Applied Biological Research 23(4): 371-378; (2021)
DOI: 10.5958/0974-4517.2021.00047.1

ANTIOXIDANT ACTIVITY, NUTRITIONAL COMPOSITION AND SENSORY EVALUATION OF MILLETS, LEGUME AND MARJORAM (*Origanum majorana* L.) LEAF-BASED BISCUITS

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(Received 20 February, 2021; accepted 11 August, 2021)

ABSTRACT

This study was carried out to evaluate the effects of varying proportions of some flour mixtures on sensory properties of biscuits; and the most acceptable types of biscuits were assessed for their nutritional properties. Based on organoleptic evaluation, it was found that the biscuits developed from blanched pearl millet: sorghum: germinated mung bean in ratio of 40:30:30 (Type III) and with marjoram or *Marwa* (*Origanum majorana* L.) leaf powder. Type VI (in ratio of 35:30:30:5) composite flours were the most acceptable products. Nutritional evaluation of value-added biscuits revealed that Type-III biscuits had higher moisture (11.56%) and crude protein (11.56%), while crude fibre (3.89%) and ash (1.08%) contents were higher in Type-VI biscuits. Dietary fibre and mineral contents were maximum in Type-VI biscuits. Type-III biscuits had significantly higher *in vitro* availability of calcium (59.69%). Phytic acid and polyphenol contents were higher in Type III biscuits. The antioxidant activity was maximum in Type-VI biscuits.

Keywords: Antioxidant content, biscuits, marjoram, nutritional composition, sensory evaluation

INTRODUCTION

Consumers' awareness and demand for high-quality and healthy foods, prepared from the whole grain flour and other functional ingredients [known as functional foods], have constantly increased during recent decades. Development and consumption of such functional foods have not only improved the nutritional status of general population but also helped those suffering from degenerative diseases associated with the changing lifestyles and environment (Ali and Rahut, 2019; Petrescu *et al.*, 2020). Among the ready-to-eat snacks, biscuits possess several attractive features including a wider consumer-base, relatively long shelf-life, more convenience and good eating quality (Patel and Rathod, 2017). The development of fortified biscuits or other composite flour bakery products is the latest trend in the bakery industry.

In India, the major cereals and millets, consumed as whole grains, are wheat, sorghum, pearl millet, finger millet and brown rice. These grains are the major source of nutrients in diets contributing to 7.3-11.6% protein, 1.3-5.0% fat, 60-72% carbohydrate and 328-361 kcal of daily energy intake (Gyawali, 2021). The perusal of literature has revealed that sorghum and pearl millet grains are nutritionally comparable or even superior to major cereals such as wheat and rice owing to the higher contents of protein with more balanced amino acid profile, vitamins, several minerals, insoluble dietary fiber leading to lower glycemic index and phytochemicals with antioxidant properties (Satankar *et al.*, 2020; Hassan *et al.*, 2021).

Mung bean is a rich source of protein and amino acid especially lysine so can supplement cereal-based human diets; and is a very good source of dietary fiber, vitamin C, vitamin K, riboflavin, folate, copper and manganese (Dahiya *et al.,* 2015). The seeds and sprouts are excellent examples of functional foods that lower the risk of various diseases. There is an increasing interest in Western countries in sprouting seeds as consumers demand minimally processed, additive-free, more natural, nutritional and healthy foods (Hou *et al.,* 2019; Shahrajabian *et al.,* 2019).

Sweet marjoram or *marwa* (*Origanum majorana* L.) is a member of the Lamiaceae family. Sweet marjoram has strong antioxidant activity, mainly because of its high content of phenolic acids, tannins, and flavonoids. Marjoram has medicinal effects as it can serve as gastrointestinal tract stimulant, tonic, carminative, diaphoretic, hypoglycemic, diuretic as well as antibacterial and antioxidant (Bina *et al.*, 2017). Sweet marjoram has been used as a remedy against asthma, indigestion, headache and rheumatism. The richness of sweet marjoram in volatile and phenolic compounds having antioxidant, antimicrobial and insecticidal activities shows that the plant has potential use in the formulation of functional foods, pharmaceutical, agro-industry and biological defense (Tripathy *et al.*, 2017). In view of above biscuits were prepared from the blends of pearl millet, sorghum, mung bean and marjoram leaf powder with the objectives to develop value-added biscuits using composite flour, study their organoleptic acceptability and assess the nutrient composition of the most acceptable biscuits.

MATERIALS AND METHODS

The sample seeds of pearl millet (*Pennisetum glaucum* cv. ‘WH-901-445’) was procured from the Bajra Section, sorghum (*Sorghum bicolor* cv. ‘HC-125’) from the Forage Section and mung bean (*Vigna radiata* cv. ‘MH-125’) from the Pulses Section and the leaves of sweet marjoram (*Origanum majorana*) from Medicinal, Aromatic and Underutilized Plants Section of the Department of Genetics & Plant Breeding, College of Agriculture, CCSHAU, Hisar (India). The seeds were cleaned and made free of dust, dirt and foreign materials prior to their processing for product development.

Processing of grains

Blanching of pearl millet was done as per the process described by Chavan and Kachare (1994). Mung bean seeds were soaked in tap water in 1:5 ratio (w/v) for 12 h at 37ºC and unimbibed water was discarded. The water-soaked seeds were germinated in sterile petri-dishes lined with wet filter paper for 48 h at 37ºC with frequent watering. The sprouts were rinsed in distilled water and dried at 50-55ºC. in boiling water for 10 min and cooled.

The sweet marjoram leaves were trimmed and dead or spoiled/diseased parts removed. The leaves were then washed and dried at -50°C in a dryer. The dried unprocessed samples of sorghum, germinated mung bean and blanched pearl millet were ground to fine powder in an electric grinder and stored in plastic containers at room temperature for future use.

Preparation of composite flours

The composite flours were prepared by mixing the blanched pearl millet, sorghum, germinated mung bean and dried sweet marjoram leaf powder, on a dry-weight basis, in variable ratios. For Type-I, Type- II, Type-III, Type-IV, Type-V and Type-VI flours, the above respective components were mixed in the ratio of 80:10:10:0, 60:20:20:0, 40:30:30:0, 75:10:10:5, 55:20:20:5 and 35:30:30:5, respectively.

Standardization and development of value-added biscuits

Various composite flour-based biscuits were standardized and developed in a conventional manner using standard recipe. For the preparation of biscuits, refined wheat flour and composite flours were sieved with baking powder and sodium bicarbonate, separately. Ghee and sugar were creamed until they became light. Sieved flours were added to ghee and sugar mixture. This mixture was rolled on the board and cut into the shape of biscuits with the help of a biscuit cutter and baked at 160°C. The composition of biscuits (recipe) was as under:

Biscuit types	RWF (g)	Blanched pearl millet flour (g)	Sorghum flour (g)	Germinated mung bean flour (g)	Marjoram leaves (g)	Milk (mL)	Ground sugar (g)	Ghee (g)
Control (RWF: 100%)	100	-	-	-	-		150	40
Type-I	-	80	10	10	-	25	150	40
Type-II	-	60	20	20	-	25	150	40
Type-III	-	40	30	30	-	25	150	40
Type- IV	-	75	10	10	5	25	150	40
Type-V	-	55	20	20	5	25	150	40
Type-VI	-	35	30	30	5	25	150	40

RWF = Refined wheat flour

Sensory evaluation

For sensory evaluation, the biscuit samples were served to a panel of ten semi-trained judges on coded paper plates for their assessment of degree of liking in terms of colour, appearance, aroma, texture, taste and overall acceptability using a 9-point Hedonic scale (Peryam and Girardot, 1952).

Chemical analysis

The most acceptable biscuits were analyzed for proximate composition as per the standard method of AOAC (2000). Total carbohydrates were calculated by difference method and total, soluble and insoluble dietary fibre constituents were determined by the enzymatic method given by Furda (1981). For calcium, iron and zinc determination, 1.0 g sample was completely digested with diacid mixture (HNO: HClO :: 5: 1, v/v). The sample was then heated to near dryness, volume made to 50 mL with double distilled water and analyzed using atomic absorption spectrophotometer (Lindsey and Norwell, 1969). Calcium, iron and zinc in samples were determined spectrophotometrically by using an atomic absorption spectrophotometer as per the method of Lindsey and Norwell (1969).

The available calcium and zinc were extracted as per the method of Kim and Zemel (1986). Available iron in sample was extracted as per the method Rao and Prabhavathi (1978). Total poly-phenols were extracted as per Singh and Jambunathan (1981). The polyphenolic compounds were estimated as tannic acid equivalent as per Folin-Danis procedure (Swain and Hills, 1959).

Antioxidant activity

For sample extraction, the samples for antioxidant activity were extracted as per the method of Serrano *et al.* (2007). The moisture-free finely ground powdered sample (300 mg) was taken in a flask and 5 mL methanol: water (80:20, v/v) extraction solvent added to it. The mixtures were shaken for 30 min using a mechanical shaker at 150 rpm, then centrifuged at 5000 rpm for 10 min at 4°C and supernatant collected. Again 5 mL of same solvent was added to each flask and the process repeated. Both the supernatants were combined, filtered using Whatman filter paper No. 1 and used to determine total phenol and total antioxidant capacity by DPPH.

The total phenolic contents were estimated as per Singleton and Rossi (1965). DPPH-free radical scavenging activity (RSA) of sample extracts was evaluated by DPPH method (Hatano *et al.*, 1988). DPPH solution was prepared daily at a concentration of 0.1 mM or 0.04 g L^{-1} in 100% ethanol. For this, in a 0.2 mL ethanolic extract of sample 3.0 mL DPPH was added and thoroughly mixed for 5 min. The control sample comprised of 0.2 mL solvent (ethanol) instead of sample extract. The absorbance of samples including control was measured at 517 nm after 30 min incubation in dark at room temperature using UV-VIS double beam spectrophotometer against a blank containing the respective solvent. Three replications were carried out for each sample. The percent DPPH radical discoloration of sample was calculated as per the equation discoloration:

DPPH RSA (%) = Absorbance in control – Absorbance in sample´ 100 / Absorbance in control

Statistical analysis

To verify the statistical significance, mean ± SD of three independent measurements were calculated. Differences between groups were tested by ANOVA using the SPSS Statistical Package. The P values of<0.05were considered significant.

RESULTS AND DISCUSSION

The mean score for overall acceptability of control biscuits was in the category of 'liked very much' while the mean scores for Type-I, II and III composite flour-based biscuits were 6.98, 7.21 and 7.82, respectively (Table 1). The biscuits prepared with supplementation of sweet marjoram leaf powder showed low overall acceptability scores *viz*., 6.88, 7.25 and 7.78 for Type-IV, V and VI biscuits, respectively, as compared to the unsupplemented biscuits and were rated as 'liked moderately'. These organoleptic acceptability scores were in close agreement with Kulthe *et al.* (2018) wherein the biscuits prepared from blanched and malted pearl millet flour were 'liked moderate'. Singh *et al.* (2020) reported that biscuits prepared from pearl millet flour and chickpea flour in different ratios were in the category of 'liked moderately' on the basis of organoleptic evaluation. Pearl millet cookies had overall a higher quality score as compared to the control cookies as reported by Mehra and Singh *et al.* (2017). Tulsi (10 g) and moringa (5 g) leaves powder supplemented biscuits were acceptable by the judges as reported by Ariful *et al.* (2014).

Table 1: Mean scores of organoleptic acceptability of biscuit based on composite flours

Levels of supplementation	Colour	Appearance	Aroma	Texture	Taste	Overall acceptability
Control (RF:100%)	8.20 ± 0.23	8.10 ± 0.18	8.00 ± 0.14	8.10 ± 0.10	8.10 ± 0.10	8.04 ± 0.11
Blanched pearl millet: sorghum: germinated mung bean						
Type I (80:10:10)	7.00 ± 0.21	7.00 ± 0.21	6.90 ± 0.23	7.05 ± 0.24	6.95 ± 0.21	6.98 ± 0.21
Type II (60:20:20)	7.40 ± 0.26	7.20 ± 0.24	7.00 ± 0.25	7.30 ± 0.21	7.15 ± 0.25	7.21 ± 0.18
Type III (40:30:30)	7.90 ± 0.29	7.90 ± 0.31	7.70 ± 0.36	7.90 ± 0.31	7.60 ± 0.37	7.82 ± 0.32
$CD_{0.05}$	0.11	0.18	0.11	0.19	0.13	0.19
Blanched pearl millet: sorghum: germinated mung bean: marjoram leaf powder						
Type IV (75:10:10:5)	6.90 ± 0.18	6.90 ± 0.18	6.80 ± 0.20	6.95 ± 0.21	6.85 ± 0.18	6.88 ± 0.18
Type V (55:20:20:5)	7.40 ± 0.26	7.20 ± 0.24	7.00 ± 0.25	7.40 ± 0.22	7.25 ± 0.22	7.25 ± 0.18
Type VI (35:30:30:5)	7.80 ± 0.23	7.90 ± 0.31	7.70 ± 0.36	7.80 ± 0.32	7.40 ± 0.37	7.78 ± 0.30
$CD_{0.05}$	0.15	0.12	0.09	0.17	0.17	0.29
$CD_{0.05}$ of all treatments	0.68	0.70	0.76	0.68	0.74	0.64

Values are mean ± SE of ten independent determinations; RF = Refined flour

Control (RF 100%), Type-III (BPM: SO: GMB 40:30:30), Type-VI (BPM: SO: GMB: MR 35:30:30:5); RF = Refined flour, BPM = Blanched pearl millet, SO= Sorghum, GMB = Germinated mung bean, MR= Marjoram leaf powder

Proximate composition

The proximate composition of biscuits showed that Type-III biscuits had significantly high moisture (3.31%) and crude protein (11.56%) contents while crude fibre (3.89%) and ash (1.08%) contents were higher in marjoram leaf powder supplemented Type-VI biscuits. Control biscuits had significantly high total carbohydrate content (Table 2). Mehra and Singh (2017) and Singh *et al.* (2020) prepared biscuits from pearl millet flour and chickpea flour and observed that the biscuits contained high protein, crude fibre and ash contents. Similar results were reported by earlier workers.

Table 2: Proximate composition of biscuit based on composite flours (% on dry weight basis)

Types of biscuit	Moisture	Crude protein	Crude fat	Crude fibre	Ash	Total carbohydrates
Control	2.59 ± 0.03^{c}	6.15 ± 0.06^{b}	25.20 ± 1.89^{c}	0.26 ± 0.03^{c}	0.42 ± 0.01^{c}	67.97 ± 1.89^{a}
Type-III	3.31 ± 0.07^{a}	11.56 ± 0.03^{a}	27.33 ± 0.41^{a}	2.26 ± 0.03^{b}	0.88 ± 0.07^{b}	57.97 ± 0.43^{b}
Type-VI	3.26 ± 0.03^{b}	11.53 ± 0.07^{a}	26.86 ± 0.37^{b}	3.89 ± 0.03^{a}	1.08 ± 0.06^{a}	56.64 ± 0.35^{c}
$CD_{0.05}$	0.01	0.01	0.02	0.01	0.19	0.14

Values are mean ± SE of three independent determinations

Control (RF 100%), Type-III (BPM: SO: GMB 40:30:30), Type-VI (BPM: SO: GMB: MR 35:30:30:5); RF = Refined flour, BPM = Blanched pearl millet, SO= Sorghum, GMB = Germinated mung bean, MR= Marjoram leaf powder

with respect to the incorporation of leaf powder for the development of biscuits increased the crude fiber and ash content (Ariful *et al*., 2014). Similar results were observed by Ivanisˇova *et al.* (2019) who reported that the biscuits with 5% chicory fiber contained high crude fiber.

Dietary fibre

Marjoram leaf powder supplemented composite flour-based biscuits had significantly higher content of total, soluble and insoluble dietary fibre, followed by unsupplemented composite flour-based biscuits and control biscuits (Table 3). Upadhyay *et al.* (2017) reported that increasing the quantity of herbal leaves increased the dietary fiber content in biscuits.

Table 3: Dietary fibre content of biscuits based on composite flours (g 100 g^{-1}, dry matter basis)

Types of biscuit	Total dietary fibre	Soluble dietary fibre	Insoluble dietary fibre
Control	10.41 ± 0.01^{c}	2.38 ± 0.06^{c}	8.03 ± 0.09^{c}
Type-III	11.82 ± 0.02^{b}	3.34 ± 0.01^{b}	8.48 ± 0.09^{b}
Type-VI	13.09 ± 0.01^{a}	3.85 ± 0.01^{a}	9.21 ± 0.01^{a}
$CD_{0.05}$	0.05	0.04	0.05

Values are mean ± SE of three independent determinations

Control (RF 100%), Type-III (BPM: SO: GMB 40:30:30), Type-VI (BPM: SO: GMB: MR 35:30:30:5); RF = Refined flour, BPM = Blanched pearl millet, SO= Sorghum, GMB = Germinated mung bean, MR= Marjoram leaf powder

Total mineral

Type-VI biscuits supplemented with marjoram leaf powder had maximum calcium (190.37 mg 100 g^{-1}), iron (9.22 mg 100 g^{-1}) contents, while maximum zinc content was found in Type-III biscuits. Type-III biscuits had significantly higher *in vitro* availability of calcium (59.69%); whereas *in vitro* iron availability was significantly high in control (Table 4). The increased quantity of minerals such as zinc, iron, calcium and potassium were found in the biscuits containing chicory inulin (Krystyjan *et al.*, 2015; Sayed and Khali, 2017). According to Talabi *et al.* (2019) biscuits developed from blends of flours had a good amount of minerals.

Table 4: Total (mg 100 g^{-1}) and available mineral (%) content of biscuit based on composite flours (dry matter basis)

Types of biscuit	Calcium		Iron		Zinc	
	Total	Available	Total	Available	Total	Available
Control	40.54 ± 0.02^{c}	51.79 ± 0.01^{c}	3.19 ± 0.02^{c}	20.16 ± 0.01^{a}	1.38 ± 0.02^{c}	68.44 ± 0.06^{a}
Type-III	96.12 ± 0.03^{b}	59.69 ± 0.04^{a}	6.10 ± 0.04^{b}	19.63 ± 0.06^{b}	3.66 ± 0.02^{a}	65.80 ± 0.05^{b}
Type-VI	190.37 ± 0.01^{a}	59.65 ± 0.09^{b}	9.22 ± 0.01^{a}	18.71 ± 0.09^{c}	3.57 ± 0.06^{b}	64.96 ± 0.34^{c}
$CD_{0.05}$	0.09	0.04	0.09	0.22	0.04	0.71

Values are mean ± SE of three independent determinations

Control (RF 100%), Type-III (BPM: SO: GMB 40:30:30), Type-VI (BPM: SO: GMB: MR 35:30:30:5); RF = Refined flour, BPM = Blanched pearl millet, SO= Sorghum, GMB = Germinated mung bean, MR= Marjoram leaf powder

Anti-nutritional factors

Both anti-nutritional factors namely phytic acid and polyphenol were higher in Type-III composite flour followed by marjoram leaf powder supplemented Type-VI composite flour-based biscuits (Table 5). This may be due to the fact that pearl millet, sorghum and mung bean had higher content of phytic acid and polyphenol. Kulthe *et al.* (2018) observed that polyphenol contents increased progressively with increasing level of pearl millet flour in the cookies prepared from pearl millet flour. However, blanching of pearl millet and germination of mung bean brought a significant reduction in phytic acid and polyphenol (Singh *et al.* 2015; Pawase *et al.*, 2019).

Table 5: Anti-nutrient content of biscuit based on composite flours (dry matter basis)

Types of biscuit	Anti-nutrient content (mg 100 g^{-1}) Phytic acid	Polyphenol
Control	209.71 ± 0.01^{c}	251.14 ± 0.02^{c}
Type-III	262.01 ± 0.01^{a}	371.34 ± 0.07^{a}
Type-VI	244.10 ± 0.09^{b}	369.12 ± 0.09^{b}
$CD_{0.05}$	0.04	0.04

Values are mean ± SE of three independent determinations Control (RF 100%), Type-III (BPM: SO: GMB 40:30:30), Type-VI (BPM: SO: GMB: MR 35:30:30:5); RF = Refined flour, BPM = Blanched pearl millet, SO = Sorghum, GMB = Germinated mung bean, MR = Marjoram leaf powder

Antioxidant activity

The antioxidant activity of biscuits differed significantly with respect to the total phenolic content and DPPH free radical scavenging activity. The supplementation of marjoram leaf powder in composite flour significantly increased the total phenolic content and DPPH radical scavenging activity. Type-VI composite flour-based biscuits had a significantly higher content of total phenolics and DPPH radical scavenging activity, followed by non-supplemented composite flour-based products. This might be attributed to the higher antioxidant activity of marjoram leaf powder (Tripathy *et al.*, 2017; Ennaji *et al.*, 2020) [Table 6]. Control products contained the lowest DPPH free radical scavenging activity and low total phenolic content. The enrichment of traditional food staples with natural food ingredients enhanced the antioxidant content in food products (Mahloko *et al.*, 2019). Starowicz, *et al.* (2021) reported higher phytochemical content and antioxidant activity in cookies supplemented with spice/herbs as compared to the oat-buckwheat cookies without condiment.

Table 6: Antioxidant activity of biscuit based on composite flours (dry matter basis)

Types of biscuit	Total phenolic content (mg GAE g^{-1})	DPPH free radical scavenging activity (%)
Control	1.13±0.09^{c}	11.48±0.09^{c}
Type-III	8.23±0.09^{b}	44.05±0.01^{b}
Type-VI	12.03±0.06^{a}	46.74±0.06^{a}
$CD_{0.05}$	0.03	0.03

Values are mean ± SE of three independent determinations Control (RF 100%), Type-III (BPM: SO: GMB 40:30:30), Type-VI (BPM: SO: GMB: MR 35:30:30:5); RF = Refined flour, BPM = Blanched pearl millet, SO = Sorghum, GMB = Germinated mung bean, MR = Marjoram leaf powder

Conclusion: Based on sensory evaluation, the biscuits developed in the ratio of 40:30:30 (Type III) and Type VI with marjoram leaf powder (in the ratio of 35:30:30:5) composite flours were most acceptable products. Biscuits supplemented with marjoram leaves were rich in crude fibre and ash content while Type-III biscuits had higher crude protein content. Control biscuits contained significantly higher total carbohydrate content. Dietary fibre was maximum in marjoram leaf powder supplemented (Type-VI) biscuits. Type-VI biscuits had highest calcium (190.37 mg 100 g^{-1}) and iron (9.22 mg 100 g^{-1}) contents while zinc was maximum in Type-III biscuits. Type-III biscuits had significantly higher *in vitro* calcium availability; whereas the *in vitro* available iron and zinc was significantly high in control biscuits. Phytic acid and polyphenols were higher in Type-III biscuits. Antioxidant activity was maximum in marjoram leaf powder supplemented Type-VI biscuits.

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