# Development and Estimation of Proximate Composition, Mineral Content, and Biochemical Analysis of Value-Added Extruded Millet Pasta

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## ABSTRACT

The present study aimed to develop food products containing millets in various combinations to determine the proximate composition and mineral content of the standard and most acceptable food products. Three different combinations of millets resulted in three types of pasta. Biochemical estimation revealed that Jowar pasta has higher moisture, protein, thiamine, and riboflavin content, whereas Bajra pasta has higher fibre, fat, and sugar content, and Kodo pasta has greater energy, carbohydrate, ash, calcium, and magnesium content, respectively.

**Key words:** Millets, proximate composition, mineral content, biochemical estimation.

## INTRODUCTION

Millets are small-seeded cereal grasses bearing coarse grains, considered as nutritional and valuable food grains since ancient times. Millets are rich in fibre, protein, iron, calcium, magnesium, zinc, vitamin B, have low glycaemic index, and show anti-oxidant, anti-inflammatory, anti-allergic, anti-carcinogenic and gastro-protective properties. In India, nine species of millets are very commonly used and large number of cultivars have been introduced by various agricultural institutes across the country to enhance the quality, size, colour, biomass, and disease resistance of these grains (Balkrishna et al., 2023).

Millets are unique among the cereals because of their richness in calcium, dietary fibre, polyphenols and protein. Millets generally contain significant amounts of essential amino acids particularly the sulphur containing amino acids (methionine and cysteine); they are also higher in fat content than maize, rice, and sorghum. In general, cereal proteins including millets are limited in lysine and tryptophan content and vary with cultivar. However, most cereals contain the essential amino acids as well as vitamins and minerals. Plant nutrients are largely used in the food industry, and cereal grains constitute a major source of dietary nutrients worldwide (Amadou I et al., 2013).

Extrusion cooking is the HTST (High temperature short-time) method, developed for the manufacturing of innovative value added ready to eat products such as produced from cereals, in which baby foods, breakfast cereals, dietary fibre, pet foods, cereal based modified starch and traditional products .It brings inactivation of raw enzymes, lower the microorganism level from the finished product, deactivation of naturally occurring toxic substances, denaturation of protein, modification of lipids and gelatinization of starch. Extruded food products have lower water activity (0.1-0.4) and due to the lower water activity of cold and hot extruded food product are preserved for long time. The extruded food products are digestible, palatable and safe to consume (Shelar GA et al., 2019).

Pasta is an excellent source of complex carbohydrates, which provide a slow release of energy. Unlike simple sugars that offer a quick, yet fleeting boost of energy, pasta helps sustain energy (K.UA et al., 2016).

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# METHODOLOGY

The methodology consists of the information related to the research design and methodological steps used for the present investigation.

**Procurement of jowar (Sorghum bicolor (L.) Moench) flour, pearl millet (bajra) (Cenchrus americanus (L.) Morrone) flour, kodo millet (Kodra, Araka) (Paspalum scrobiculatum L.) flour, whole wheat flour, semolina flour.**

Jowar (Sorghum bicolor (L.) Moench) flour, pearl millet (bajra) (Cenchrus americanus (L.) Morrone) flour, kodo millet (Kodra, Araka) (Paspalum scrobiculatum L.) flour, whole wheat flour and semolina flour were procured from sector 32 market, Chandigarh.

**Preparation and mixing of jowar (Sorghum bicolor (L.) Moench) flour with whole wheat flour and semolina flour, pearl millet (bajra) (Cenchrus americanus (L.) Morrone) flour with whole wheat flour and semolina flour, kodo millet (Kodra, Araka) (Paspalum scrobiculatum L.) flour with whole wheat flour and semolina flour** (Fig 2.1)

**Figure 2.1**: Development of Millet Pasta using Pasta Extruder Machine


Different types of millet flours were used to prepare extruded pasta products. The ingredients were collected, weighed and mixed in different proportions such as, 100 grams of jowar (Sorghum bicolor (L.) Moench) flour was added to 200 grams of whole wheat flour and 200 grams of semolina, similarly 100 grams of pearl millet (bajra) (Cenchrus americanus (L.) Morrone) flour was added in 200 grams of whole wheat flour and 200 grams of semolina, and 100 grams of kodo millet (Kodra, Araka) (Paspalum scrobiculatum L.) flour was added in whole wheat flour and 200 grams of semolina. The combined mixtures were sieved through a mesh sieve to obtain a fine powder. Water was added to the three flour mixtures in various quantities to obtain the desired consistency for the development of pasta. In the first mixture i.e., for jowar pasta 210 ml of water was added, in the second

mixture i.e., for bajra pasta 190 ml of water was added whereas for the third mixture i.e., for kodo pasta 190 ml of water was added. All three mixtures were sieved again and added to the pasta extruder machine simultaneously which resulted in the formation of a spiral and ribbon shaped pasta. After the desired shape is obtained the pasta were kept in three different trays and were placed in a tray dryer for about one hour. The pasta samples were cooled, packed and stored in a cool and dry place. The products were developed as per the details given below.

**Jowar pasta**

**Preparations of flour mix for Jowar Pasta**

A formulation was prepared by mixing Jowar (Sorghum bicolor (L.) Moench) flour with whole wheat flour and semolina flour in different proportions. A combination of the flours is depicted in the Table 2.2 A.

**Table 2.2** A Proportion of Jowar Pasta

| SNO. | INGREDIENTS | SAMPLE 1 |
|---|---|---|
| 1. | Jowar Flour | 20% |
| 2. | Whole Wheat Flour | 40% |
| 3. | Semolina | 40% |

**Bajra pasta**

**Preparations of flour mix for Bajra Pasta**

A formulation was prepared by mixing pearl millet (bajra) (Cenchrus americanus (L.) Morrone) flour, whole wheat flour and semolina flour in different proportions. A combination of the flours is depicted in Table 2.2 B.

**Table 2.2** Proportion of Bajra Pasta

| SNO. | INGREDIENTS | SAMPLE 1 |
|---|---|---|
| 1. | Bajra Flour | 20% |
| 2. | Whole Wheat Flour | 40% |
| 3. | Semolina | 40% |

**Kodo pasta**

**Preparations of flour mix for Kodo Pasta**

A formulation was prepared by mixing kodo millet (Kodra, Araka) (Paspalum scrobiculatum L.) flour, with whole wheat flour and semolina flour in different proportions. A combination of the flours is depicted in Table 2.2 C.

**Table 2.2**Proportion of Kodo Pasta

| SNO. | INGREDIENTS | SAMPLE 1 |
|---|---|---|
| 1. | Kodo Flour | 20% |
| 2. | Whole Wheat Flour | 40% |
| 3. | Semolina | 40% |

**Estimation of proximate composition and mineral content of standardized acceptable millet-based extruded food products**

The standard and most acceptable food products out of the ratios were evaluated to assess proximate and mineral composition as per the parameters mentioned in the next step.

**Biochemical analysis of millet-based extruded food products**

The millet pasta samples were analysed by the following parameters:

**Moisture**

Moisture content was determined by employing the standard method of analysis. (AOAC, 2000) Procedure: Ten gram of the sample was weighed in a petri dish and dried in an oven at 105 ˚C for six hours or till a constant weight was obtained. The sample was weighed after cooling it in desiccators.

$$\text{Moisture (\%)} = \frac{\text{Loss in weight (gram)}}{\text{Weight of the sample (gram)}} \text{X } 100$$

**Energy**

This method is based on analysis of the sample for total protein, fat and carbohydrates and then multiplying the protein and carbohydrate content by 4 and that of fat by 9 and adding up to give energy in Kcal. This method gives us the metabolizable energy and not the total or gross energy of the sample and is used in Food Composition Tables (Hira CK *et al.,* 2008).

**Carbohydrate**

Add up to the values of moisture, crude protein, crude fat, crude fibre and ash and subtract from 100. The difference will give value of available carbohydrates (Hira CK *et al.,* 2008).

**Fibre**

Crude fibre in the sample was determined by standard method of analysis (AOAC, 2000)

**Reagents**

1. Hydrochloric acid (%) v/v
2. Sulphuric acid stock solution (10%) v/v: Diluted 55 ml concentrated sulphuric acid to 1 L.
3. Sulphuric acid working solution (1.25%): Diluted 125 ml of stock solution to 1L.
4. Sodium hydroxide stock solution (10%) w/v: Dissolved 100 gram of NaOH in distilled water and diluted to 1 L.
5. Sodium hydroxide workine solution (1.25%): Diluted 125 ml stock solution to 1 L with distilled water. 6.Antifoam (2%): Silicon in $CCI_4$

**Procedure:** Two gram fat free dried sample was put in 1L tall beaker and 200 ml 1.25 per cent $H_2SO_4$ and a few drops of antifoam were added. The solution was kept for boiling for 30 minutes under bulb condenser. Beaker was rotated occasionally to mix the contents and remove the particles from sides. The contents were filtered into the beaker through Buchner funnel. The sample was washed back into the beaker with 200 ml 1.25 per cent NaOH and again boiled for exactly 30 minutes. All the insoluble mass was transferred to the crucible (G-1) by means of boiling distilled water till acid free. Washed twice with alcohol and thrice with acetone, and then dried at 100 ˚C to constant weight. The dried material was ashed in a muffle furnace at 550 ˚C for one hour. The crucible was cooled in a desiccator and weighed.

$$\text{Crude fibre (\%)} = \frac{W_2 - W_3}{W1} \text{ x100}$$

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Where,

W1= weight (g) of sample

W2 =weight (g) of insoluble matter (wt. of crucible-insoluble matter- wt. of crucible)

W3 = weight (g) of ash (crucible + ash - wt. of crucible)

**Protein**

The total nitrogen was estimated by a standard method of (AOAC, 2000). The crude protein was calculated by using the conversion factor of 6.25.

**Reagents**

1. Hydrochloric acid (N/100)
2. Boric acid (4%)
3. Sodium hydroxide (40%)
4. Digestion mixture: 10 gram $K_2SO_4$, 0.5 gram $CuSO_4.6H_20$ and 2 gram $FeSO_4$
5. Mixed indicator solution: Dissolved 0.1 gram methyl red and 0.5 gram bromocresol green in 100 ml of 95% ethanol and the solution was adjusted with drops of dilute NaOH to bluish purple colour.

**Procedure:** Two hundred milligrams sample was taken and digested with 20 ml concentrated $H_2SO_4$ and a pinch of digestion mixture. The nitrogen, as ammoniacal salt, was diluted with 40 per cent NaOH in a Microkjeldahl apparatus. The ammonia thus liberated was absorbed in 10 ml boric acid solution containing a few drops of mixed indicator and was titrated against standard HC1(N/100). The end point was indicated by the change of colour from bluish-green to pink.

$$\text{Crude protein (\%)} = \frac{0.00014\ x Vx\ (S-B) x 100}{V1 X w}\ xF$$

Where,

W = weight (g) of sample taken

V = volume (m1) made

V1 = volume (ml) of aliquot taken for distillation

S = volume (m1) of HCI (N/100) used in titration for blank

B = volume (ml) of HC1 (N/100) used in titration for blank 0.00014 = 10 ml of 0.1 N HCI neutralize 0.00014 gram of nitrogen

F= factor for converting N to protein (6.25)

**Fat lipid**

Crude fat was estimated by a standard method of (AOAC, 2000) using the soxhlet extraction apparatus.

**Procedure:** Five gram of moisture free sample was taken and transferred to an extraction thimble and then weighed. The thimble was placed in a soxhlet extractor fitted with a condenser and flask containing sufficient petroleum ether. The extraction was carried out for six hours. After the extraction thimble was removed with the sample from the desiccator and weighed. The loss in weight of the thimble was the estimate of the ether extract in the sample.

$$\text{Crude fat (\%)} = \frac{\text{Loss of weight (gram)}}{\text{Sample weight (gram)}} \text{ x100}$$

**Ash**

It was estimated by employing the standard method of analysis (AOAC, 2000)

Procedure: Five gram of oven dried sample was weighed in the silica crucible. It was ignited till no charred particles remained in the crucible. The crucible was put in muffle furnace (550˚C) for 5 6 hours or till a white ash was obtained. The crucible was then cooled in a desiccator and weighed.

$$\text{Ash (\%)} = \frac{\text{Weight of ash (gram)}}{\text{Weight of the sample (gram)}} \text{ x}100$$

**Sugar**

The total sugars are estimated by the phenol-sulphuric acid method of Dubois *et al.* (1956).

**Reagents**

1. 95.5% sulphuric acid
2. 5% phenol in glass distilled water

**Procedure:** For the determination of total sugars, take 0.2 ml of the test extract in each test tube and make the volume to 1 ml. Glucose standards (10-60g) and blank are taken simultaneously. After adding 1 ml of 5% phenol to each tube, the tubes are then placed in ice cold water and 5 ml of 95.5% sulphuric acid is added swiftly, the stream of acid being directed against the liquid surface rather than against the side of the test tube to obtain good mixing. The contents are mixed and equilibrated to room temperature. The absorbance of the pink colour developed is read at 490 nm in a Bausch and Lomb spectrophotometer-20. The concentration of total sugars from the test extracts is then calculated as glucose from the standard glucose curve (Hira CK *et al.,* 2008).

**Total minerals**

**Calcium**

Calcium in the sample was determined by standard method of analysis Titrimetric method (AOAC, 2000).

**Reagents**

1. Saturated ammonium oxalate solution.
2. Dilute hydrochloric acid (1 part HCl + 4 part water).
3. Methyl red indicator (0.5% in absolute alcohol).
4. Potassium permanganate solution 0.1 N.
5. Dilute ammonium hydroxide (1-part $NH_4OH$ + 1 part water).
6. Dilute sulphuric acid (10%).
7. Oxalic acid solution 0.1N: Sodium oxalate is dried in an oven at 100 degree Celsius for 12 hours. Exactly 6.7 gram is dissolved in distilled water, 5 ml of conc. $H_2SO_4$ is added and solution made up to 1L, after it has cooled down.

Standardization of potassium permanganate solution: 10 ml of 0.1 N oxalic acid solutions is transferred to a conical flask. One ml conc. $H_2SO_4$ is added, warmed to about 70 degrees Celsius titrated against $KMnO_4$ solution, till the faint pink colour remains.

**Procedure:** Take 50 ml of clear sample filtrate prepared from ash into a beaker. Add 10 ml of saturated ammonium oxalate solution. Boil and add two drops of methyl red indicator. The contents are neutralized with dilute ammonium hydroxide and boil the contents again to have coarse crystalline precipitate. Add a few drops of dilute hydrochloric acid until the colour is adjusted to faint pink. The solution is allowed to stay overnight.

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The precipitates are filtered through Whatman filter paper and washed thoroughly with hot distilled water till the precipitates are free of oxalates. The precipitates along the filter paper are added in the original beaker and dissolved in 20 ml of 10 % sulphuric acid. The contents are heated to about 70 ˚C and titrated against 0.1 N potassium permanganate solutions to a faint pink colour. A blank is also run using similar procedure. Calculations: I ml of 0.1 N $KMnO_4$ used = 0.002 gram Calcium

$$\text{Calcium (\%)} = \frac{\text{ml of N KMnO4 used x 0.002x A/B}}{\text{Weight of sample (grams)}} \text{x100}$$

**Magnesium**

Magnesium is converted to magnesium pyrophosphate, which is estimated gravimetrically (Raghuramulu N *et al.,* 2003).

**Reagents**

a. 10% Ammonium phosphate solution
b. 10%, Sodium citrate solution
c. 0.1% Methyl red indicator
d. 1:4 and 1:10, ammonia: water solution

**Procedure:** To the calcium-free filtrate (obtained from the filtrate after precipitation of calcium as oxalate refer calcium by titrimetric method) is added 30 ml of conc. $HNO_3$ and the solution evaporated completely on a boiling water bath. Five mi of conc. HCl and 100 ml of water are then added and the solution stirred well with a glass rod. It is followed by the addition of 10 ml of 10% ammonium phosphate solution and 5 ml of 10% sodium citrate solution and the mixture stirred. After adding 2 or 3 drops of methyl red indicator, the solution is neutralised with the addition of 1:4 dilute ammonia. Strong ammonia (25 ml) is then added, stirred vigorously and the mixture left to stand overnight, filtered through Whatman No. 40 or 44 filter paper and washed free from chlorides using 1:10 dilute ammonia (tested with HNO, + silver nitrate solution). The funnel with the precipitate on the filter paper is dried in an oven. The filter paper is then transferred to a weighed crucible (the crucible is previously heated, cooled and weighed) and ashed slowly over a burner. It is then kept in a muffle furnace at 600°C for 2 h. The crucible and the contents are cooled in a desiccator and weighed to get magnesium as its pyrophosphate.

**Calculation:**

$$\text{Mg of magnesium/100 g of sample} =$$

$$\text{weight of ash x } \frac{48.6}{222.6} \text{ x } \frac{100}{\text{volume taken for estimation}} \text{ x1000}$$

**Total vitamins**

**Thiamine**

This is based on the oxidation of thiamine to thiochrome, which fluoresces in UV light. Under standard conditions and in the absence of other fluorescing substances, the fluorescence is proportional to the thiochrome present, and hence, to the thiamine originally in solution (Raghuramulu N *et al.,* 2003).

**Reagents**

a. Basic lead acetate solution: Lead acetate, 180 g, is dissolved in about 700 ml of distilled water and the solution boiled. To the boiling solution is added, in small quantities, lead oxide (litharge) 180 g and the solution boiled for 30 min. It is then cooled, made up to 1 L with distilled water and filtered.
b. Thiamine solution: 25 mg of thiamine dissolved in 250 ml of 0.01 N HCl. 1 ml of this stock solution is diluted to 100 ml to get a working standard containing 1 ug/ml.

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**Procedure:** Preparation of vitamin extract: About 10-20 g of the well-mixed, ground food sample is taken and treated with 1.00 ml of acetate buffer, pH 4.0-4.2, (made by mixing 30 ml of 1 M sodium acetate solution with 70 ml of 1 N acetic acid and making up to 500 ml) and the mixture blended in a waring blender, if necessary, for thorough mixing. To the slurry, 5 ml of enzyme suspension containing 150 mg of taka-diastase and 75 mg of papain in 5 ml of acetate buffer are added. An enzyme blank containing the buffer and enzymes, but without the sample, is also run simultaneously. A few drops of toluene are added and the mixture is incubated overnight at 37°C. The enzymes are inactivated by heating in a water bath at about 80°C for

5-10 min, the solution cooled, the mixture centrifuged and the clear centrifugate collected. Aliquots of this solution are used for estimating thiamine and riboflavin.

Twenty ml of the vitamin extract is treated with 10 ml of basic lead acetate solution, mixed well and centrifuged. A suitable aliquot of the supernatant (20 or 25 ml) is treated in another centrifuge tube with 3 ml of 30% $H_2SO_4$ and 12 ml of water (or enough water to make the total volume to 40 ml) mixed well and centrifuged. The supernatant serves as the thiamine extract. Two mi of 40% NaOH is added to three, 100 ml: separating funnels (one of them serving as a blank, the other as the experimental and the third as recovery.

Add 0.8 ml of 2% potassium ferricyanide solution to the experimental and recovery funnels, shake well and 10 ml of thiamine extract is added to all the three separating funnels. To the recovery funnel, 1 ug of thiamine standard solution is added and the contents allowed to stand for 2 min after shaking. Then 15 ml of isobutyl alcohol (water saturated) is added and shaken for 2 min. The aqueous layer is rejected. If the alcohol layer is turbid, 1 ml of ethyl alcohol is added to clear the solution. The fluorescence of the isobutyl alcohol layer is measured in a fluorimeter using the excitation wavelength of 370 m and emission wavelength of 445 nm. The calculation is done after making due allowance for the dilution and the blank.

**Calculation:**

$$\mu g \text{ of Thiamine}/100g \text{ of sample} = \frac{\text{Experimental reading} - \text{Blank reading}}{\text{Recovery reading} - \text{Experimental reading}} \text{ x Dilution x } \frac{100}{\text{Wt.of the sample}}$$

**Riboflavin**

The native fluorescence of riboflavin in neutral pH is used for the chemical estimation (Raghuramulu N *et al.,* 2003).

**Reagents**

a. Riboflavin standard solution: 25 mg of riboflavin is dissolved in 300 to 400 ml of water, adding 1.2 ml of glacial acetic acid and warming at a low temperature to aid solution. After riboflavin is dissolved, the solution is cooled and made up to 1 L. This stock solution has a concentration of 25 g/ml. Two ml of this stock solution is diluted to 50 ml to give a working standard of 1 ug per ml.
b. 4% Potassium permanganate
c. 10: 1:1 H202: water mixture.

**Procedure:** To about 25 ml of the vitamin extract (refer: preparation of vitamin extract under thiamine), one or two drops of caprylic alcohol is added, followed by 3 ml of freshly prepared 4% potassium permanganate solution. The mixture is stirred well and within 2 min, 3 ml of 1:1 $H_2O_2$: water solution is added to discharge the permanganate colour and the pH adjusted to 7.0 with NaOH. The volume is made up to 35 ml and the solution is filtered and the fluorescence of the filtrate is measured in a fluorimeter using excitation wavelength of 475 nm and emission wave length of 530 nm.

**Calculation:**

The fluorescence of a known aliquot of the filtrate is - A.

1 µg of riboflavin is added and the reading noted - B.

A small pinch of sodium hydrosulphite is added to destroy the riboflavin only and recorded - C.

Then the quantity of riboflavin in the solution taken for fluorimetry = $\frac{A-C}{B-A}$ X 1µg

From this, the riboflavin content of the sample is calculated, making due allowance for dilution and weight of the sample.

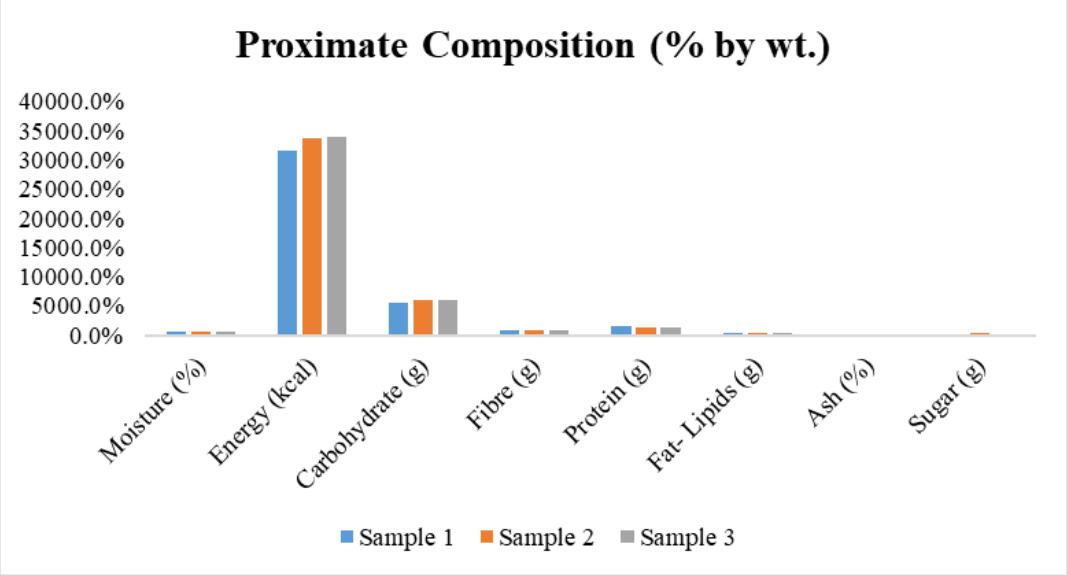
**Results**

3.1 Estimation of proximate composition and mineral content of standardized acceptable millet (jowar (Sorghum bicolor (L.) Moench) flour, pearl millet (bajra) (Cenchrus americanus (L.) Morrone) flour, kodo millet (Kodra, Araka) (Paspalum scrobiculatum L.) flour, whole wheat flour and semolina flour) based extruded food products.

**Table 3.1A:** Proximate composition, mineral and vitamin content of Jowar pasta, Bajra pasta, Kodo pasta

| **Proximate composition** | **Sample 1** | **Sample 2** | **Sample 3** |
|---|---|---|---|
| Moisture (%) | 7.20 | 5.07 | 5.20 |
| Energy (kcal) | 316 | 336 | 339 |
| Carbohydrate (g) | 55.0 | 60 | 61 |
| Fibre (g) | 9.0 | 9.34 | 9.23 |
| Protein (g) | 15.10 | 13.90 | 13.71 |
| Fat- Lipids (g) | 4.05 | 4.56 | 3.60 |
| Ash (%) | 1.78 | 1.46 | 1.90 |
| Sugar (g) | 2.15 | 2.90 | 2.02 |
| Calcium (mg) | 89 | 70 | 112 |
| Magnesium (mg) | 14 | 24 | 29 |
| Thiamine (µg) | 0.78 | 0.34 | 0.42 |
| Riboflavin (µg) | 0.45 | 0.23 | 0.33 |

**Figure 3.1** A: Proximate composition, mineral and vitamin content of Jowar pasta, Bajra pasta, Kodo pasta



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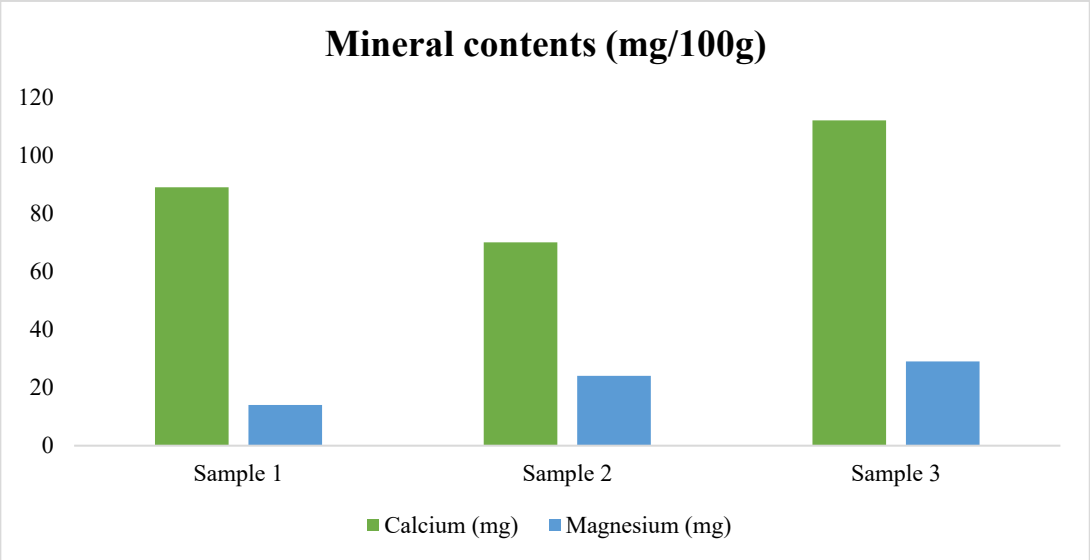


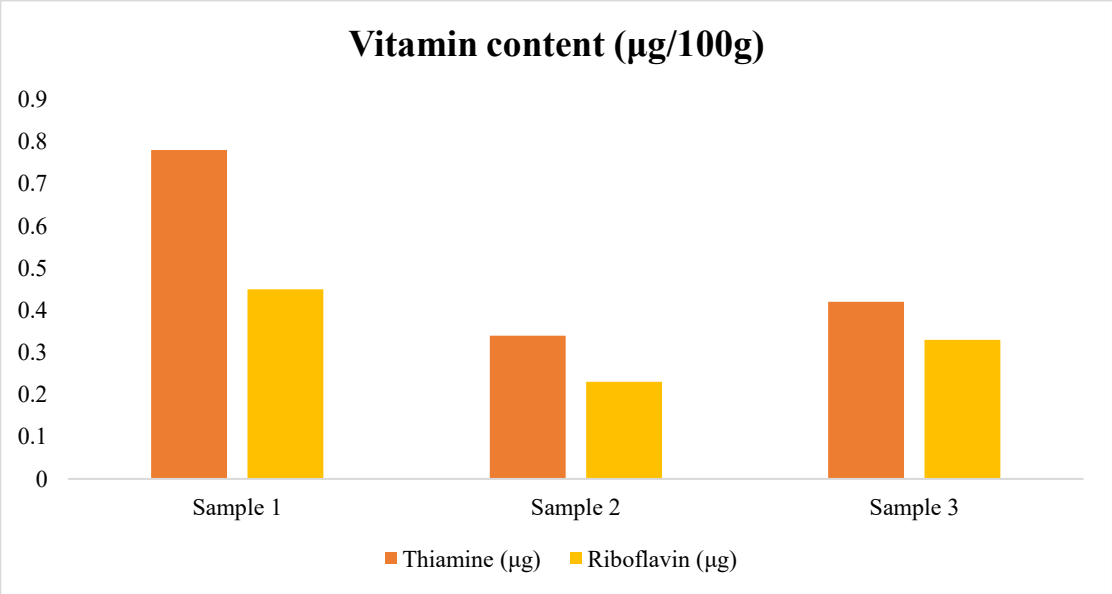


## CONCLUSION

The data in respect to proximate composition, mineral and vitamin content of Jowar pasta (Sample 1), Bajra pasta (Sample 2) and Kodo pasta (Sample 3) and pictorial representation was depicted in Table 3.1 A and Figure 3.1 A. It was observed that Sample 1 has more moisture i.e. 7.20%, protein i.e. 15.10 g, thiamine i.e. 0.78 µg, riboflavin i.e. 0.45 µg than Sample 2 which has 5.07%, 13.90 g, thiamine 0.34 µg, riboflavin 0.23 µg and Sample 3 has 5.20%, 13.71 g, thiamine 0.42 µg, riboflavin 0.33 µg. However, Sample 2 has more fibre i.e. 9.34 g, fat 4.56 g, sugar 2.90 g, than Sample 1 which has 9.0 g, 4.05 g, 2.15 g and Sample 3 has 9.23 g, 3.60 g, 2.02 g. whereas, Sample 3 has more energy i.e. 339 kcal, carbohydrate 61 g, ash 1.90%, calcium 112 mg, magnesium 29 mg than Sample 1 which has 316 kcal, 55 g, 1.78%, 89 mg, 14 mg and Sample 2 has 336 kcal, 60 g, 1.46%, 70 mg, 24 mg respectively. Biochemical estimation of millet pasta revealed that Jowar pasta has more moisture, protein, thiamine and riboflavin content whereas Bajra pasta has more fibre, fat and sugar content where Kodo pasta has more energy, carbohydrate, ash, calcium and magnesium content respectively.

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