

Development and Evaluation of Value-Added Iron Rich Spirulina Millet Mix Flour

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Abstract: Millet's, called 'nutria cereals' are grains that are long forgotten. They can be revived and value-added with iron rich ingredients like Spirulina to combat Iron deficiency anemia, currently a global public health issue. The objectives were to develop a value-added iron rich Spirulina Millet Mix Flour (SMMF); to evaluate its proximate principles- Iron and Calcium, study its shelf life and to study the sensory characteristics of traditional recipes incorporated with SMMF. The product was developed using sprouted Finger Millet, Foxtail Millet, Kodo Millet and Spirulina. The standardized SMMF was, evaluated and incorporated into traditional recipes at different levels, followed by sensory evaluation. The addition of 2.5g Spirulina had an overall acceptability of 6.86±0.83 per serving of sieved millet mix flour muffins. SMMF had a rich nutrient profile of 5.4g moisture, 2g ash, 378.84 kcals energy, 72.63g carbohydrates, 16.07g protein, 2.66g fat, 1.24g fiber, 299.6g calcium and 20.16g of Iron per 100g. A serving of SMMF can deliver 6.08mg of Iron that would be highly bioavailable. It had an excellent shelf life of a month, and a peroxide content-4.6 meq/kg. The sensory acceptance of SMMF at 0,25,50,75 and 100% into traditional recipes were significant for Dhokla ($p < .05$), Burfi ($p < .10$) and Chapathi ($p < .01$). SMMF incorporated Onion pakora and Muffin had no significant difference ($p < .10$). Of the traditional recipes modified, 50% SMMF Chapathi and Burfi, 75% SMMF Dhoklas and Onion Pakoras and 100% SMMF muffins were the most accepted. Thus, by efficient combination of Spirulina and the humble Millet's, SMMF can reach households and help tackle public health issues of Iron deficiency anemia.

Keywords: Iron, Spirulina, Millet, Millet mix flour, Anemia

1. Introduction

Iron deficiency anemia is a common prevalent nutritional problem and currently a public health issue in the world. As infants, young children, adolescents, women of childbearing age and pregnant women are the most vulnerable group; it has awakened the need for iron rich functional foods [1]. Alvarez-Uria et al., 2014 reported that the World Health Organisation (WHO) claims that two billion people are affected by anemia in the world, half of which are anemia due to Iron deficiency. A low-cost, effective intervention is needed to treat this condition at large [2].

Millets, which are high in iron and cost-effective, should be encouraged for consumption in India and eventually aim at negating Iron Deficiency Anemia. Millets are claimed to be the oldest grown grain. Known as Gods own crops 'Miracle Grains'/'Adbhut Anaj' 'Nutri Cereals' these grains owe it to the nutrient richness they possess. The term "Millet" is the name given to a group of cereals other than wheat, rice, maize & barley. (Food and Agriculture Organisation-FAO and The International Crops Research Institute for the Semi-Arid Tropics-ICRISAT, 1996).

Currently, India is the largest producer of a variety of Millets. It still is a staple crop. The millets cultivated in India are- Finger Millet, Foxtail Millet, Kodo Millet, Little Millet, Proso Millet, Barnyard Millet, Pearl Millet and Sorghum. Among these Millets, Finger Millet, Kodo Millet, and Foxtail Millet were selected for the study because of their Iron content and acceptability.



Arthrospira (Spirulina) is a microscopic, filamentous prokaryote. *Spirulina platensis* is commercially available in the form of powder, tablets, and capsules. Spirulina powder is a uniform powder which is dark blue-green in color having a distinct mild seaweed taste and smell.

It was brought to notice around the 1940's by a phycologist Dangeard who described in a journal about 'dihé', which was consumed by the Kanembu people near Lake Chad in Africa as hardened cakes from dried lakes. Dihé were broken into small pieces and consumed without any further treatment. It was used to make sauces accompanying the regular Millet meal. Locals consumed this meal during seasons of drought and famine during which the lakes dried up thus keeping them safe from malnutrition.

Spirulina is one among the most concentrated nutritious food having 55% protein and containing ample amounts of antioxidants, phytonutrients, essential fatty acids, etc, therefore it is even considered an ideal food for the 21st century by the Food and Agriculture Organization (FAO).

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Table 1: Nutrient profile of Spirulina (Antenna Nutritech Foundation)

Nutrient	Amount /100g
Energy (kcal)	290
Carbohydrate (g)	15.25
Protein (g)	55.70
Fat (g)	4.7
Moisture (g)	3.7
Calcium (mg)	1000
Iron (mg)	180

(Obtained from Antenna technology via correspondence).

It is cultivated, processed and marketed worldwide. Other flavoring substances can mask the taste and smell. FAO suggests a dosage of **0.5 to 3 grams** of Spirulina **per serving size**. The recommendations are adopted in the current study.

Spirulina has high dietary iron with enhanced bioavailability. Being a concentrated source of iron both for supplementation and fortification, Spirulina has been chosen as one of the main ingredients in the product to be developed.

The selected ingredients are loaded with nutrients and can be incorporated into other products easily. They are specifically rich in iron, have high bioavailability and are feasible. Another need for including these specific ingredients are to revieve the use of these whole foods. Due to ignorance or unawareness, Spirulina is still being discovered. Millets on the other hand are losing its place in the production and consumption food basket of India since the green revolution due to factors like low monetary benefits, lack of government initiatives and subsidies, subsidized cereals like rice and wheat and change in consumer preferences. In recent years, there has been some effort toward reviving the underutilized Millets.

Thus, these Millets along with appropriate amounts of Spirulina can be combined to obtain an Iron rich mix. The incorporation aims to provide the necessary nutrients to people of all walks of life on a day to day basis and in particular to the vulnerable section affected by anemia.

The objectives of the study were

- To develop an Iron rich Millet mix flour with Spirulina
- To evaluate the proximate principles, mineral composition (Iron and Calcium) and shelf life principles of variations of Spirulina Millet Mix Flour.
- To incorporate Spirulina Millet Mix Flour (SMMF) into traditional recipes and evaluate the sensory characteristics.

2. Materials and Methods

2.1 Selection and procurement of ingredients

The Millets and Spirulina were selected based on their organoleptic quality, availability and nutrient content (Iron). Foxtail and Kodo Millet were procured in the rice form (de-husked) from Madurai. Finger Millet was procured at Bengaluru. Spirulina powder was obtained from Antenna Nutritech Foundation, Madurai.

2.2 Preparation of Millet mix flour

Finger Millet taken as a full grain form was germinated and dried. The three Millets were then roasted and milled in 1:1:1 ratio. A predetermined amount of Spirulina was mixed manually with the sieved flours.

2.3 Standardization of the level of acceptance of Spirulina using muffins

To determine the acceptable level of Spirulina per serving, a standard muffin recipe was modified by addition of 2 grams, 2.5 grams and 3 grams per serving size.

Plate 1: Standardization of acceptance of Spirulina incorporation per serving (30g) 21 semi-trained panelists conducted the sensory analysis with 9 point: hedonic scale and the responses were statistically analyzed by mean, standard deviation, and analysis of variance using SPSS and Microsoft Excel software.

2.4 Proximate and mineral analysis of the flours

Sieved Millet mix flour, SMMF (2g/serving), SMMF (2.5g/serving) and SMMF (3g/serving) were analyzed for their proximate principles. Estimation of Moisture, Ash, Protein, Fat, Calcium and Iron were conducted according to AOAC 2000, Energy and Carbohydrate by AOAC 1995 and Crude fiber by AOAC 1980 method.

2.5 Shelf life Analysis

The products microbial analysis [3] and the Peroxide test (AOAC, 2000) was conducted on day 1, 15 and 30 respectively.

2.6 Product Incorporation

Based on Spirulina acceptance level test, 2.5g Spirulina was used to develop SMMF. SMMF was then incorporated into five traditional recipes: Dhokla, Burfi, Chapathi, Pakora, Muffin at 0,25,50,75 and 100% and subjected to sensory analysis according to 9 point hedonic scale.

3. Results and Discussion

3.1 Sensory evaluation

3.1.1 Standardization of Spirulina by using muffins

Refer Table 2: Standardization of Spirulina by using muffins

The means of the sensory evaluation of Spirulina muffins at different levels of addition reveal that the 3 g addition per serving size (30g) had disagreeable sensory characters with an overall mean acceptability of 5.45. The overall mean score of 2 g and 2.5g Spirulina incorporated muffin deviated by a mean difference of 0.05. Thus the present study has used 2.5 g of Spirulina per serving size. This addition falls within the limit of the FAO and by FDA standards (2002) that have the same recommendations for a medium-end consumer [4].

There was a significant difference ($p < .01$) between the standard muffin and muffins with Spirulina. This difference

was due to their strong odor, taste, and color. These parameters also affect the appearance of the muffin and hence its overall acceptability.

3.2 Sensory evaluation of products developed

The acceptance is represented statistically by mean, standard deviation, and analysis of variance. The value closest to the standard product was taken as the most accepted variation.

3.2.1 Sensory evaluation of Dhokla

Refer Table 3: Sensory evaluation of standard and SMMF Dhokla.

In dhoklas, SMMF addition at 75% was the best accepted with an overall acceptability score of 7.04 on the hedonic scale. The dhoklas had good mean scores for its aroma, color, and texture. The taste of 100% addition had the lowest scores because of high amounts of Spirulina. There was a significant difference ($p < .05$) found in the overall acceptability of variations and standard.

3.2.2 Sensory evaluation of Burfi

Refer Table 4: Sensory evaluation of standard and SMMF Burfis

Burfi's at all variations had a good acceptance. The sweetness of the burfi masked the strong odor and taste of Spirulina. The mean overall acceptability was highest in the standard (no Spirulina) followed by 50% addition. The means also indicate that 100% variation had high overall acceptance. On comparison, there was a significant difference at $p < .10$ in the overall acceptability among the standard and the variations. The mean acceptances for each product were different.

3.2.3 Sensory evaluation of Chapathi

Refer Table 5: Sensory evaluation of standard and SMMF Chapathi

In a study done by Iyer *et al.*, in 2007 Spirulina was incorporated into chapathi's with vegetables at 1g, 2.5 and 5g per serving [5]. The overall acceptability scores were with respect to the vegetable added. The attributes such as taste, color, texture, were comparable within groups but the best acceptable was up to 2.5g. There was a significant difference ($p < .01$) between the five types of chapathi prepared. The addition of Spirulina changed the sensory quality of chapathi to a great extent. The 100% addition had a mean acceptability score of 4.90 which was rated as 'dislike slightly' on the hedonic scale. 50% addition and lower showed acceptance as close to the standard.

3.2.4 Sensory evaluation of Onion Pakora

Refer Table 6: Sensory evaluation of standard and SMMF Onion Pakora

The color sensory parameter of the standard pakora gained the best mean score, while the score is the least for 100% addition. The overall accepted product closest to the standard is the 75% addition. This percentage addition is recommended for incorporation into traditional pakora recipe. Though qualities like color and aroma varied between

the five pakoras, the statistics state there is no significant difference ($p < .10$) among the products. The deviations for the mean scores signify that there is a slight difference among the panelists, however; the overall acceptability means show no significant difference.

3.2.5 Sensory evaluation of Muffins

Refer Table 9: Sensory evaluation of standard and SMMF Muffins

The overall mean scores for 25% and 100% were the same, having the highest level of acceptability after the standard muffin. For the benefit of the study, the 100% addition was taken as the acceptable level based on the minimum standard deviation.

There was a no significant difference ($p < .10$) between the standard and the variations. The muffins overall means showed varied acceptance by the panelists. The color of the muffins played a huge difference in the acceptance. The addition of SSMF changed the entire sensory parameters of a standard muffin.

3.3 Proximate Analysis

The value of a new product developed depends on its nutritional content. Proximate analysis was conducted to determine the nutritional value of four variations. The variations were: Sieved Millet Mix Flour, SMMF 1: Spirulina Millet Mix Flour 1 (6.6g Spirulina/100g MMF), SMMF 2: Spirulina Millet Mix Flour 2 (8.33g Spirulina/100g MMF), SMMF 3: Spirulina Millet Mix Flour 3 (10g Spirulina/100g MMF).

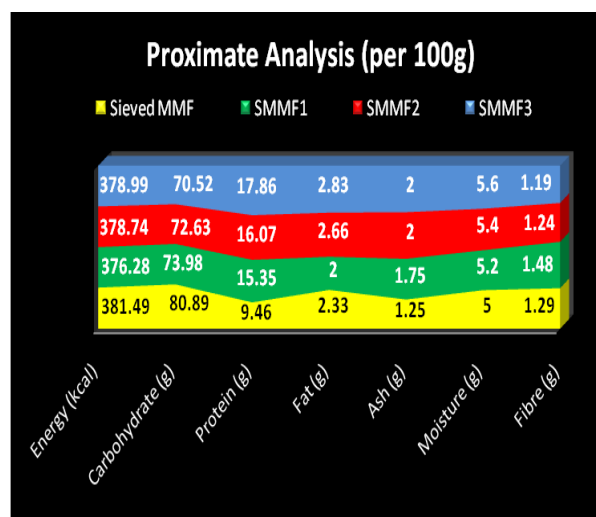


Figure 1: Proximate content of five variations (per 100g)

By the addition of Spirulina at increasing concentrations, the values of energy, protein, ash and moisture increases, while carbohydrate, fat, and fiber decrease moderately.

A significant increase in protein is because Spirulina contains 60% protein containing all essential amino acids and has a high biological value only lesser to the egg [6]. On overall analysis, variation 3: SMMF3 showed high nutrient composition. The final standardized product chosen as the product was SMMF Variation two: 2.5g Spirulina/serving in sieved Millet mix flour.

The Iron and calcium are significantly high in Spirulina and Millets. The values increase due to germination of Finger Millet and by incorporating Spirulina into the Millet flour. Studies say that Spirulina has the same amount of calcium in gram per gram basis as milk.

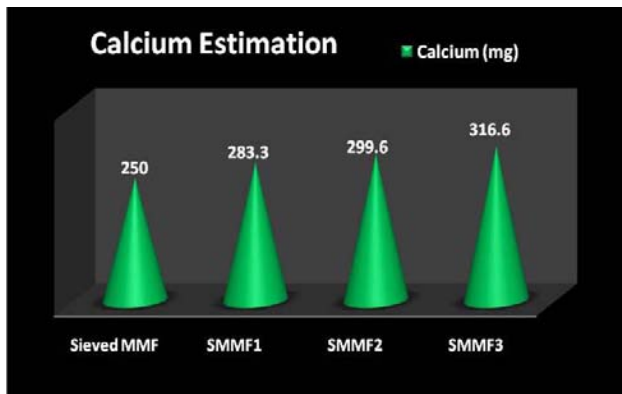


Figure 2: Calcium content five variations (mg/100g)

The calcium content increases on each subsequent addition of Spirulina. The calcium content of SMMF (2) was 299.6g/100g. This high amount of calcium of the selected flour can help improving the nutritional status of the consumer.

Iron estimation: According to the National Institute of Nutrition the Iron content of Finger Millet is 3.9mg, 2.8mg in Foxtail millet and 0.5mg in Kodo Millet. Spirulina was reported to have 180 mg of Iron per 100g.

Various studies on iron bioavailability from Spirulina have shown that it not is a only a good source of iron but more importantly, the iron is highly bioavailable [7].

Puyfoulhoux et al., 2001 proposed that Iron from Spirulina is about 6.5 times more bioavailable than that from beef [8].

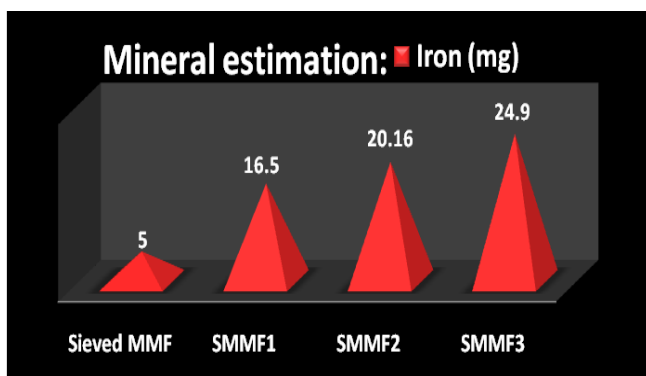


Figure 3: Iron content five variations (mg/100g)

The iron content of sieved Millet mix flour increases five times on addition of approximately 10% Spirulina. (5 mg to 25 mg). Millets, when mixed with Spirulina, have added benefits. A study was done by Simpure et al., 2006 showing supplementation of Misola (Millet, Soja, and Peanut) and Spirulina to prevent malnutrition among children in Burkina Faso, Africa. [9] The Millets in the study were milled. By milling Millets, the bio-availability of iron is better than the corresponding un-milled grains which can be attributed to the removal of interfering substances such as phytate, tannin,

and fiber. Germination or Malting increases the vitamin C and folic acid content of legumes and also degrades the anti-nutrients present. Kapoor *et al.*, in 1998 suggested that Spirulina can serve as a useful supplementary food during these crucial periods to combat anemia [10].

Thus, SMMF (100g) provides 20.16mg of Iron thus a cupcake or any recipe of one serving size (30g) would yield 6.08 mg of Iron. This amount will adequately meet the daily Iron recommendation by 25%.

3.4 Shelf Life Study

The developed product: SMMF was stored in three different aluminum pouches and analyzed for their peroxide value on day 1, day 15 and day 30 respectively.

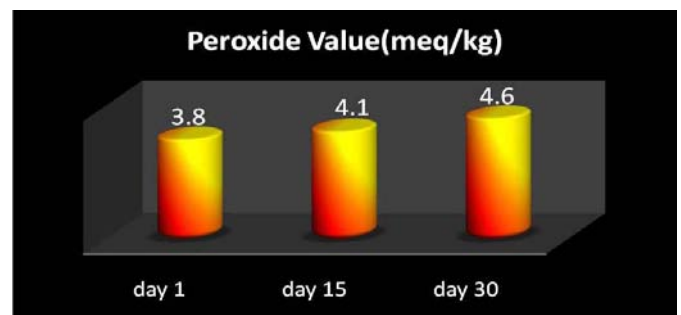


Figure 4: Peroxide value of SMMF on day 1, 15 and 30.

The results obtained for day 30 were well within the limits of fresh oils: 10 meq/kg and far from the rancid level of 30 meq/kg. The peroxide value of the product shows a slow rise in the peroxide content thus signifying that the product has less moisture and reacts slowly. More importantly, the product promises safety and a long shelf life. The results correlate with a study on the shelf life of milled and sieved Finger Millet by Chandru *et al.*, 2010 where the proposed shelf life was said to be 5 to 9 months [11]. Spirulina contains a three double bond Poly Unsaturated Fatty Acid (PUFA) - Gamma Linolenic Acid (GLA). GLA is prone to oxidation in the presence of light, oxygen, trace minerals and also due to processing and storage temperatures. In the study done by Tiburcio *et al.*, in 2007 evaluated the shelf life of spray dried Spirulina. The study concluded that the predicted shelf life of Spirulina is 8.6 months at 30°C based on GLA degradation [12].

3.5 Microbial Analysis

To render the products safety SMMF was subjected to microbial analysis on day 1, day 15 and day 30. Triplicates of the sample were analyzed to avoid error in results.

There was no growth of bacterial or fungal colonies. The product, being a dry powder is devoid of moisture, and thus the safety and quality of the product are increased. The product was considered safe for up to a month based on the microbial analysis conducted.

4. Conclusion

In light of the extent of malnutrition, iron deficiency anemia and the grip of green revolution, Spirulina and Millets were

used in this study. Spirulina is a wholesome, nutritious product of biological origin. The nutritious quality and bioavailability it offers far exceeds any food at even minute amounts. To meet the growing health demands of the population and combat the threatening public health problem of Iron deficiency anemia which accounts for half of the anemic cases in India, it is necessary to combine the underutilized Millets like Kodo and Foxtail Millet with the infamous cyanobacteria Spirulina, to provide the optimal nutrient package.

Most people have minimal access to iron rich foods. The shift in trends has also seen many women opting for convenience foods and preference for packaged foods. The current study considers this aspect too. By formulating a mix, it is easy to incorporate into dishes commonly prepared at home.

The SMMF developed meets a good proportion of the daily recommended allowance. It has an excellent nutrient profile providing 378.84 kcals of energy. The protein content of the SMMF is 16.07g which is higher than that of the hen's egg-13.3g, and Cow's milk -3.2g (Nutritive value of Indian Foods, ICMR). This product can hence be used to alleviate problems of malnutrition, which is India's biggest health concern. By the addition of Spirulina to the nutritious milled Millets: Foxtail, Finger and Kodo Millet, the nutrient profile is of high value. The SMMF mix provides 5.4g moisture, 2g ash, 378.84 kcals energy, 72.63 g of carbohydrates, 16.07 g protein, 2.66g fat and 1.24g fiber. The iron and calcium content per 100g was 16.5 mg and 299.6mg respectfully. The recommended daily allowance of Iron for an adult man is 17mg per day and 21mg per day for an adult woman, a serving of SMMF flour would deliver 4.95mg of Iron. Spirulina does have its share of controversy in the market due to its safety. However, the study has used Spirulina from a reputed source where safety, heavy-metal free ponds were used. Furthermore, the FDA board recommends Spirulina for a medium-end consumer [4]. The study has ensured quality and quantity usage of Spirulina.

The SMMF is a nut packed product with a humble intent to provide nourishment to the consumer. Through this product, the benefits of Millets- Finger Millet, Foxtail Millet and Kodo Millet can be obtained. With this intention, the study seeks to fulfill its objectives of providing Iron rich foods and thus improve the nutritional status of the consumer.

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Table 2: Standardization of Spirulina by using muffins: grams/serving size

<i>Spirulina added/serving</i>	<i>Appearance</i>	<i>Taste</i>	<i>Aroma</i>	<i>Color</i>	<i>Texture</i>	<i>Overall acceptability</i>	<i>Anova/ p value</i>
Standard-Maida	7.33±0.79	7±0.63	6.66±0.96	6.76±0.88	7.19047619	7.09±0.43	24.9 <.01*
2g	6.72±0.93	6.54±0.96	6.86±0.83	6.5±0.91	6.90±0.75	6.81±0.66	
2.5	6.54±0.96	6.36±1.00	6.40±0.95	6.45±0.91	6.54±0.96	6.86±0.83	
3g	5.22±0.75	5.54±0.73	5.22±0.86	5.59±0.59	5.5±0.85	5.45±0.73	

*significant at p < .01

Table 3: Sensory evaluation of standard and SMMF Dhokla

Dhokla							
Variation	Appearance	Taste	Aroma	Color	Texture	Overall acceptability	ANOVA p value
Mean ±Std Dev							6.13 <.05*
Standard	7.71±0.78	7.42±0.87	7.09±0.76	7.14±0.72	7.04±0.74	7.61±0.74	
25%	6.66±0.96	6.76±0.94	6.76±0.88	6.66±0.85	6.61±0.58	6.80±0.51	
50%	6.47±0.95	6.23±0.92	6.33±0.99	6.52±0.73	6.66±0.99	6.76±0.92	
75%	7.52±0.81	7.23±0.94	7.19±0.81	7.95±0.66	7±0.92	7.04±0.86	
100%	6.76±0.88	5.71±0.95	6.52±0.92	6.42±0.87	6.42±0.97	6.52±0.74	

*Significant at p < .05

Table 4: Sensory evaluation of standard and SMMF Burfi

Burfi							
Variation	Appearance	Taste	Aroma	Color	Texture	Overall acceptability	ANOVA p value
Mean ± Std Dev							4.17 <0.10*
Standard	7.61±0.86	6.80±1.36	6.61±1.39	6.90±1.54	7.33±0.96	7.85±0.85	
25%	6.66±0.12	6.52±1.36	6.38±1.20	6.19±1.20	6.42±1.20	6.61±1.16	
50%	7.04±0.92	7.52±1.12	7±1.14	7.14±1.23	7.14±1.10	7.52±0.87	
75%	6.66±1.49	6.57±1.74	6.52±1.50	6.42±1.74	6.28±1.67	6.57±1.77	
100%	7.23±1.33	7.09±1.70	6.76±1.81	6.66±1.62	7.28±1.34	7.28±1.45	

*Significant at p < .10

Table 5: Sensory evaluation of standard and SMMF Chapathi

Chapathi							
Variation	Appearance	Taste	Aroma	Color	Texture	Overall acceptability	ANOVA p value
Mean ± Std Dev							16.31 <.01*
Standard	7.57±1.28	7.428±1.28	7.19±1.32	7.8±1.85	7.38±1.43	7.47±1.16	
25%	7.47±1.05	7.09±1.30	6.76±1.22	7.80±0.87	7.42±1.12	7.42±1.13	
50%	6.47±1.16	6.52±1.28	6.52±1.43	6.85±1.35	6.66±1.27	7±1.14	
75%	5.85±1.35	6.14±1.31	5.66±1.01	5.76±1.17	5.71±1.10	6.04±1.02	
100%	4.47±1.12	4.61±1.24	4.66±1.35	4.42±1.56	4.61±1.65	4.9±1.64	

*Significant at p < .01

Table 6: Sensory evaluation of standard and SMMF Onion Pakora

Onion Pakora							
Variation	Appearance	Taste	Aroma	Color	Texture	Overall acceptability	ANOVA p value
Mean ± Std Dev							1.68 <.10* NS
Standard	7.33±1.01	6.90±1.51	7.23±1.13	7.47±0.81	7.14±1.23	7.14±1.15	
25%	6.19±1.40	6.47±1.43	6.66±1.59	6.23±1.64	6.95±1.56	6.71±1.41	
50%	6.76±1.41	7.23±1.44	6.85±1.42	6.61±1.53	7.38±1.16	7.19±1.24	
75%	6.57±1.85	6.76±1.51	6.80±1.50	6.42±1.74	7.38±1.11	7.23±1.33	
100%	6.14±1.68	6.61±1.32	6.04±1.65	5.95±1.85	6.90±1.04	6.42±1.12	

* NS –Not significant at p <.10

Table 7: Sensory evaluation of standard and SMMF Muffins

Muffin							
Variation	Appearance	Taste	Aroma	Color	Texture	Overall acceptability	ANOVA p value
Mean ± Std Dev							2.37 <.10* NS
Standard	6.71±1.34	7.38±1.20	7.19±1.20	6.80±1.43	7.52±0.98	7.42±1.02	
25%	6.28±1.48	6.80±1.59	6.61±1.55	6.19±1.67	6.95±1.46	6.90±1.54	
50%	6.09±1.67	6.52±1.28	6.19±1.60	6±1.84	6.85±0.96	6.38±1.16	
75%	6.71±1.05	6.85±0.91	6.71±1.14	6.76±0.88	6.85±6.85	6.76±0.76	
100%	6.66±1.23	6.57±1.46	6.61±1.32	6.57±1.28	6.57±1.32	6.90±0.99	

*NS- Not Significant at p <.10