Effect of Whey Protein Concentrates (WPC) on Microbial and Textural Evaluation of Foxtail and Finger Millet Papad

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ABSTRACT

Whey Proteins Concentrate (WPC) is an excellent source of dietary nitrogen and branched chain amino acids. Protein Concentrate (WPC) at different levels of substitution i.e. control (T₀), 2.5 parts (T₁), 5 parts (T₂), 7.5 parts (T₃) and 10 parts (T₄). In related to the microbial evaluation of raw foxtail and finger millet papad prepared from treatments T₀ to T₄ content, The total plate count, yeast and mold count of all samples of raw foxtail and finger millet papad was under acceptable limits. Textural analysis revealed that 10 parts of WPC supplemented foxtail and finger millet fried papad showed maximum Hardness (1.84 N) and Fracturability (0.96 mm).

Keywords
WPC, Foxtail and finger millet, Papad, Microbial and textural properties

Introduction

Papad is a popular and tasty food item in the Indian diet in many centuries, which is regularly consumed as a meal, accompaniment, after roasting or frying or as adjunct along with vegetable soups and curries (Chowdhury et al., 2009). It is also known as Appalam and essentially a thin wafer like product, circular in shape, rolled and nutritious as well.

Millets are important crops of Asia and Africa (especially in India and Nigeria), with 97 percent of millet production in developing countries. Various traditional foods and beverages such as roti, bread (fermented or unfermented), porridge, snacks and fast foods, baby foods, millet wine, millet nutrition powder etc. are made up of millets (Chandrasekara and Shahidi, 2012) and it contains 60-70 per cent carbohydrates, 7-11 per cent proteins, 1.5-5 per cent fat, and 2-7 per cent crude fibre and are also rich in vitamins and minerals. They are excellent source of vitamin B, magnesium, and antioxidants (Singh et al., 2012). Millets are also rich sources of micronutrients and phytochemicals (Liu, 2007). It is an alkaline forming food, alkaline based diet is often

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recommended to achieve optimal health, meaning when it combines with digestive enzymes. The soothing alkaline nature of millet helps to maintain a healthy pH balance in the body, crucial to prevent illnesses.

Foxtail millet (*Setaria italica*), a member of the family *Poaceae*, is one of the oldest cereal crops. Foxtail millet grain is rich in protein (12.3%) and iron (2.8mg/100g) as compared to rice (6.8 % protein and 1.8mg iron/100g grain) and rich in fat 4.3 per cent which is superior to rice and wheat. The grain is good source of beta - carotene, which is the precursor of Vitamin A (Murugan and Nirmalakumari 2006). Foxtail millet is mixed with legumes to make porridge and also mixed with soybean to make mixed flour. Foxtail millet has low glycemic index (GI), used for preparation of low GI biscuits and *burfi*, a sweet product and it is an ideal for people suffering from diabetes (Anju and Sarita, 2010) and also fermented to make vinegar, yellow wine, maltose, beer and other related products.

Whey proteins are an excellent source of dietary nitrogen and branched chain amino acids which are used to fuel working muscles and stimulate protein synthesis. Whey protein has antimicrobial, antiviral and antioxidant properties, and they also act as techno-functional ingredients in many formulated food systems due to their good solubility, surface activity and gelling properties. In addition, whey proteins and their associated peptides display significant functional food ingredient potential (Chatterjee and Kanawjia, 2010).

**Materials and Methods**

**Millet grains**

Minor millets such as foxtail millet (DHF-1) and finger millet (CO-9) are procured from the Indian Institute of Millet Research (IIMR), Rajendranagar, Hyderabad.

**Whey Protein Concentrate (WPC)**

Whey Protein Concentrate (WPC-70) required for study was purchased from Modern dairy limited, Karnal.

**Ingredients**

Salt, alkaline salt (*papad khar*), red chilly powder, cumin, sesamum, asafoetida and fortune soybean oil was procured from the local market of Parbhani (Table 1 and 2).

**Chemicals and glassware’s**

Chemicals (Analytical grade) were procured from standard firms and glassware’s required for conducting chemicals analysis and preparation of product utilized from laboratory of department of AHDS, VNMKV, Parbhani.

**Treatment details**

During this *papad* preparation two minor millets viz. Foxtail millet (DHF-1) and Finger millet (CO-9) were utilized with WPC.

- **T<sub>0</sub>** – 70 parts Foxtail millets + 30 parts finger millet
- **T<sub>1</sub>** – 67.5 parts of Foxtail millets + 30 parts finger millet + 2.5 parts of WPC
- **T<sub>2</sub>** – 65.0 parts of Foxtail millets + 30 parts finger millet + 5.0 parts of WPC
- **T<sub>3</sub>** – 62.5 parts of Foxtail millets + 30 parts finger millet +7.5 parts of WPC
- **T<sub>4</sub>** – 60.0 parts of Foxtail millets + 30 parts finger millet +10.0 parts of WPC
Flow diagram for preparation of protein enriched foxtail and finger millet Papad

Foxtail millet grains                      Finger millet grains

Cleaning of grains                       cleaning of grains

Grinding                                  Grinding

Sieving                                    Sieving
  (60 mesh sieve)                          (60 mesh sieve)

Flour                                  Flour

Mixing of flour
  (Foxtail millet (as per treatment) + 30% Finger millet)

Addition of WPC-70 as per treatment

Addition of ingredients
  (Salt + Alkaline Salt + Red chilly powder + Cumin + Sesamum + Asafoetida)

Add above mixture in boiling water and Stirring well
  (Flour: water - 1:2)

Kneading

Preparation of soft dough

Divide dough in to small portion (15 gm each ball)

Place these balls in plastic sheet

Take these balls in Papad press machine

Pressed into 0.10 or 0.15 cm thick sheet

Dried at room temperature (8-10)

Packed in to LDPE bag

Storage at room temperature

Preparation of protein enriched foxtail millet and finger millet papad
Methodology

Microbiology of Papad

All samples were analyzed for different microbial counts such as total plate count and yeast and mold count by adopting standard procedures.

Total plate count

The total plate count of protein enriched foxtail and finger millet papad was determined by using total plate count method using Nutrient Agar. The dilution where made up to 10^-6 and the 1 ml of aliquot was used for the isolation. All processes were carried out in a sterile area with the help of laminar air flow. Plate was incubated at 37°C for 48 hr. and results noted in CFU/ml. Total plate count (TPC) of papad were examined at 1 to 6 months (Chandru et al., 2010).

Yeast and mould count

Yeast and mould count was determined by the method cited in ISI (IS: 5403) 1969 using potato glucose agar up to 1 to 6 months.

Textural characteristics of Papad

Stable Micro System TAST2 plus Texture Analyzer was used for texture profile analysis (TPA) of protein enriched minor millet papad prepared by lab sample. A spherical-end probe of 6.35 mm of diameter with test speed of 1 mm/sec. of pre-test and post-test speeds; and 50 % compression was taken for TPA analysis. TPA is “two-bite” test, which includes the first and second compression cycles. The first and second compression cycles indicate the force vs. time data during the first and second compression of the product by the instrument probe. There were two replications of the instrumental analysis conducted on two separate days. For each replication, minor millet papad incorporated with different proportions of WPC were evaluated.

Statistical analysis

The data obtained was analysed using Completely Randomize Design (Panse and Sukhatme, 1985).

Results and Discussion

Effect of WPC on microbial qualities of finger millet raw Papad

It is revealed from table 3 that after 30 days of storage total plate count was found in T_3 and T_4 and not detected in rest of treatments. After 60 days of storage the TPC was increased from T_2 (0.58×10^2 CFU/g). From Table 3, it can be concluded that after 90 days of storage the samples of foxtail and finger millet papad with 10 pars of WPC supplemented (T_4) tend to have higher mean Total plate counts than those with T_3, T_2, T_1 and T_0. The maximum Total plate count on nutrient agar was observed in T_4 (10 parts WPC supplemented papad) and minimum in T_0 (control) for 90, 120, 150 and 180 days of storage period. Results revealed that the total plate count of all samples does not differ significantly. The increase in microbial load of WPC supplemented foxtail and finger millet papad as compared to control may be due to increase in moisture content with increasing WPC supplementation level. The growths of TPC were in increasing order as storage period increased. As per the WHO (1994) guidelines the total plate count should be less than 2 x 10^5 per gram. The result of this study in respect of TPC was in acceptable limit.

From table 4, it was observed that the yeast and mould growth was not detectable up to 30 days of storage in all treatments and after 60 days of storage yeast and mould count was
observed only in treatment T₄ (0.65×10² CFU/g). After 90 days of storage yeast and mould count was observed in T₂, T₃ and T₄ and absent in T₀ and T₁. After 120 days of storage the samples of foxtail and finger millet papad with 10 parts of WPC supplemented (T₄) tend to have higher mean yeast and mould counts than those with T₃, T₂, T₁ and T₀. The maximum yeast and mould count was observed in T₄ (10 pars WPC supplemented papad) and minimum in T₀ (control) for 120, 150 and 180 days of storage. Results of this study revealed that the yeast and mould count of all samples does not differ significantly. The increase in yeast and mould count of WPC supplemented millet papad as compared to control may be due to increase in moisture content with increasing WPC supplementation level. As per the WHO (1994) guideline the Yeast and mold count should be less than 1×10⁴ per gram. Above results related to yeast and mould count was within the acceptable limit.

Maximum hardness was observed for foxtail and finger millet fried papad prepared using 10 parts of WPC (1.84 N) and minimum (1.61N) for foxtail and finger millet fried papad prepared by without addition of WPC. Hardness of foxtail and finger millet fried papad in peak positive force was increases as the incorporation of WPC increases from 0 to 10 parts. Variation in hardness of protein enriched foxtail and finger millet fried papad samples might be due to protein content; it hold recipe water and binds other ingredients and increases the breaking strength or hardness of the product. Present results are in agreement with Wani et al., (2015) indicated that, the highest peak force was observed in 6 (%) WPC supplemented cookies (55.3 N), followed by control cookies (50.2 N). Fracturability of foxtail and finger millet fried papad was ranged from 0.76 to 0.96 mm. Fracturability of fried papad increased as the incorporation of WPC increases from 0 to 10 parts. It is may be due to the increasing hardness of developed product. The fracturability of the foxtail and finger millet fried papad with 10 parts WPC (T₄) was significantly (P<0.05) superior over all treatments.

**Table.1** Recipe for preparation of protein enriched minor millet Papad

| Sr. No | Ingredients                      | Quantity for 100 gm |
|--------|----------------------------------|---------------------|
| 1      | Salt                             | 2 gm                |
| 2      | Alkaline Salt (Papadkhar)        | 3 gm                |
| 3      | Red chilly powder                | 1 gm                |
| 4      | Cumin                            | 1.5 gm              |
| 5      | Sesamum                          | 2 gm                |
| 6      | Asafoetida                       | 0.5 gm              |
| 7      | Water                            | 200 - 220 ml        |
| 8      | WPC- 70                          | As per treatment combinations |
Table 2 Chemical composition of WPC, foxtail and finger millet grains

| Sr. no | Composition        | WPC Powder (%) | Foxtail millet Grains (%) | Finger millet Grains (%) |
|--------|--------------------|----------------|---------------------------|--------------------------|
| 1      | Total Solid (%)    | 96.96          | 91.95                     | 90.10                    |
| 2      | Ash (%)            | 6.20           | 1.09                      | 2.40                     |
| 3      | Moisture (%)       | 3.04           | 9.42                      | 9.50                     |
| 4      | Protein (%)        | 70.85          | 13.0                      | 8.80                     |
| 5      | Fat (%)            | 6.50           | 3.67                      | 3.90                     |
| 6      | Carbohydrate (%)   | -              | 74.19                     | 75.00                    |
| 7      | NPN (%)            | 0.18           | -                         | -                        |
| 8      | Calcium (%)        | 1.70           | -                         | -                        |
| 9      | Chloride (%)       | 1.57           | -                         | -                        |
| 10     | Acidity (%)        | 1.62           | -                         | -                        |

(Rathour et al., 2016; Nazni and Bhuvaneswari, 2015; Kamtkar et al., 2015)

Table 3 Effect of WPC on total plate count (×10² CFU/g) of foxtail and finger millet raw papad

| Treatment | Storage period in days (×10² CFU/g) |
|-----------|-----------------------------------|
|           | 00 | 30 | 60 | 90 | 120 | 150 | 180 |
| T₀        | ND | ND | ND | 0.45 | 1.00 | 1.75 | 2.50 |
| T₁        | ND | ND | ND | 0.64 | 1.20 | 2.75 | 3.75 |
| T₂        | ND | ND | 0.58 | 0.90 | 1.75 | 3.20 | 4.65 |
| T₃        | ND | 0.65 | 0.82 | 1.50 | 2.50 | 4.50 | 5.75 |
| T₄        | ND | 0.85 | 1.20 | 2.50 | 3.75 | 5.50 | 6.75 |
| SE        | 0.00 | 0.09 | 0.05 | 0.04 | 0.05 | 0.05 | 0.03 |
| CD at 5%  | 0.00 | 0.27 | 0.15 | 0.12 | 0.15 | 0.15 | 0.09 |

Table 4 Effect of WPC on yeast and mould count (×10² CFU/g) of raw foxtail and finger millet papad

| Treatment | Storage period in days (×10² CFU/g) |
|-----------|-----------------------------------|
|           | 00 | 30 | 60 | 90 | 120 | 150 | 180 |
| T₀        | ND | ND | ND | ND | 0.25 | 0.75 | 1.25 |
| T₁        | ND | ND | ND | ND | 0.45 | 1.20 | 1.75 |
| T₂        | ND | ND | ND | 0.25 | 0.75 | 1.75 | 2.50 |
| T₃        | ND | ND | ND | 0.75 | 1.25 | 2.50 | 3.15 |
| T₄        | ND | ND | 0.65 | 1.25 | 2.00 | 2.75 | 3.95 |
| SE        | 0.00 | 0.00 | 0.01 | 0.03 | 0.03 | 0.07 | 0.06 |
| CD at 5%  | 0.00 | 0.00 | 0.03 | 0.09 | 0.10 | 0.21 | 0.18 |
Table 5 Effect of WPC on textural properties of foxtail and finger millet fried *Papad*

| Treatments | Hardness (N) | Fracturability (mm) |
|------------|-------------|---------------------|
| T₀         | 1.61ᵃ       | 0.76ᵃ               |
| T₁         | 1.67ᵇ       | 0.82ᵇ               |
| T₂         | 1.72ᶜ       | 0.87ᶜ               |
| T₃         | 1.77ᵈ       | 0.92ᵈ               |
| T₄         | 1.84ᵉ       | 0.96ᵉ               |
| SE         | 0.01        | 0.01                |
| CD at 5%   | 0.03        | 0.03                |

Hence concluded, in view of experimental results obtained during the present investigation, as supplementation of WPC increases microbial quality such as TPC and yeast and mold increased in foxtail and finger millet *papad*. The TPC and Yeast and mold count in all samples for period of six months was found within acceptable limits. WPC supplementation affected textural characteristic of fried *Papad*, the hardness and fracturability increases with increase in the WPC level.

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