conclusions were drawn. The result showed that the macronutrient content of pumpayarind powder was significant at 5% level. The essential amino acids of pumpayarind powder were not significant but branched chain amino and non- essential amino acid were significant at 10% level and 5% level respectively. Thus it can be concluded that pumpayarind powder was found to be high in protein and also had a good profile of amino acids especially branched chain amino acid. Hence this protein could be an alternate source of protein supplement for the athletes.

Keywords:- *Macronutrient, Amino Acids, Pumpayarind Powder.*

I. INTRODUCTION

Protein is an irreplaceable nutrient for all animals, including humans, and is involved in all physiological functions. An adequate dietary supply of protein, as a source of amino acids and nitrogen, is required to allow the regular turnover of tissue and functional body proteins like enzymes (Elmadfa and Meyer, 2017). A higher protein intake has been recommended for athletes compared with healthy non-exercising individuals (Kato et al, 2016).

Athletic performance is the culmination of genetics, proper training, adequate nutrition, hydration, desire, and rest. Understanding sport-specific physiologic requirements for training and competition is integral to obtaining sufficient energy, optimal levels of macronutrients and micronutrients, and adequate levels of fluids (International Olympic Committee [IOC], 2011).

Nitrogen balance studies in endurance athletes suggest a range of 1.2 g/kg to 1.4 g/kg/day, and for strength athletes 1.2 to 1.7 g/kg/day for protein intake, with the higher end of the range recommended early in the competitive season (Phillips, 2012).

An acute exercise stimulus, particularly resistance exercise, and protein ingestion both stimulate muscle protein synthesis (MPS) and are synergistic when protein consumption occurs before or after resistance exercise. For building muscle mass and for maintaining muscle mass through a positive muscle protein balance, an overall daily protein intake in the range of 1.4–2.0 g protein/kg body weight/day (g/kg/d) is sufficient for most exercising individuals (Jager et al, 2017).A large number of athletes and exercisers have turned to protein supplements to meet these excess demands on the body (Wilborn et al, 2013).

Two of the most popular forms of protein supplement on the market are whey and casein. Both proteins are derived from milk but each protein differs in absorption rate and bioavailability (Wilborn et al, 2013).

A 2010 review of 24 commercially available protein supplements revealed lead contamination in two protein supplements; at the levels of contamination found, these products would deliver a daily dose of 6 to 18 µg of lead. The cumulative effects of lead intake are potentially harmful. Three of the products were also found to contain levels of heavy metals like mercury, arsenic, cadmium and lead in excess of the safe levels proposed by the US Pharmacopeia. A more significant concern is raised by the reports of FDA about the problems with supplements, with many of these reports relating to the presence in a wide range of supplement products of undeclared allergens, microbiological contamination, or foreign objects. (Maughan et al, 2018)

Considering, the negative effects of commercially available protein supplements and the high demand of protein for the athletes, the current study is designed to develop protein mix powder from the plant seeds without any strenuous processing techniques.

- *Objectives*

The objectives of the study were to evaluate the macronutrients (carbohydrate, protein, fat and energy) and amino acid profile of the formulated pumpayarind powder.

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II. MATERIALS AND METHOD

A. Purchasing, Drying and Grinding of Seeds:

Papaya and pumpkin seeds were washed thoroughly. Papaya seeds were sun dried for 3 days and pumpkin seeds were sun dried for 2 days. Once the seeds were dried the shell of the pumpkin seed was removed. Tamarind seeds were dry roasted for 5-10 minutes at medium flame to remove the seed coat. After the removal of seed coat and seed shell, the seeds were ground separately in a mixer and sieved through a siever.

B. Formulation of Pumpayarind Powder:

After the seeds have been ground pumpayarind powder was formulated. To prepare 100g of pumpayarind powder 50g of pumpkin seed flour, 40g of tamarind seed powder and 10g of papaya seed flour were mixed together. The grounded seed powder was weighed using a digital weighing machine and appropriate amount of seed powder such as 40g of tamarind seed powder, 10g of papaya seed powder and 50g of pumpkin seed powder was measured. The seed powders were then put together in a bowl and mixed, until they get evenly mixed. The pumpayarind powder prepared was then dry roasted in low flame to mask the off flavour.

C. Analysis of Pumpayarind Powder:

Nutritional analysis of pumpayarind was done at SGS India Private limited, laboratory. Nutrients like Energy, Carbohydrate, Protein and Fat and Amino acid content of the pumpayarind powder was estimated at this laboratory. 250g of pumpayarind powder was the requisite amount for analysis and it was prepared and packed in a sealed polythene packet and was given to the laboratory. The standard procedures used in the laboratory for estimation of nutrients were according to Association of Analytical Communities (AOAC)

D. Statistical Tools Used

The results of the analysis were compared with reference protein, egg and conclusions were drawn. ‘t’ test was the statistical used to check the significance.

III. RESULTS AND DISCUSSION

A. Macronutrient Composition of Pumpayarind Powder

In this study macronutrients like carbohydrate, protein, fat and energy were analysed. The macronutrient compositions of the pumpayarind powder are given in table 1.

S.no	Nutrient	Egg*	Pumpayarind powder
1	Energy kcal/100g	173	506.45
2	Carbohydrate g/100g	-	32.97
3	Protein g/100g	13.3	27.29
4	Fat g/100g	13.3	29.49

Table 1:- Macronutrient composition of pumpayarind powder per 100g
*Gopalan et al, 2016

From table 1 it can be seen that the energy content of pumpayarind powder was found to be 506.45kcal/100g. Carbohydrate and fat content of pumpayarind powder were found to be 32.97g/100g and 29.49g/100g. From these values it can be concluded that pumpayarind powder has a rich profile of macronutrients.

Energy needs of female athletes are high to maintain a high level of training, building and repair of body tissues, cover the energy costs of daily living, prevent illness and maintain reproductive function. Research suggests that female athletes need energy dense food to meet their requirement (Manore, 2017). Through nutrient analysis it was found that the pumpayarind powder is an energy dense food. So thus pumpayarind powder can provide the above mentioned benefits.

The protein content of pumpayarind powder was 27.49g/100g. The protein content of egg is 13.3g/100g and is generally considered as reference protein (Gopalan et al, 2016). Comparing the protein content of pumpayarind powder (27.29 g/100g) with the reference protein, the protein content of pumpayarind powder was found to be high. In this context pumpayarind powder can be considered as a good source of protein.

Macronutrients of reference protein and pumpayarind powder were subjected to statistical analysis and the data is given in table 2.

S. no	Nutrient	Egg	Pumpayarind powder	t value	Level of S	S
1.	Macro nutrients	8.86 ± 7.67 g/100g	29.89 ± 2.82 g/100g	4.451	.011**	S

Table 2:- Mean difference in macronutrient composition of pumpayarind powder and egg
**significant at 5% level

From table 2 it can be seen that the macronutrient composition of pumpayarind powder showed a 5% level of significance higher than that of the reference protein.

B. Essential Amino Acid Profile of Pumpayarind Powder

Essential amino acids are those that cannot be synthesized in the body and has to be taken through diet. In the present study, all the nine essential amino acids were estimated and the composition is tabulated in the table 3.

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S.no	Amino acid	Egg*	Pumpayarind powder
1	Methionine g/100g	0.4	0.2
2	Phenylalanine g/100g	0.68	1.3
3	Tryptophan g/100g	0.19	0.27
4	Histidine g/100g	0.32	0.65
5	Lysine g/100g	0.93	0.97
6	Threonine g/100g	0.56	0.81
7	Valine g/100g	0.816	1.35
8	Leucine g/100g	1.11	1.98
9	Isoleucine g/100g	0.661	1.20

Table 3:- Essential amino acid profile of pumpayarind powder per 100g
*USDA National nutrient database for standard reference, 2016

Table 3 shows that all the essential amino acid present in pumpayarind powder is more than that of the egg. Leucine is the amino acid that is present in major proportion in pumpayarind powder and it was found to be 1.98g/100g. Similarly methionine is the amino acid that is present in lower proportion and it was found to be 0.2g/100g.

Nutrient analysis reveals that pumpayarind powder contains all the nine essential amino acid in a balanced proportion and hence it is a complete protein. According to Wu (2016) it is said that for a food to be an ideal source of protein, it must contain all the nine essential amino acid.

Ingestion or infusion of amino acids stimulates an increase in skeletal muscle protein synthesis an effect that is enhanced by prior resistance exercise. Essential amino acids (EAAs) stimulate increased rates of myofibrillar protein synthesis. On an average, 20–25 g of high- quality protein contains 8–10 g EAAs,

Which are essential for the regulation of muscle protein synthesis (Churchward- Venne et al, 2012).

With reference to the above mentioned study done by Churchward- Venne et al (2012), it can be assumed that pumpayarind powder which has protein content of 27.29g/100g of protein and 8.73g/100g of EAAs would be sufficient to cause increase in muscle protein synthesis.

Essential amino acid profile of reference protein and pumpayarind powder was subjected to statistical analysis and the data is given in table 4.

S. no	Nutrient	Egg	Pumpayarind powder	t valve	Level of significance	S
1.	Essential amino acid	0.62 ± 0.29 g/100g	0.97 ± 0.56 g/100g	1.604	0.128	NS

Table 4:- Mean difference in essential amino acid profile of pumpayarind powder and egg

t test was the statistical tool used to analyse the essential amino acid profile between pumpayarind powder and egg. From table 13 it can be noted that the level of significance is greater than 0.1 and therefore there is no significance in the level of EAA of egg and pumpayarind powder at 1%, 5% and 10% level. Therefore it can be presumed that the essential amino acid content of egg is more than the essential amino acid content of pumpayarind powder.

C. Branched Chain Amino Acid Profile of Pumpayarind Powder

In this study, all the three BCAAs, leucine, isoleucine and valine were estimated. The BCAAs content of the pumpayarind powder obtained through analysis are tabulated in table 5

S.no	Amino acid	Egg*	Pumpayarind powder
1	Valine g/100g	0.816	1.35
2	Leucine g/100g	1.11	1.98
3	Isoleucine g/100g	0.661	1.20

Table 5:- Branched chain amino acid profile of pumpayarind powder per 100g
*USDA National nutrient database for standard reference, 2016

Branched-chain amino acids serve not only as substrates for—but are also potent stimulators of—skeletal and liver protein synthesis, which would position them as important dietary components for athletes during recovery (Kato et al, 2018).

Table 5 shows that BCAAs value of pumpayarind powder was 4.53g/100g. But whereas BCAAs content of egg was 2.58g/100g which is less than that of pumpayarind powder. Leucine content of pumpayarind powder was found to be 1.98g/100g, valine was found to be 1.35g/100g and isoleucine was 1.35g/100g. This shows that pumpayarind powder holds a good profile of BCAA.

Leucine content of pumpayarind powder was found to be 1.98g/100g and it is the major BCAA of pumpayarind

powder. Leucine has been reported to activate the mammalian target of rapamycin signaling pathway, thereby promoting muscle-protein synthesis. It has also been suggested that they could enhance mitochondrial biogenesis and reactive oxygen species scavenging leading to potential benefits in skeletal muscle energy metabolism (Foure and Bendahan, 2017). Thus high concentration of leucine in pumpayarind powder helps the athletes to promote muscle synthesis and also helps in the production of energy.

t test was used to statistically analyse the significance of branched chain amino acid profile of reference protein and pumpayarind powder and the data is given in table 6.

S. no	Nutrient	Egg	Pumpayarind powder	t value	Level of s	S
1.	Branched chain amino acid	0.86 ± 0.22g/100g	1.51 ± 0.41 g/100g	2.374	.076***	S

Table 6:- Mean difference between branched chain amino acid profile of pumpayarind powder and egg
***significant at 10% level

Mean difference analysis revealed that BCAA of pumpayarind powder is significantly higher than that of BCAA of reference protein. The significance was at 10% level.

D. Non-Essential Amino Acid Profile of Pumpayarind Powder

Non-essential amino acids (NEAAs) are those that can be synthesized inside the body but can also be obtained through the diet. In the current study, non- essential amino acids like alanine, arginine, aspartic acid, cysteine .glutamate, cysteine, glycine, proline, serine and tyrosine were estimated. The results of the analysis are tabulated in the table 7.

S.no	Amino acid	Egg*	Pumpayarind powder
1	Alanine g/100g	0.72	1.12
2	Arginine g/100g	0.812	3.37
3	Aspartic acid g/100g	1.302	3.20
4	Cystine g/100g	0.28	0.24
5	Glutamic acid g/100g	1.649	5.11
6	Gycine g/100g	0.432	1.41
7	Proline g/100g	0.497	1.72
8	Serine g/100g	0.983	1.23
9	Tyrosine g/100g	0.535	0.97

Table 7:- Non-essential amino acid profile of pumpayarind powder per 100g
*****USDA National nutrient database for standard reference, 2016

From table 7, it can be depicted that the overall non-essential amino acid composition of pumpayarind powder was 18.37g/100g but whereas NEAA of egg was only 7.21g/100g. Glutamic acid, arginine and aspartic acid are the three NEAA that forms major portion of NEAA of pumpayarind powder. Glutamic acid of pumpayarind powder was found to be 5.11g/100g, arginine was found to be 3.37g/100g and aspartic acid was found to be 3.20g/100g.

Earlier it was thought that NEAA can presumably increase EAA availability for whole-body protein synthesis only when diet deficient in EAA was consumed. But study done by Kato et al, (2018) showed that NEAA helps in whole body protein synthesis even when EAA are supplemented in adequate amount. Thus through this study it can be concluded that exogenous NEAA are dispensable for whole-body protein synthesis during recovery from endurance exercise. Pumpayarind powder which holds good profile of NEAA can therefore be effective to increase whole body protein synthesis Essential amino acid profile of reference protein and pumpayarind powder was subjected to statistical analysis and the data is given in table 8.

S. no	Nutrient	Egg	Pumpayarind powder	t value	Level of significance	S
1.	Non-essential amino acid	0.80 ± 0.44 g/100g	2.04 ± 1.53 g/100g	2.323	.034 **	S

Table 8:- Mean difference in non-essential amino acid profile of pumpayarind powder and egg
**significant at 5% level

Table 8 shows that the mean NEAA of egg was 0.80 ± 0.44 g/100g which is less than the mean NEAA of pumpayarind powder was 2.04 ± 1.53 g/100g. t test was used to assess the significant difference of non-essential amino acid profile of egg and pumpayarind powder. The result showed that the non- essential amino acid content of pumpayarind powder was significantly higher than that of egg.

IV. CONCLUSION

In the present study protein supplement, pumpayarind powder was formulated from agro waste. Nutrient analysis of this protein supplement showed that it is energy dense and provides 506.45kcal/ 100g. The protein content of the formulated pumpayarind powder was found to be 27.29g/100g and hence it becomes an excellent source of protein. Pumpayarind powder is a complete protein with all the nine essential amino acid in a balanced proportion. Pumpayarind powder has a good profile of branched chain amino acid which are the most essential nutrient for athletes and it also has a good profile of non-essential amino acid which helps in whole body protein synthesis. Through this nutrient analysis it can be concluded that pumpayarind mix powder could be an alternate source of protein supplement for athletes.

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