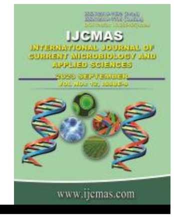
Int.J.Curr.Microbiol.App.Sci (2023) *12*(09): 164-175

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 12 Number 9 (2023)**
Journal homepage: http://www.ijcmas.com



Original Research Article https://doi.org/10.20546/ijcmas.2023.1209.016

Physical, Functional and Nutraceutical Properties of Composite Flour Cookies

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Keywords

Wheat, millet, texture, color, antioxidants, *Elusine coracana*

Article Info

Received:
20 July 2023
Accepted:
24 August 2023
Available Online:
10 September 2023

ABSTRACT

Composite flours obtaining by blending cereal or legume flours and other functional ingredients can be used in the preparation of baked foods with superior quality. In this study cookies made from finger millet, little millet, horse gram and refined wheat flour were characterised for their physical, functional and chemical properties. Color values of composite flour, finger millet and horse gram flour were darker and yellower compared to little millet flour and refined wheat flour. Sprouting increased water solubility and water binding capacity of flours. Oil absorption capacity was higher in composite flour compared to individual flours. As the proportion of composite flour increased, there was a gradual decrease in the diameter and increase in the thickness, hardness, darkness, total phenolic content and antioxidant activity of the cookies. Cookies were acceptable up to 50% level of incorporation of composite flour in cookies. Polyphenol and antioxidant activity were positively and significantly correlated. Further research is needed to better understand the impact of intervention on improvement in the nutritional profile of school children. Cookies made from germinated composite flours can be an alternative to wheat flour cookies. Incorporation of millet based composite flour in cookies improves the nutritional quality. Significance and novelty: Consumption of millets could overcome the problem of poor nutrition among school children and helps to combat micronutrient deficiencies.

Introduction

Millets are a group of cereal crops that are cultivated around the world in a wide range of soils and climate either for food or fodder. This group of cereal crops includes finger millet (*Elusine coracana*), little millet (*Panicum miliare*), kodo millet (*Paspalum scrobiculatum*), foxtail millet

(*Setaria italica*), barnyard millet (*Echinochloa frumentacea*) and proso millet (*Panicum miliaceum*). Millets are generally accepted to have a better nutritional quality when compared to rice and wheat, as millets are relatively high in dietary fiber, antioxidants, sulphur containing amino acids and essential fatty acids.

Although millets have some antinutritional factors viz., tannin, phenols, oxalate, phytate, protease and amylase inhibitors that diminish their nutritional virtues by adversely affecting nutritional bio-availability, these factors can be hindered by simple and traditional processing methods.

For example, germination (also known as sprouting or malting) is a biochemical process which involves hydration of grains in ambient conditions and modification of grain constituents by endogenous enzymes (Naddem *et al.,* 2010). Such simple traditional technologies can improve nutritional quality as well as functionality.

Composite flours, replacing part of the wheat with other cereal or legume flours and other functional ingredients have been widely used in the preparation of baked food products. Besides improving the texture and nutritional properties of baked foods, composite flours were reported to impart functional and therapeutic value to the product as well (Goldberg, 1994; Sheeby and Morissey, 1998). One way to incorporate such products into our daily diets is through consumption of snack foods such as cookies.

The use of composite flours made from wheat and other cereals including minor millets in bakery products is becoming more popular due to its economic and nutritional advantages. If sourced locally, they have benefits including ease of handling, packing and storage stability and providing easily digestible sugars and amino acids especially for children. Accordingly, the objective of the study was to develop composite flour cookies from wheat and a malted flour blends of finger millet, little millet and horse gram flour and to analyze the functional, physical, sensory and nutritional properties of these cookies.

Materials and Methods

Processing of Grains

Finger millet, little millet, horse gram and refined wheat flour were obtained from local markets in Dharwad, Karnataka (India), and were subjected to the traditional processing methods such as germination or fermentation. Germinated finger millet and horse gram flour was prepared according to Guddam *et al.,* (2016), while fermented little millet was prepared following the procedure of Kamat and Yenagi (2012). Moisture content of flour samples was determined according to air oven method (44-16.04) of AACCI (1999).

Cookie Making

Cookies were prepared according to micro-cut method (10-54.01) of AACCI (1999). Wheat flour (100%) was used as control and replaced with composite flour at levels of 25, 50, 75, and 100% (Table 1).

Water Solubility and Water Binding Capacity

Water solubility and water binding capacity were determined following Singh and Singh (2003) as modified by Masatcloglu *et al.,* (2014). Ground sample (1 g) was mixed with 10 ml distilled water and vortexed (Reax Top, Heidolph, Schwabach, Germany) for 15 s every 5 min. After 1 h the mixture was centrifuged(NF 1200 model, Nüve, Ankara, Turkey) at 4000 ×g for 10 min. Supernatant was dried in an air circulated oven (ED-E2 model, BinderGmbH, Tuttlingen, Germany) at 100 °C and water solubility was calculated according to the following equation:

Solubility (%)

$$= \text{weight of dried supernatant} \times \frac{100}{\text{weight of sample}}$$

Precipitate was weighed and then dried at 100°C overnight. Water binding capacity was calculated according to the following equation:

$$\text{Water binding capacity (\%)} = (\text{weight of wet precipitate} - \text{weight of dried precipitate}) \times \frac{100}{\text{weight of sample}}$$

Oil absorption capacity

Ground sample (1 g) was mixed with 10 ml of vegetable oil in pre-weighed centrifuge tubes following the method of Sosulski *et al.,* (1976). The tubes were stirred for 1 min for complete dispersion of sample in the oil. After 30 min of holding time at room temperature (27°C), the sample was centrifuged at 3000 rpm for 25 min.

The separated oil was then removed and tubes were inverted on oil absorbent paper for 25 min to drain the oil prior to reweighing. The oil absorption capacity was expressed as grams of oil absorbed per gram of the sample.

Pasting properties of Flour and Cookies

Pasting properties of the flours and cookies were determined using a Rapid Visco Analyzer (RVA 4, Newport Scientific, Warriewood, Australia). In this assay, 3 g (14% moisture basis) sample was dispersed in 25 ml distilled water in an aluminum sample canister.

The RVA pasting curve was obtained by a 20 min test profile: initial equilibrium at 25 °C for 2 min, heating to 95 °C over 5 min, holding at 95 °C for 3 min, cooling to 25 °C over 5 min and holding at 25 °C for 5 min.

Pasting parameters were determined from the RVA curves using the data analysis software (Thermocline for Windows, Newport Scientific, Australia). The results are reported as average of triplicate analyses for each sample.

Color

Color was determined by overflowing flour samples onto an empty dish, striping the excess flour from the top of the dish using a ruler, while making sure that the bottom layer of the dish is fully covered (Di Matteo *et al.*, 2004). A Minolta Chroma Meter CR-400 (Canada) colorimeter was used and the results obtained were expressed in terms of L* (lightness), a*(redness/greenness) and b* (yellowness/blueness) values. Color measurements of top and bottom surfaces of cookies were carried out 24 h after baking using the same equipment as for flours.

Textural characteristics

Textural analysis of the cookies was conducted using a TA. XTPlus Texture Analyzer (Stable Micro Systems, New York, USA), equipped with a 3-point bending rig (Probe: HDP/3PB), and a 25 kg load cell.

In compression mode, texture analyzer pre-test speed was 1.5 mm/s, test speed during compression was 2.0 mm/s and post-test speed was 10.0 mm/s. Textural properties were determined 24 h after baking and the maximum force in the Force vs. Time graph was expressed as the cookie hardness value.

Physical characteristics of cookies

Weight, diameter, thickness, spread ratio and % spread factor of cookies were estimated as per AACCI, 10-50.05 (1999) method.

Sensory Evaluation

The cookies were evaluated for sensory attributes by semi-trained judges at the college of Community Science, University of Agricultural Sciences Dharwad, Karnataka (India). A 9 point Hedonic scale (Larmond, 1991) ranging from like extremely to dislike extremely was used for sensory attributes like appearance, color, texture, taste, flavor and overall acceptability. Acceptability index was calculated by adding the average scores of appearance, color, texture, flavour, taste and overall acceptability divided by six (measured sensory attributes).

Nutraceutical characteristics of flours and cookies

Total phenolic content

Total phenolic content (TPC) of the samples was determined as described by Apea-Bah *et al.,* (2016) and Xiang *et al.,* (2019) using a 96-well ELX800 microplate reader (BioTek Instruments Inc., Winooski, VT, USA) with ferulic acid as the standard. The total phenolic content was expressed as milligrams ferulic acid equivalents per 100grams of sample (mg FAE/100g) on dry weight basis.

DPPH radical scavenging activity

This assay was based on the method of Li and Beta (2007) and Xiang *et al.,* (2019) using a 96-well micro plate reader. Ten µL of appropriately diluted crude extract (or fractions) was added to 190 µL of freshly made DPPH radical solution (60µM). After 30 min of incubation at room temperature, the absorbance at 515 nm was measured. DPPH free radical scavenging activities of crude methanol extracts and different fractions were expressed as micromole Trolox equivalents per gram of sample (µmol TE/g) on dry weight basis.

Statistical Analysis

Statistical analysis of the results was conducted using statistical software SPSS version 16. The data reported in all the tables are an average of triplicate observations and were subject to one-way analysis of variance (ANOVA).

Results and Discussion

Physical and functional properties of the raw materials are presented in Table 2. L* values were highest for refined wheat flour followed by little millet. The values for finger millet, horse gram, and composite flour were lower, meaning that they were darker. The a* values were highest in finger millet followed by composite flour. The b* values were highest in horse gram meaning it was yellower followed by finger millet. Color values of composite flour, finger millet and horse gram flour were darker and yellower in line with previous studies (Thilagavathi *et al.,* 2015). Significant differences ($p<0.01$) were found in the color attributes of processed and composite flour. This difference in color could be due to the composition of flours and botanical origin of the plant responsible for color pigment characterization that constitutes differences in color (Aboubakar *et al.,* 2008).

Water solubility, an indicator of the degree of starch granule dispersion after cooking, varied significantly among raw materials, in line with the literature (Bhupender *et al.,* 2013). A germinated finger millet and horse gram had the highest solubility, possibly due to sprouting, as sprouting increases the amount of soluble materials such as carbohydrates (Nazni and Shobana, 2016). Differences in starch granule dispersion after cooking can be attributed to the different chain length distribution of starch in different raw materials (Perez *et al.,* 2000).

Water binding capacity was highest in horse gram followed by finger millet and composite flour. Flours with higher water binding capacity could be good ingredients in bakery applications (Ma *et al.,* 2011). For example, flours with higher water binding capacity enables bakers to add more water to dough, improves the handling characteristics of doughs and maintains freshness of bread for longer (Wolf, 1992).

Oil absorption capacity was higher in composite flour followed by little millet and finger millet flour. Higher oil absorption capacity is desired in most bakery products since it adds to mouth feel, palatability and shelf life (Aremu *et al.,* 2007). Variation in oil absorption capacity of different millet flours may be attributed to variation in protein concentration, their degree of interaction with water and oil and possibly their conformational characteristics (Butt and Batool, 2010). The lower oil absorption may also be due to the presence of large proportion of hydrophilic groups and polar amino acids on the surface of the protein molecules.

The pasting properties of the raw materials varied widely. Peak viscosity, the ability of starch to form a paste on cooking, ranged from 563.67 to 3097.33 cP being lowest in finger millet and highest in little millet. A low peak viscosity indicates a product is better suited for applications requiring low gel strength and elasticity like carrageenan gel (Bolarinwa *et al.,* 2015; Abioye *et al.,* 2018).

Trough viscosity is the measure of the ability of a paste to withstand breakdown during cooling. The high trough viscosity was seen in refined wheat flour (1499.00cP) followed by little millet flour (611.33 cP) and composite flour (229.33 cP). The low trough viscosity was found in horse gram flour (69.67 cP). The significantly high trough viscosity observed in this study indicates the tendency of the refined wheat flour and little millet flour to breakdown during cooking.

Breakdown viscosity is caused by disintegration of gelatinized starch granules structure during continued stirring and heating (Zhang and Hamauzu, 2004). The relatively lower breakdown viscosity of composite, finger millet and horse gram flours compared to refined wheat flour indicates starch stability under elevated temperatures.

Final viscosity is the quality of particular starch-based flour since it indicates the ability of the flour to form a viscous paste after cooking and cooling (Adebowale *et al.*, 2005). The high final viscosity of the little millet and refined wheat flours indicates their high resistance to shear stress during stirring (Zhang and Hamauzu, 2004). The variations in the final viscosity might be due to the simple kinetic effect of cooling on viscosity and the reassociation of starch molecules in the flour samples. The setback viscosity is an index of retrogradation, i.e., the lower the setback viscosity, the lower is the rate of retrogradation. Among the raw materials studied, finger millet had the lowest set back viscosity, meaning that finger millet flour in the composite flour mix would cause a reduction in the retrogradation rate and thus a prolonged shelf life (Karim *et al.,* 2007).

Pasting temperature is an indication of minimum temperature required for cooking the samples. Pasting temperature of flours ranged from 76.28 to 87.38°C being highest in refined wheat flour and lowest in little millet. It is observed that less time and low temperature is required to cook the composite flour. Awolu (2017) reported pasting temperature of refined wheat flour was 88.9°C, similar to our findings. Peak time of flours ranged from 6.07 to 7.00min.

Physical parameters of cookies incorporated with composite flour are presented in Table 3. Weight of the cookies ranged from 5.00 to 5.30 g. Diameters of the cookies ranged from 42.65 to 48.29 mm, highest in control cookies and lowest in cookies prepared with 100% composite flour. Thickness of cookies ranged from 43.70 to 46.28 mm, highest being in 100% composite flour cookies, lowest in control cookies. As the proportion of composite flour increased, there was a gradual decrease in the diameter and increase in the thickness of the cookies.

Table.1 Composition of composite flour

Ingredients	Composition (%)
Germinated finger millet flour	50
Fermented little millet flour	30
Horse gram flour	10
Refined wheat flour (RWF)	10

Table.2 Physical and functional properties of raw materials

Samples /Attributes	Finger millet flour	Little millet flour	Horse gram flour	Refined wheat flour (RWF)	Composite flour (CF)
L*	78.83±0.18^{e}	93.53±0.05^{b}	85.61±0.17^{c}	93.78±0.10^{a}	82.19±0.11^{d}
a*	3.29±0.02^{a}	0.42±0.02^{e}	1.68±0.04^{c}	0.58±0.01^{d}	2.73±0.05^{b}
b*	9.13±0.06^{c}	5.80±0.04^{e}	10.99±0.09^{a}	9.91±0.07^{b}	8.85±0.19^{d}
Water solubility (%)	7.84±0.04^{b}	0.52±0.00^{e}	19.02±0.18^{a}	5.28±0.22^{d}	6.52±0.09^{c}
Water binding capacity (%)	166.74±3.83^{b}	139.88±2.39^{d}	205.34±8.85^{a}	100.69±4.01^{e}	155.03±3.58^{c}
Oil absorption capacity (g oil/g)	0.93±0.01^{b}	0.94±0.03^{b}	0.86±0.02^{c}	0.88±0.02^{c}	1.13±0.02^{a}
Pasting properties (RVA)					
Peak viscosity(cP)	563.67±6.51^{e}	3097.33±75.61^{a}	828.67±15.04^{d}	2497.00±15.62^{b}	1106.67±2.08^{c}
Trough viscosity (cP)	144.33±17.67^{d}	611.33±6.03^{b}	69.67±2.08^{e}	1499.00±6.24^{a}	229.33±4.04^{c}
Breakdown viscosity (cP)	407.67±5.51^{e}	2514.00±34.04^{a}	771.67±3.06^{d}	998.00±20.22^{b}	889.00±17.09^{c}
Final viscosity (cP)	1313.67±29.54^{d}	5534.00±135.75^{a}	1342.67±30.37^{d}	2992.67±23.09^{b}	2340.00±8.54^{c}
Set back viscosity (cP)	1169.33±16.20^{e}	4950.67±87.79^{a}	1299.33±27.02^{d}	1493.67±27.79^{c}	2122.67±7.09^{b}
Peak time (min)	7.00±0.00^{a}	7.00±0.00^{a}	7.00±0.00^{a}	6.07±0.07^{b}	7.00±0.00^{a}
Pasting temperature (°C)	78.68±0.60^{c}	76.28±0.08^{d}	81.82±0.08^{b}	87.38±0.49^{a}	78.05±0.05^{c}

Mean ± SD, Means with the same superscript letters within a row are not significantly different

Table.3 Physical properties of cookies incorporated with composite flour

Samples	Physical Properties					
	Weight of cookie (g)	Bakeloss (%)	Diameter (mm)	Thickness (mm)	Spread ratio (%)	Percent spread (%)
Control (RWF)	5.30±0.48	15.96±1.15	48.29±0.14[a]	43.70±0.50[b]	1.12±0.00[a]	100.00±0.01[a]
25%	5.30±0.48	15.96±1.15	48.12±0.06[a]	43.15±0.14[b]	1.11±0.01[a]	99.58±0.22[a]
50%	5.20±0.42	16.19±1.01	44.96±0.66[b]	43.10±0.65[b]	1.05±0.01[b]	93.14±0.37[b]
75%	5.00±0.47	16.77±1.36	44.31±0.22 [b]	43.20±0.05[b]	1.00±0.01[c]	89.18±0.68[c]
100%	5.00±0.47	16.77±1.36	42.65±0.57[c]	46.28±0.30[a]	0.96±0.00[d]	85.48±0.16[d]

Mean ± SD, Means with the same superscript letters within a row are not significantly different, Control-refined wheat flour

Table.4 Textural and color attributes of composite flour incorporated cookies

Samples	Hardness(N)	L*	a*	b*
Control (RWF)	3.54±0.04[c]	63.85±0.01[a]	10.16±0.00[a]	30.81±0.02[a]
25%	3.36±0.01[d]	57.70±0.41[b]	8.96±0.10[c]	25.42±0.01[b]
50%	3.44±0.06[d]	55.42±0.14[c]	8.44±0.01[e]	22.45±0.05[c]
75%	3.75±0.03[b]	52.57±0.40[d]	8.65±0.08[d]	20.75±0.41[d]
100%	4.12±0.09[a]	45.55±0.06[e]	9.76±0.03[b]	19.54±0.02[e]

Mean ± SD, Means with the same superscript letters within a row are not significantly different Control-Refined wheat flour

Table.5 Organoleptic evaluation of composite flour cookies

Combinations	Sensory Attributes					
	Appearance	**Color**	**Texture**	**Flavor**	**Taste**	**Overall**
Control (RWF)	7.90±0.71[a]	8.23±0.73[a]	7.93±0.74[a]	7.33±0.83[a]	7.77±0.82[a]	7.83±0.65[a]
75%	7.40±0.76[b]	7.63±0.80[b]	7.60±0.72[ab]	7.40±0.67[a]	7.57±0.67[a]	7.60±0.56[ab]
50%	7.17±0.99[bc]	7.27±0.94[bc]	7.43±0.82[b]	7.30±0.88[a]	7.50±0.73[a]	7.43±0.63[b]
25%	6.73±0.98[cd]	6.80±1.06[cd]	6.93±0.94[c]	6.70±0.83[b]	6.60±0.71[b]	6.80±0.76[c]
100%	6.30±1.12[d]	6.47±1.11[d]	6.60±0.93[c]	6.40±0.84[b]	6.27±0.83[b]	6.50±0.68[d]

***Significant at 0.1 % level, Means with the same superscript letters within a column are not significantly different, Control-refined wheat flour

Table.6 Correlation of physico chemical and nutraceutical properties of composite flour cookies

Parameters	**Hardness**	**L***	**a***	**b***	**Bakeloss**	**Thickness**	**Polyphenol content**	**Antioxidant activity**
Hardness	1	-0.788**	-0.679**	-0.589*	-0.367	0.756**	0.855**	0.850**
L*		1	0.919**	0.935**	0.082	-0.693**	-0.967**	-0.965**
a*			1	0.499	-0.263	0.475	-0.128	-0.149
b*				1	0.012	-0.425	-0.911**	-0.918**
Thickness						1	0.655**	0.611*
Polyphenol content							1	0.989**
Antioxidant activity								1

*Significant at 5% level, **Significant at 1 % level

Fig.1 Total Polyphenol content and Antioxidant activity of processed and composite flour

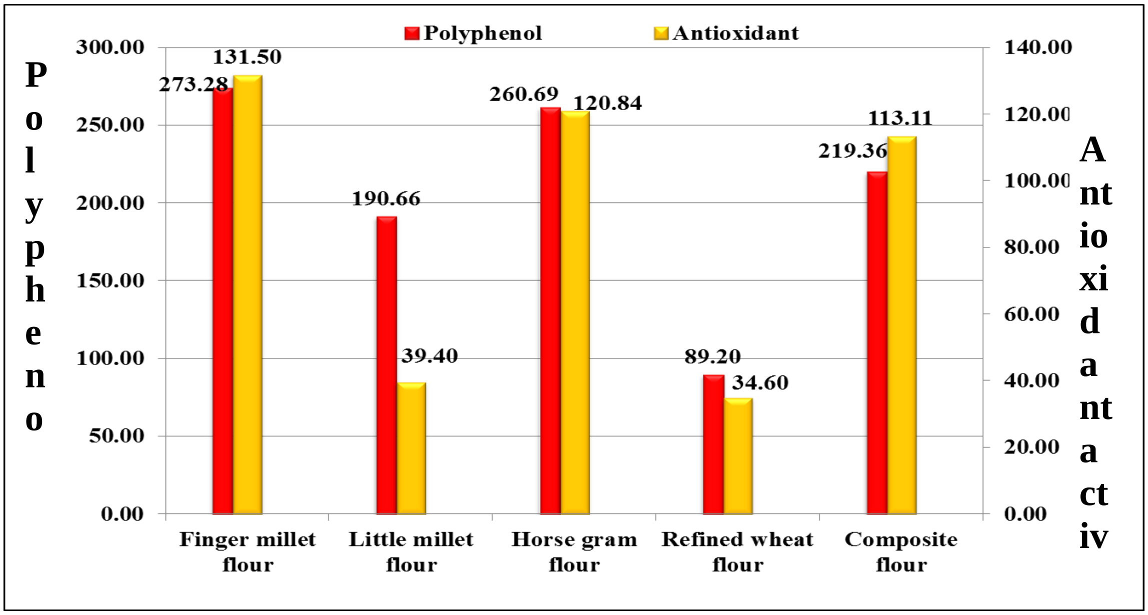
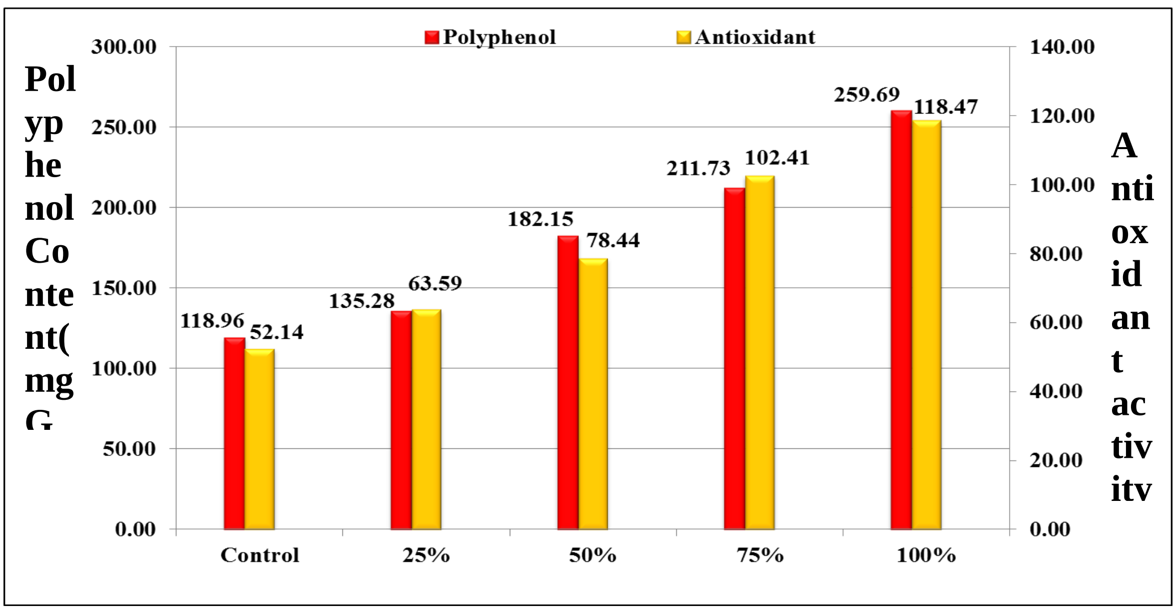


Fig.2 Total Polyphenol content and antioxidant activity of composite flour incorporated cookies



The quality of cookies is widely determined by spread factor is the ratio of width by thickness (in six cookies) (Pareyt *et al.,* 2009). Spread ratio and spread factor of cookies ranged from 0.96 to 1.12 and 85.48 to 100%, respectively.

A decreasing trend was observed for the spread ratio and the spread factor with an increase in the level of composite flour incorporation, possibly due to the higher water holding capacity of composite flour compared to wheat flour (see Table 2). These results are in line with the literature (Banusha and Vasantharuba, 2014; Ashwath Kumar *et al.,* 2016; Fuhr, 1962).

With increasing levels of composite flour in cookies, a significant increase in hardness was observed. This increase may be attributed to dilution of wheat proteins with millet based composite proteins made cookies compact thus, increasing the cookie hardness.

Hardness is caused by the interaction of protein and starch by hydrogen bonding. The increase in protein content could also be the promising reason of the harder texture detected due to its interaction during dough development (Cheng and Bhat, 2016). And also this would be due to functionality of wheat proteins, compared with other proteins, which makes cookies compact and this would affect the texture of the cookies.

Color development occurs largely during the later stages of baking (Wade, 1988), caused by maillard reactions between sugars and proteins (Bertram, 1953). Color is an important element for initial acceptability of baked product by consumers (Agrahar Murugkar *et al.,* 2015). Cookies incorporated with composite flour were evaluated for their color in terms of L*, a* and b*. The darkness of cookies increased with the increase in the proportion of composite flour from 25to 100 per cent. The L*, a*, b* value of all composite flour based cookies were lower than control sample indicating darker, less reddish, less yellowish color due to the nature of ingredients. Others factors that might contribute to the color of final products are ingredients' composition and time of baking.

The total polyphenol content and antioxidant activity of processed and composite flours are presented in Figure 1. The total polyphenol and antioxidant activity of flours ranged from 89.20 to 273.28 mg/100g and 39.40 to 131.50 µmoltrolox/g respectively. Higher values of total polyphenol and antioxidant activity were found in finger millet, whereas, the lowest values were found for refined wheat flour. Accordingly, cookies prepared with increasing levels of composite flour showed an increasing trend in total phenolic content and antioxidant activity (Figure 2).

The organoleptic acceptability of cookies on a 9 point Hedonic scale is presented in Table 5. The scores obtained for overall acceptability for control and 25 % composite flour incorporated cookies were not significantly different. Texture, flavor, taste were found to be statistically not significant for 25 and 50 % composite flour incorporated cookies. Composite flour cookies which were darker in color recorded the lowest score for color and appearance. However, they were acceptable up to 50 % level of composite flour incorporation. Acceptability index ranged from 71.37 to 87.02, being highest in control and lowest in 100 % composite flour cookies.

Correlation of physical and nutraceutical properties of composite flour cookies were reported in Table 6. Hardness of cookies was negatively correlated with color attributes. Whereas, significant positive correlation was found with thickness, polyphenol and antioxidant activity. Thicknesses of cookies had significant positive correlation with antioxidant and polyphenol content. In addition, polyphenol and antioxidant activity were positively significantly correlated. Increase in the level of incorporation of composite flour showed increasing trend in antioxidant and polyphenol activity of cookies.

Composite flour cookies were found to be superior to the refined wheat flour with respect to functional, physical, textural, nutraceutical properties and sensorially accepted. Composite flour cookies had lower spread ratio, higher protein and fiber content, were harder. Even though the composite flour cookies were darker in color due to basic nature of the ingredients, they had higher organoleptic acceptability on the 9 point hedonic scale. At 50 per cent level of substitution of composite flour better performance in sensory, color and physical attributes of cookies were observed. Incorporation of composite flour improved the polyphenol content and antioxidant activity of cookies. Thus, it can be concluded that cookies made from composite flours can be a more nutritious alternative to wheat flour cookies.

Acknowledgements

The authors would like to thank the University of Manitoba start-up funds and the Queen Elizabeth Diamond Jubilee II fellowship program. Technical assistance from Alison Ser of the Department of Food and Human Nutritional Sciences is greatly appreciated.

Conflict of Interest

The authors declare no conflict of interest.

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How to cite this article:

Akkavva Wadakappanavar, S., Jinie Xiang, Trust Beta, Filiz Koksel and Nirmala B. Yenagi. 2023. Physical, Functional and Nutraceutical Properties of Composite Flour Cookies. *Int.J.Curr.Microbiol.App.Sci.* 12(09): 164-175. **doi:** https://doi.org/10.20546/ijcmas.2023.1209.016