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Development and Physico-chemical Analysis of *Ocimum sanctum* extract based Food products

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Abstract

Positive health benefits of *Ocimum sanctum* aka Tulsi are well known and it has been widely consumed in India from ancient times as prasadam or as a medicinal herb to improve immunity and to cure respiratory tract infections and fever. The present research work was done to evaluate the organoleptic qualities and nutrition composition of maize, oat and Tulsi leaves based food products. Three types of flour blends were prepared from maize and oats in ratio of 42.5:7.5, 37.5:12.5 and 32.5:17.5. Then one of the best standard was selected from these three after sensory evaluation and further Tulsi was incorporated in it. Then sensory evaluation was done and nutrition composition was calculated of best acceptable Tulsi incorporated product from each product category i.e. traditional bhakhari, baked biscuit and extruded vermicelli. Results showed that overall acceptability was highest for type I bhakari in which maize and oats flour were mixed in the ratio of 42.5:7.5. Type V bhakhari was found best acceptable after incorporation with Tulsi leaves at 37.5:12.5:10 level. Type 2 biscuits were found best acceptable (maize & oats in the ratio of 37.5:12.5. After incorporation of Tulsi leaves, all types of Tulsi incorporated samples were liked similarly. Type II vermicelli were liked the most with maize and oats in the ratio of 37.5:12.5 while after incorporation of Tulsi leaves in this sample, all levels of Tulsi incorporation were liked similar. Nutritive value of all the three types of products was seen, it was found that bhakhari contained crude protein, fibre and mineral content in highest amount while highest amount of fat and zinc were noted in biscuits although calcium, zinc and iron content of the developed three products was not statistically significantly different.

Keywords

Tulsi leaves, *Ocimum sanctum*, product development, physico-chemical analysis, organoleptic properties

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Introduction

Ayurveda is a conventional Hindu medicine system which emphasizes the use of endemic plant-based medicines for the medication of the diseases. According to Hindu mythology, Dhanvantari, the God's physician, is attributed to the origin of Ayurvedic medicine. Most of the ingredients of Ayurvedic and Homeopathic medicines find their origin to plants or plant based extracts. Medicinal herbs are widely used to cure many human ailments due to their minor side effects (Basgel & erdemoglu, 2006). Demand for the medicinal plants increased in the recent years due to expeditious growth in the pharmaceutical industries. According to WHO Report (2011), almost 70% of the world population still depends on traditional medicines while about 50% population of the developed countries use it as an alternative for health care (Bodeker, 2012). Parts of various plants (root, leaves, fruit, flower and seed) or whole plant of many varieties of the medicinal herbs are used for the preparation of the medicines. Only in India, over 2000 drugs used are of plant origin Chaudhary and Sekhon (2011), world over it may be higher.

As herbs are natural, these are considered more safe for human beings as well as for the environment in comparison to synthetic or manmade products. In recent era, importance of herbal medicines have been raised tremendously as these have global impact on health as well as on trade. The elemental status of the medicinal herb is determined by its soil's properties, type of plant to absorb the minerals and pollution (Queralt *et al.,* 2005).

Tulsi, the Holy Basil

Holy Basil in English, Tulsi in Hindi belongs to Lamiaceae family of plants. It is known for its medicinal value. Nomenclature of Tulsi was borrowed from the Sanskrit word that means the "matchless one". In India, Tulsi is grown worldwide including India, Malaysia, Australia, Africa and Arab countries (Verma, 2016). Tulsi is an erect, soft hairy aromatic herb of 0.5 to 1.5 m height including leaves dimension of 2 cm to 4 cm (Skaria *et al.,* 2007). Tulsi belongs to *Ocimum* species of Lamiaceae family and this family is very important because of therapeutic capability (Yang *et al.,* 2002). *Ocimum sanctum, Ocimum gratissium and Ocimum camphora are* the different varieties of Basil. *Ocimum americanum* earlier known as *Ocimum canum* covers wild species in India but it is also cultivated in the Indonesia commercially. Various species of *Ocimum basilicum including Ocimum kilim and scharicum* generally called African blue Basil are popular for its camphor-like smell. As per the Ayurveda, Tulsi is of three types known as Rama/ green leaf Tulsi (*Ocimum gratissimum*), Shyama/ dark leaf Tulsi (*Ocimum sanctum*) and Vana/ wild leaf Tulsi (*Ocimum canum*). All the three types of Tulsi have their significance in Ayurveda, but the Rama and Shyama are the most widely used. Both the varieties also have common medicinal properties but the Rama Tulsi has greater medicinal importance and it is commonly used for worship (Sampath *et al.*, 2015). Both varieties have similar chemical properties and they have a significant role in the field of science from ancient times to the modern scientist due to their many vital medicinal properties (Shafqatullah *et*

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al., 2020). Tulsi has been described in many writings of different cultures around the world such as Greeks, Romans, Egyptians and Indians. Ancient scriptures of Indians show that benefits for Tulsi were known *for* centuries with its curative and healing properties (Iqbal et. al, 2014).

Tulsi is being used for the healing properties from the thousands of years and it is characterized in the Charaka Samhita (Puri, 2002). In the present world, Tulsi and its varieties are universally used for human nutrition and as a spice for improving aroma in vegetable and other meals. However, primarily it is used in health treatments worldwide (Rusaczonek *et al.,* 2007). Conventionally Indian people are widely using Tulsi for the treatment of various diseases from the ancient time, but even satisfactory knowledge is not present in the mode of action of Tulsi. Tulsi is a panacea for a lot of diseases for example fever, common cold, cough, throat aching, vascular diseases, stress, insect bites, skin problems, anti-inflammatory action, antibiotic, food additive etc. (Rathore & Shekhawat, 2013). Scientific analysis received a momentum in 20th century that was being done on medicinal values of holy Tulsi (Sampath *et al.,* 2015). Studies proved that Basil can be used to balance food sugar level and cholesterol level (Chandra *et al.,* 2011). The leaves of Tulsi are hypoglycemic, immune modulatory, anti-stress, analgesic, anti-inflammatory, anti-ulcerogenic, antitumor, antipyretic, and antibacterial in nature and found good for boosting up the immune system (Mohan et al, 2011). It can protect us from nearly all types of infections from viruses, bacteria, fungi, and protozoa. Tulsi is considered as an adaptogen, which balances different processes in the body, and helpful in treating with stress.

Elujoba (2000) quoted positive impact of Tulsi in HIV and AIDS. The importance of the mineral content of the Basil and its varieties was identified in several studies (Ghani et al, 1997, Karumari et al, 2014).

Importance of immunity has been widely understood after the COVID-19 pandemic all over the world. Better immunity is associated with better health. Immunity can be developed over time through proper diet and lifestyle.

Many recent researches have shown anti-bacterial, anti-viral and anti-fungal properties of Tulsi. It has many defensive action in infectious diseases. The AYUSH Ministry has incorporated Tulsi in Ayush kwath to boost immunity. It is a natural way to boost immunity by inculcating Tulsi in food products that are consumed in daily life which provide variety in life. Therefore, present investigation has been planned to develop value added immunity booster food products from Tulsi leaves.

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Objectives of the study

- To develop and analyse Physico-chemical properties of *Ocimum sanctum* extract based Food products

  The above objective of the study was met by the following thebelow mentioned process -
  - Develop flour blends of maize-oats
  - Develop food products from developed flour blends
  - Finding out acceptability of products developed from flour blends
  - Incorporate Tulsi at three levels in the best accepted flour blend products from different categories
  - Finding out acceptability of products after incorporation of Tulsi leaves at different levels
  - Assess the nutrient composition and shelf life of best acceptable Tulsi incorporated food products

Research Methodology

1. Collection and processing of leaves
2. Development of flour blends
3. Product development with three levels of flour blends
4. Sensory evaluation of developed product
5. Tulsi incorporated products development at three levels of supplementation in the best acceptable flour blend product
6. Sensory evaluation for Tulsi incorporated products
7. Nutritional composition evaluation of best acceptable Tulsi incorporated products
8. Statistical analysis

The detailed process of Research Methodology -

1. **Collection and processing of Tulsi leaves**

   Fresh Tulsi leaves of Rama were collected from the Medicinal garden of National Institute of Ayurveda, Amber, Jaipur (Raj).

   Tulsi powder was developed by drying method in order to minimize the loss on nutritional and pharmacological properties.

2. **Development of flour blends**
   Material was procured in single lot to avoid varietal difference.

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Flour	Maize	Oats
Type I	42.5	7.5
Type II	37.5	12.5
Type III	32.5	17.5

Table 1. Development of 3 types of flour blends

Maize and oats are important crops in India. In present study, various combinations of these two ingredients were tried. Ground maize and oat flour were used to prepare blend flour. Three types of blend flour were developed. These were termed as type I, type II and type III in which maize and oats were mixed in the ratio of 42.5: 7.5, 37.5: 12.5 and 32.5: 17.5.

3. Product development with three levels of flour blends

In the present investigation, food products were selected on the basis of following criteria :

- Taste wise acceptable to majority of population
- Available in all seasons
- Have nutritive value equal to or higher than fresh foods
- It allows fermentation / roasting / steam cooking

Various flour blends based food products were standardized and prepared using traditional methods *i.e* traditional product (Bhakhari), bakery product (biscuit) and extruded product (Vermicelli). Detailed recipes have been given as following -

Ingredients								
Level of supplementation	**Maize flour (g)**	**Oats flour (g)**	**Sour curd (g)**	**Turmeric (tsp)**	**Chilli powder (tsp)**	**Asafoetida**	**Salt (g)**	**Fat (ml)**
Type I (42.5:7.5)	42.5	7.5	115	½	1	A pinch	To taste	25
Type II (42.5:7.5)	37.5	12.5	115	½	1	A pinch	To taste	25
Type I (42.5:7.5)	32.5	17.5	115	½	1	A pinch	To taste	25

Table 2: Traditional product bhakhari

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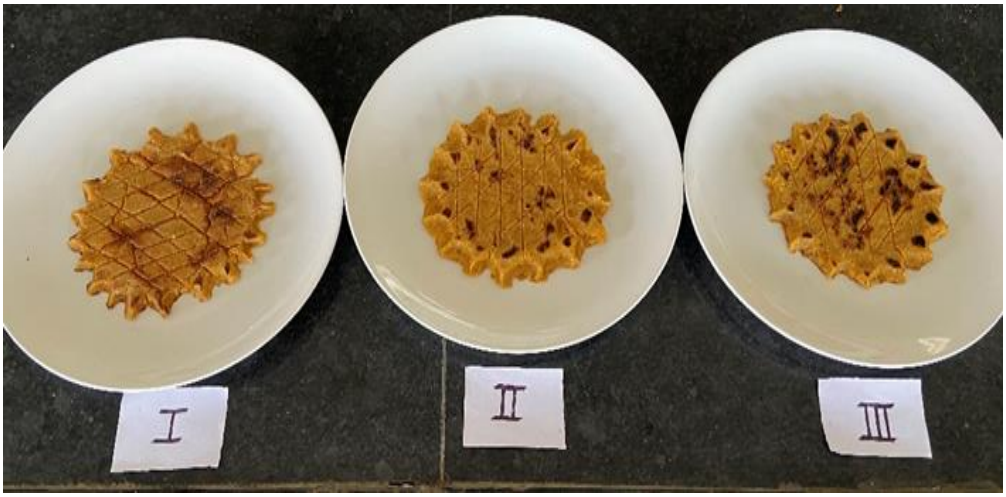

Figure 1: Standard bhakhari at 3 levels of mixture of maize flour & oats

Ingredients								
Level of supplementation	**Maize flour(g)**	**Oats flour(g)**	**Butter (g)**	**Castor sugar (g)**	**Salt**	**Ajwain seeds (tsp**	**Ammonium bicarbonate**	**Milk**
Type I (42.5:7.5)	42.5	7.5	25	5	½ tsp	¼	A pinch	To make dough
Type II (37.5 : 12.5)	37.5	12.5	25	5	½ tsp	¼	A pinch	To make dough
Type III (32.5: 17.5)	32.5	17.5	25	5	½ tsp	¼	A pinch	To make dough

Table 3: Bakery product biscuit

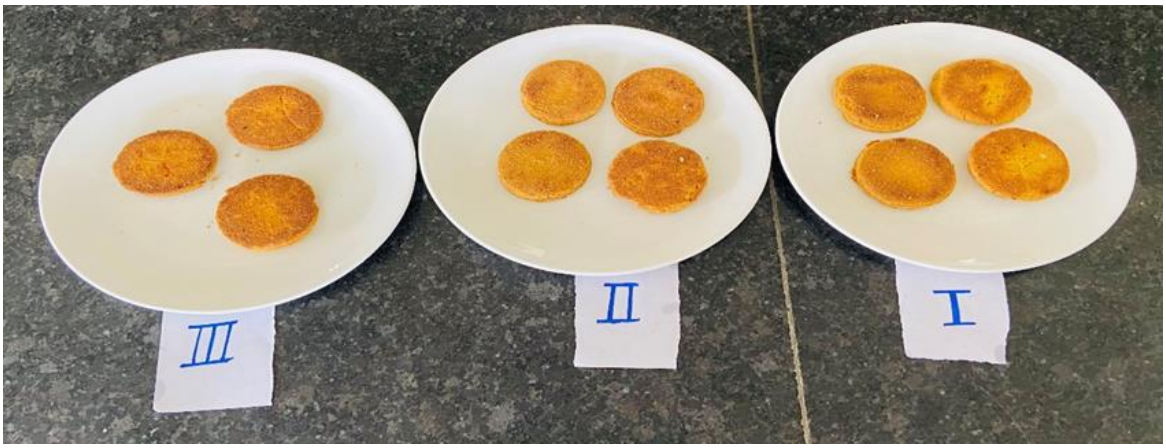

Figure 2. Standard biscuits at three levels of flour mixtures

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Ingredients			
Level of supplementation (Maize: Oat)	**Maize flour (g)**	**Oat flour (g)**	**Water (ml)**
Type I (42.5:7.5)	42.5	7.5	7
Type II (37.5 : 12.5)	37.5	12.5	7
Type III (32.5: 17.5)	32.5	17.5	7

Table 4: Extruded product vermicelli

Figure 3: Standard vermicelli after three levels of flour mixtures

4. Sensory Evaluation of developed products

Selection of panel members: Threshold test was conducted to select 30 panel lists (Gupta, 1976).

Threshold test

It is described as sensitivity test that assess the ability of an individual to identify minute changes in the sensory aspects of any food stuff (Srilakshmi, 2010).

Organoleptic evaluation of developed products

Nine point hedonic scale was used for the evaluation of organoleptic qualities of develop product. It rates the sensory trait considering 9 for excellence and 1 for disliking extremely. The score was used for evaluating the products quality viz. appearance, color, taste, flavor, texture and acceptability (Srilakshmi, 2010).

5. Tulsi incorporated products development at three levels of supplementation

After doing sensory evaluation of three products developed from three different blends of maize and oats; the selected products were enriched with Tulsi powder. Jamshidi and Cohen (2017) reported favourable outcomes and no adverse effects

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after Tulsi administration in metabolic and cardiovascular diseases. Sharma (1983) examined that Tulsi leaves incorporation in daily diet thrice a day relieved symptoms of asthama. From the selected food products, various percentages of Tulsi powder were incorporated which is given follow:

Product	**E1**	**E2**	**E3**
Bhakhari	5	10	15
Biscuits	5	10	15
Vermicelli	5	10	15

Table 5: Tulsi incorporation at 3 levels

Figure 4: Tulsi bhakri at 3 levels of supplementation

Figure 5: Tulsi biscuits at three levels of incorporation

Figure 6: Tulsi vermicelli at three levels of incorporation

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6. Sensory evaluation

It was done again to know acceptability of Tulsi incorporated food products in which physico-chemical analysis was done for the best acceptable product from the all three categories, i.e. traditional bhakri, baked product biscuit and extruded product vermicelli.

7. Physico-chemical evaluation of developed products

Proximate Composition: Once selected, products were analyzed for their proximate composition including moisture, ash, crude protein content. The analysis was done using the standardized method.

- Moisture
- Crude fat
- Crude Protein
- Ash content
- Fibre content
- Carbohydrate

- **Moisture:** 10 gram food sample was weighed in a petri plate, dried in oven at 100 degree C for 4 to 5 hours and cooled in desiccators. Sample was weighed on an electrical balance of 0.01 accuracy. The moisture percentage was calculated using following formula -

$$\text{Moisture (g/100gm)} = \frac{\text{Initial weight (W1) -- Final weight (W2)}}{\text{Weight of the sample (gm)}} \times 100$$

- **Crude fat:** Crude fat content was estimated by standard method of analysis (AOAC 1965).

  Fat was analyzed by ether extractable method. This includes true fat, phospholipids, essential oils, sterols, waxes and pigments.

  The percent of fat will be calculated as follows -

$$\text{Fat content (g/100gm sample)} = \frac{\text{weight of fat (gm)}}{\text{weight of the sample (gm)}} \times 100$$

- **Crude protein:** Crude protein of the sample will be estimated by the microkjeldahl's method, AOAC (1984).

  The sample will treated with conc. H_2SO_4, in presence of a catalyst and help in the conversion of nitrogenous compounds to ammonium sulphate. In the

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presence of the strong alkali, liberate ammonia will be estimate. Chemically most proteins had at least 16% nitrogen in their composition i.e. 6.25mg proteins was equal to 1mg protein.

- **Ash content:** The ash is known as the inorganic residue that remains after decomposition of organic matter.

- **Crude fibre:** Fibre is an insoluble and indigestible component of plants. Cellulose, hemicelluloses and lignin are the usual components of fibre

- **Carbohydrate:** The carbohydrate content of the sample was calculated by difference method as follows:

  Carbohydrates = 100 - (% Moisture+ % Protein + % Fat + % Total ash+ % Crude fibre) (g/ 100g)

8. Statistical Analysis

In this part of the study, collected data was organized, analyzed, interpreted, and presented after applying suitable statistical test.

Mean: In the study, Mean and Standard deviation were calculated for the 3 consecutive readings by following methods -

(X) values were calculated by the following formula -

$\bar{x} = (\Sigma xi) / n$

Where

$\bar{x}$ = Observation

Σ= Sum of all values of variable

n= Number of observation

Standard Deviation, $\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \mu)^2}$

Then ANOVA was applied to find out if there is any statistically significant difference is present or not between samples.

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Result & discussion

The present investigation was carried out to develop blend flours based products by maize and oats alone and with incorporation of Tulsi leaves powder. All the products were evaluated for their organoleptic characteristics through sensory evaluation method named hedonic scale test and most acceptable products were analyzed for their nutritional parameters.

Utilization of blend flours for product development

Various blend flours based products were developed from maize flour, oat flour and Tulsi leaves' powder. Traditional product is bhakhari, extruded product vermicelli and baked product biscuits were prepared by maize and oat flour and incorporating Tulsi leaves powder in different proportions.

Organoleptic acceptability of flour blends based food products

Organoleptic acceptability of the developed blend flour products was analyzed by judges using a 9-point hedonic rating scale.

Bhakhari

Level of supplementation (Maize: Oat)	Colour	Flavour	Texture	Taste	Appearance	Overall acceptability
Type I (42.5:7.5)	8.3±0.72	7.75±0.9	8.05±1.7	7.76±0.9	8.15±0.99	8±0.06
Type II (37.5:12.5)	7.9± 1.1	7.15±1.0	7.35±1.11	6.75±1.06	7.3 ± 0.8	7.29±0.10
Type III (32.5:17.5)	7.75±0.9	7.3±0.8	7.55±0.88	7.6± 0.09	7.15±0.09	7.47±0.01

Table 6: Mean scores of organoleptic characteristics of maize-oat flour based blend bhakhari

Table 6 shows that when bhakhari was prepared using three types of maize and oats flour blends and when its sensory evaluation was done, type 1 bhakhari was found most acceptable in terms of colour, appearance, aroma, texture, taste and overall acceptability. While the type 2 and type 3 bhakharis were also acceptable and scored as good although the difference was not statistically significant.

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Level of supplementation (Maize, oats, Tulsi)	Colour	Appearance	Aroma	Texture	Taste	Overall acceptability
Type IV (42.5:7.5:5)	7.5±0.16	7.5±0.16	7.9±0.1	7.7±0.15	7.8±0.13	7.68±0.09
Type V (37.5:12.5: 10)	8±0.07	7.6±0.16	8 ±0.11	8 ±0.12	8 ±0.07	7.92 ±0.03
Type VI (37.5:12.5:15)	7.10±0.10	7.10±0.10	7.3 ±0.15	7.4 ±0.16	7.4 ±0.16	7.26 ±0.11
(P<0.05)	0.17	0.26	0.15	0.18	0.16	0.27

Table 7: Mean scores of sensory evaluation of maize, oats & Tulsi blends for Bhakhari

Similar type of research was done by Dendile et al (2014) who also found better acceptability in shrikhand after incorporation of cardamom and saffron.

Biscuits

Level of supplementation (Maize: Oat)	Colour	Flavour	Texture	Taste	Appearance	Overall acceptability
Type I (42.5:7.5)	7.65±0.72	7.5±0.9	7.25±1.7	7.6±0.9	7.75±0.99	7.88±0.06
Type II (37.5 : 12.5)	7.85± 1.1	7.8±1.0	7.9±1.11	7.65±1.06	7.6±0.8	8.30±0.10
Type III (32.5: 17.5)	7.6±0.9	7.8±0.8	7.75±0.88	7.65±1.06	7.65±0.09	7.68±0.01

Table 8: Mean scores of organoleptic characteristics of maize-oat flour based blend biscuits

Table 8 shows acceptability of biscuits in three types of flour blends. it was found that all types of biscuits were statistically equally acceptable and were rated in the range of 7.25 - 8.3; that means termed as good.

Level of supplementation (Maize, oats, Tulsi)	Colour	Appearance	Aroma	Texture	Taste	Overall acceptability
Type IV (42.5:7.5:5)	7.6 ±0.21	7.4 ±0.21	7.51 ±0.24	7.67 ±0.27	7.71±0.21	7.57 ±0.21
Type V (37.5:12.5: 10)	7.7 ±0.23	7.52 ±0.23	7.57 ±0.23	7.71 ±0.23	7.78 ±0.21	7.65 ±0.22
Type VI (37.5:12.5:15)	7.6 ±0.22	7.5 ±0.26	7.5 ±0.26	7.46 ±0.22	7.5 ±0.16	7.54 ±0.21
(P<0.05)	0.15	0.17	0.11	0.18	0.21	0.25

Table 9: Mean scores of organoleptic maize oats Tulsi flour blends for Biscuits

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When Tulsi was incorporated at three levels of supplementation in biscuits prepared from best acceptable flour blend, all types of biscuits were found equally acceptable as good ranging from 7.4 - 7.7.

Vermicelli

Level of supplementation (Maize: Oat)	Colour	Flavour	Texture	Taste	Appearance	Overall acceptability
Type I (42.5:7.5)	7.8±0.13	7.8±0.21	7.78±0.13	7.68±0.9	7.56±0.99	7.72±0.06
Type II (37.5 : 12.5)	7.91± 1.1	7.9±1.0	7.8±1.11	7.8±1.06	8 ± 0.16	8.3±0.10
Type III (32.5: 17.5)	7.5±0.9	7.1±0.8	7.75±0.88	7.85± 0.09	7.65±0.09	7.68±0.01

Table 10: Mean scores of organoleptic characteristics of maize-oat flour blend Vermicelli

Extruded product vermicelli was also prepared from three ratios of flour blends using maize and oats. Table 10 is showing that all types of vermicelli were equally acceptable in the range of 7.1 to 8. it means it was acceptable as 'Good'.

Level of supplementation (Maize, oats, Tulsi)	Colour	Appearance	Aroma	Texture	Taste	Overall acceptability
Type IV (42.5:7.5:5)	7.17 ±0.41	7.06 ±0.30	7.32 ±0.24	7.47±0.24	7.6 ±0.23	7.32 ±0.26
Type V (37.5:12.5: 10)	7.21 ±0.23	7.18 ±0.23	7.5 ±0.26	7.66 ±0.26	7.73 ±0.26	7.45 ±0.23
Type VI (37.5:12.5:15)	7.11 ±0.29	7 ±0.29	7.15 ±0.16	7.3 ±0.6	7.29 ±0.24	7.17 ±0.21
(P<0.05)	0.15	0.18	0.21	0.16	0.19	0.27

Table 11: Mean scores of organoleptic maize oats Tulsi flour blends vermicelli

It is evident from table 11 that Tulsi was incorporated in best acceptable vermicelli at three levels and it was found that all concentrations of Tulsi were well accepted in the range of 7 - 7.7. It shows that Tulsi leaves flavour is very popular among Indian community therefore all levels of supplementations were equally acceptable.

Nutritive value of products

Finally best acceptable Tulsi incorporated products from all the three categories i.e. traditional product bhakhari, baked product biscuit and extruded product vermicelli were evaluated for their nutritive value. In this, proximate composition and minerals like calcium, iron and zinc were evaluated. It was found that nutritive value of the developed products was very much closer to the nutritive value of the original maize and oats. In fact, crude protein content was fairly good. Besides this, after Tulsi incorporation the products became

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rich in antioxidants as well. Table 12 is depicting the nutritive value of all three types of best acceptable developed products.

Product	Moisture	Crude protein	Crude fat	Crude fibre	Ash	Total carbohydrate	Calcium	Iron	Zinc
Bhakhari Type III	26.83	11.02	3.33	1.59	2.46	81.5	46.6	21.5	66.7
Biscuits Type II	1.56	6.4	27.4	0.2	0.4	65.7	52.1	20.14	68.4
Vermicelli Type I	49.8	9.05	4.86	0.22	1.13	84.7	60.8	40.22	67.5

Table 12. Nutritive value of best acceptable three types of developed products

David (2015) a:so prepared basil incorporated shrikhand and found that 4% basil incorporation showed maximum moisture, protein, ash, antioxidant and acidity content of the sample not contained basil was high in fat, total solids and pH content.

Conclusion

Thus on the basis of the present study. it can be concluded that blend flour of maize oat alone and in combination with Tulsi leaves had better nutritive values and higher antioxidant content as compared to unsupplemented ones. Hence these blend flour products can be commercially manufactured for human consumption. These can be developed at household level as well to enrich nutrition of immediate family members.

The present study was done to develop evaluate the organoleptic properties, nutrition composition, of maize, oat and Tulsi leaves, their blend flour and various value added products prepared using blend flour. Three types of blend flours were prepared using maize:oats in ratio of 42.5:7.5, 37.5:12.5 and 32.5: 17.5. and then one of the best standard was selected from these three and further Tulsi was incorporated in it at three levels of supplementation. Results showed that overall acceptability was highest in type I bhakari in which maize and oats flour were mixed in the ratio of 42.5:7.5. Type V bhakhari obtained best acceptability scores after incorporation with Tulsi leaves at 37.5:12.5:10 level. Type 2 biscuits were found best acceptable (maize & oats in the ratio of 37.5:12.5. After incorporation of Tulsi leaves, all types of Tulsi incorporated samples were liked similarly. Type II vermicelli was liked the most with maize and oats in the ratio of 37.5:12.5 while after incorporation of Tulsi leaves in this sample, all levels of Tulsi incorporation were equally acceptable. Nutritive value of all the three types of products was analysed and it was found that bhakhari contained crude protein, fibre and mineral content in highest amount while highest amount of fat and zinc were found in biscuits although calcium, zinc and iron content of the developed three products was not statistically significantly different.

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