J Food Sci Technol (December 2016) 53(12):4278–4288
DOI 10.1007/s13197-016-2423-5

ORIGINAL ARTICLE

Wheat-water chestnut flour blends: effect of baking on antioxidant properties of cookies

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Revised: 8 September 2016 / Accepted: 28 November 2016 / Published online: 17 December 2016

Abstract Proximate composition, mineral content, functional, pasting and antioxidant properties of water chestnut flour (WCF) were compared with refined wheat flour. WCF showed higher phenolic (4.25 gGAE/1000 g), flavonoid (1.92 g QE/1000 g) and mineral content (K, Mg, Zn, Cu) than wheat flour. WCF showed greater retrogradation tendency but lower peak viscosity than wheat flour. Wheat flour - WCF blends and cookies were evaluated for water activity, physical & textural properties. Water activity of cookies decreased significantly (0.415–0.311) with increase in level of WCF in wheat flour. Total phenolic content, flavonoid content and antioxidant activity (DPPH• scavenging capacity, FRAP) of WCF - wheat flour blends as well as their cookies was also determined. Baking led to a greater increase in DPPH• scavenging capacity of WCF cookies (33.8%) than WF cookies (25%). Baking had a similar effect on FRAP value. Wheat flour cookies showed a decrease of 51%, and 62% while WCF cookies showed a decrease of 36%, and 34% in TPC and TFC values respectively. WCF cookies thus showed better retention of antioxidant activities suggesting greater stability of WC phenolics than wheat phenolics. Sensory analysis showed cookies made from water chestnut (100%) had fair acceptability due to their characteristic flavor. Thus, water chestnut flour serves both as a gluten free as well as antioxidant rich flour for production of cookies.

Keywords Water chestnut · Nutritional composition · Antioxidants · Cookie characteristics

Introduction

One of the major challenges in baking industry is the production of gluten free products. Gluten, important for dough development, is usually associated with gluten intolerance and gluten sensitivity that have recently been categorized as two different medical conditions and are associated with the consumption of gluten protein. Usually those gluten free sources are preferred for incorporation in bakery products that are rich in starch. Keeping in view, various under-utilized food sources have been incorporated in bakery products. Incorporation of buckwheat, amaranths, quinoa, and barley has been thoroughly studied due to absence of gluten and rich antioxidant profiling. Among such gluten free sources is water chestnut that is not only rich in starch but has also been reported for its high flavonoid content. It also contains plentiful B vitamins (including B1, B2, B5 and B6), E, A and ascorbic acid. Water chestnut (*Trapa natans*) possess strong antioxidant, antimicrobial and anticancer activities, which have been attributed to their bioactive components, such as polyphenols, flavonoids and alkaloids (Yu et al. 2013; Chiang and Ciou 2010).

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Water chestnut extracts are also reported to have high inhibitory activity against glycolytic enzymes such as α-amylase that inhibits blood glucose elevation and a unique feature of reducing insulin secretion and hence can be effective food additives in managing type 2-diabetes (Yasuda et al. 2014).

Recent researchers are focusing on alternatives that can cut down production cost as well as improve nutritional as well as nutraceutical status of food items (Baba et al. 2016). Water chestnut is available in abundance in south East Asia, China and northern India during the months of November to March and can be a good option to achieve such objectives. In addition, use of natural sources of antioxidants as food additives is gaining interests due to economical and safety concerns associated with synthetic additives. Water chestnut fruit being a rich source of starch with no gluten, its flour can be used to replace wheat flour for the production of gluten free products. In addition, flour production being a size reduction process may alter the structure, surface area and functional properties of flour resulting in some new applications (Chiang and Ciou 2010).

Although some basic work regarding characterization of water chestnut flour and production of water chestnut cookies (Sarabhai and Prabhasankar 2015; Singh et al. 2011) has been carried out but the antioxidant potential of water chestnut flour and effect of baking on its antioxidant activity during cookie production has not been reported so far. Hence, this study was conducted to report the physicochemical, pasting properties of the flour blends and effect of baking on its antioxidant potential during cookie production.

Materials and methods

Procurement of water chestnut and wheat flour

Dried fruits of water chestnut (*T. natans*) were purchased from Sopore, Jammu & Kashmir, India. Dried fruits (80 °C) were ground (Sujata Powermatic plus) and flour obtained was passed through 120 mesh standard sieve, manually. Refined wheat flour (WF) was procured from local market of Srinagar, Kashmir. All sieved samples were packed in amber glass bottles and stored at 5 °C. All the chemicals and reagents used were of analytical grade.

Formulation of flour blend

Blends of water chestnut flour (WCF) were made and refined wheat flour (WF) was taken as control. Six blends were prepared by mixing WCF with WF in the percentage proportions of 00:100, 20:80, 40:60, 60:40, 80:20 and 100:00 respectively.

Cookie, preparation and evaluation

Cookies were prepared according to formula described by Jan et al. (2015). The cookies' formula based on flour weight was: 100 g flour, 53 g sugar, 26.5 g shortening, 1.1 g sodium bicarbonate, 0.89 g sodium chloride and 12 cm^3 water. Prepared dough was then sheeted to a thickness of 6 mm with a rolling pin. Cookies were cut round in shape with a cookie die of diameter 5.5 cm and transferred to a tray lined with aluminum foil and were baked at 150 °C for 15 min in an electric oven (Dollar). The baked cookies were cooled to room temperature and packed in airtight containers for further analysis.

Proximate composition of WF and WCF

The proximate composition of WF and WCF viz; crude ash, crude fat, crude fiber and crude protein were determined according to the methods described by Bazaz et al. (2016). The nitrogen conversion factor used for crude protein calculation was 6.25. The carbohydrate content (%) was calculated by subtracting the contents of crude ash, fat, fiber and protein from 100% of dry matter.

Mineral estimation

Samples were dry-ashed according to AOAC (1990) Aliquots were analyzed for mineral components of calcium (Ca), magnesium (Mg), zinc (Zn) and copper (Cu) using atomic absorption spectrophotometer (ECIL Atomic Absorption Spectrophotometer-4141). Phosphorus (P) was determined using a spectrophotometer (Systronics, UV–Vis 108), and Potassium (K) by flame photometer (Systronics-130).

Physical and functional properties of WF and WCF

Bulk density (loose and packed)

For loose bulk density, an empty and dried 50 ml measuring flask was taken and weighed. Flour sample was allowed to fall freely into it up to the mark, with a gentle tapping. The flask was weighted again along with the sample. For packed bulk density, sample was tapped inside the measuring flask and more sample added up to the mark before weighing. Bulk density was calculated as weight of sample per unit volume of sample and results were reported as g/ml.

Water and oil absorption capacity (WAC and OAC)

Flour samples (1.0 g each) at ambient temperature were mixed with 10 ml distilled water or refined mustard oil and stored for 30 min. Samples were centrifuged (Eppendorf

Centrifuge 5810R, Germany) for 10 min at 2000 g. The aqueous supernatant or clear oil obtained after centrifuging was decanted and the test tubes were inverted and allowed to drain for 5 min on a paper towel. By weighing the residue, WAC and OAC were calculated and expressed as percentage of water or oil absorbed per gram of sample, respectively.

Swelling power (SP)/Solubility(S)

For determining swelling power and solubility of the flour samples, known amount of dry flour (0.5 g) was dispersed in 50 ml of water and the dispersion was heated under mild agitation at 90 °C for 30 min. The gelatinized dispersion was then centrifuged (3000*g*) for 15 min and the supernatant was decanted and dried at 100 °C up to a constant weight. The swelling power and solubility were determined using the given standard equations and the results were expressed as g/g of dry flour.

Color analysis of flour and cookies

Color characteristics of WF, WCF and cookie blends were measured using a Hunter's Lab color analyzer (Mini Scan XE Plus, Model 45/0-S, Inc., Reston, VA, USA). Color values, L^*, a^* and b^*, were recorded, each value being the average of four measurements.

Pasting properties

Pasting properties of the WF and WCF were evaluated with the Rapid Visco Analyzer (Tech Master, Perten Instruments Pty Ltd, Australia). Samples were held at 50 °C for 1 min, heated to 95 °C at 12 °C/min cooling rate. Samples were then held at 95 °C for 2.5 min, and cooled to 50 °C at 12 °C/min heating rate and held at 50 °C for 2 min. Parameters recorded were pasting temperature, peak time, peak viscosity, trough viscosity (minimum viscosity at 95 °C), final viscosity (viscosity at 50 °C), breakdown viscosity and setback viscosity.

Physical characteristics of cookies

Thickness (T) of cookies was measured by a Vernier Caliper and the diameter (W) was determined using a scale placing them edge-to-edge. From the measurements taken, spread ratio (W/T) was calculated. Cookies weight was determined using an electronic weighing balance (Kern EMB 1000-2).

Texture analysis of cookies

Textural properties of the final products were investigated using Texture analyzer (TA XP plus Exponent Stable Micro System, Haslemere, UK). The hardness was measured using a 3-point Bending Rig and 5 kg load cell. The distance between two beams was 60 mm. Another identical beam was brought down from above (pre-test speed of 1.0 mm/s, test speed of 3.0 mm/s, post-test speed of 10.0 mm/s, distance: 5 mm) to contact the cookie. The downward movement was continued until the cookie broke. Peak force was reported as hardness (g). Ten representative samples were analyzed from each formulation.

Water activity of cookies

Water activity of cookies was studied for a period of 90 days of storage using an AQUALAB instrument (Decagon, SN: PRE000197).

Antioxidant activity of WF, WCF and Cookie blends

Preparation of extracts

Each sample (0.3 g) was dissolved in 20 ml of 70% methanol. After stirring for 2 h on a magnetic stirrer, it was centrifuged (3500 rpm) for 10 min. The supernatant was filtered and stored at −18 °C.

Total phenolic content

Total phenolic content (TPC) was determined by Folin–Ciocalteu's spectrophotometric method (Jan et al. 2015), with some modifications. The results were expressed as Gallic acid equivalents (gGAE/1000 g) of sample.

Total flavonoid content

Total flavonoid content (TFC) was determined spectrophotometrically, according to standard method (Hosu et al. 2014). Briefly, 0.5 ml of each extract was mixed with 0.4 ml of 25 g/l $AlCl_3$, 0.5 ml of 100 g/l CH_3COONa and 4 ml of distilled water. The sample was incubated for 30 min at room temperature. Absorption readings at 430 nm were taken against a blank. TFC was expressed as g QE/1000 g, using Quercetin calibration curve.

DPPH• (2,2-diphenyl-1-picrylhydrazyl) scavenging activity

DPPH• scavenging activity of the extracts was determined according to the method described by Baba et al. (2014). The absorbance at 517 nm was measured after incubating samples for 30 min. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Percentage inhibition was calculated by using the formulae

Table 1 (a) Proximate composition of wheat and water chestnut flour and (b) mineral analysis of flours

Samples	Protein (%)	Fat (%)	Carbohydrate (%)	Ash (%)	Crude fiber (%)
(a)					
WF	10.94 ± 0.1^{a}	2.72 ± 0.1^{a}	73.59 ± 0.2^{a}	1.53 ± 0.1^{a}	0.53 ± 0.3^{a}
WCF	4.18 ± 0.2^{b}	0.52 ± 0.2^{b}	81.25 ± 0.1^{b}	2.32 ± 0.1^{b}	1.51 ± 0.2^{b}

Samples	P (%)	K (%)	Ca (ppm)	Mg (ppm)	Zn (ppm)	Cu (ppm)
(b)						
WF	0.08 ± 0.05^{a}	0.85 ± 0.05^{a}	1122 ± 0.5^{a}	733 ± 0.5^{a}	28.4 ± 0.1^{a}	6.8 ± 0.1^{a}
WCF	0.20 ± 0.03^{b}	3.85 ± 0.03^{b}	356.4 ± 0.5^{b}	1179 ± 0.5^{b}	38.01 ± 0.1^{b}	22.4 ± 0.1^{b}

Values in the same column with different lower letters are significantly different ($P < 0.05$)

$$\%\,\text{inhibition} = \left[\text{A}_{\text{control517}} - \text{A}_{\text{sample517}}/\text{A}_{\text{control517}}\right] \times 100$$

where $\text{A}_{\text{control517}}$ is the absorbance of the control and $\text{A}_{\text{sample517}}$ is the absorbance of the extract.

FRAP (Ferric reducing antioxidant power)

FRAP value was determined according to the method described by Jan et al. (2015). Briefly 2.4 ml of TPTZ reagent (*ferric*-2,4,6-*tripyridy-5-triazine*) was mixed with 0.1 ml of each sample extract. After 1 h at room temperature, the absorbance was read at 593 nm. Using ascorbic acid as standard the antioxidant capacity (FRAP) was expressed as (g AA/1000 g) of sample.

Sensory evaluation of cookies

Cookies prepared from WF, WCF and their blends were subjected to sensory evaluation by a trained sensory panel of ten people. Before the sensory evaluation was conducted, the panels were trained by using commercial cookies to get familiar with the use of rating method, terminology for each attribute and sensory characteristics. The judges rated the quality characteristics of each sample on a nine-point hedonic rating scale. The judges evaluated randomly coded cookies in terms of color, appearance, flavor, texture, taste and overall acceptability.

Statistical analysis

Experiments were performed in triplicates. The data was analyzed using one way analysis of variance (ANOVA) and Duncan test by SPSS (version 21).

Results and discussion

Proximate composition of flours

The proximate composition of WCF and WF is presented in Table 1(a). The protein content of WCF and WF was found to be 4.18 and 10.94% respectively. These values were lower than the results reported by Ahmed et al. (2016) (8.4%) where-as Bala et al. (2015) and Singh et al. (2011) reported fairly similar protein content of water chestnut. The variation in the protein content of WCF could be attributed to different processing and environmental conditions. The fat content of WCF (0.52%) was lower than the WF (2.72%) where as ash content of WCF (2.32%) was higher than the WF (1.53%). Similar results were also reported by Bala et al. (2015) for water chestnut. The total carbohydrate content of WCF (81.25%) was found to be higher as compared to WF (73.59%). Our results were in agreement with Bala et al. (2015) who reported 83% carbohydrate content of water chestnut. Water chestnut kernels are abundant source of starch (Singh et al. 2011). Crude fiber content of WCF and WF were found to be 1.51 and 0.52%, respectively. Ahmed et al. (2016) also reported similar crude fiber content of WCF.

Mineral content

The mineral content of the WCF and WF samples are presented in Table 1(b). WCF contained significantly higher amounts of potassium (3.85%), phosphorus (0.20%), magnesium (1179 ppm), zinc (38.01 ppm) and copper (22.4 ppm) than WF. Mir et al. (2015) also reported presence of minerals such as potassium, phosphorus and magnesium in water chestnut. The result of WF was in accordance with the findings of Heshe et al. (2016) who reported 0.077% of phosphorus. Phosphorus is required for proper development and protection of bones and teeth, as part of DNA and RNA and helps to convert food into energy (Yellavila et al. 2015). Incorporation of WCF in our daily diet can help to achieve the recommended daily allowance of 700 mg of phosphorus required for proper functioning of the body. Mann et al. (2012) also reported that *Trapa bispinosa* is rich in potassium (98.2 ppm). Potassium rich diet seems to lower blood pressure and may benefit bones apart from maintaining cellular water balance and pH regulation in the body (Yellavila et al. 2015).

Mortality and morbidity rates are greatly enhanced due to micronutrient deficiency. It reduces the quality of life for all those affected, diminishes cognitive abilities in children and lowers labor productivity. Deficiency of micronutrients, such as iron and zinc, is critical and major problem worldwide (Heshe et al. 2016). Zinc content of WF (28.4 ppm) was significantly lower than WCF (38.01 ppm). Mann et al. (2012) reported *T. bispinosa* an important source of microelement zinc. Hence addition of WC flour can be a good option for improving mineral profile of foods.

Physical and functional properties of flour

Physical and functional properties of WF and WCF are shown in Table 2. Bulk density (loose) of WCF and WF was 0.52 and 0.47 g/ml respectively where as bulk density (packed) was found to be 0.79 and 0.71 g/ml respectively. Bulk density depends on the particle size of the samples. It is a measure of heaviness of a flour sample. It is important for determining packaging requirements (Singh et al. 2011); material handling and application in wet processing in the food industry (Yellavila et al. 2015). Increase in bulk density is desirable since it offers greater packaging advantage by allowing packaging of greater quantity within constant volume.

Water absorption capacity (WAC) of flour plays an important role in food preparation due to its influence on other functional and sensory properties. WAC is the ability of a product to associate with water under limiting conditions. WAC of WCF (102.8%) was significantly higher than the WF (66.44%). Variation in the WAC between the flours might be due to the difference in protein structures and the presence of hydrophilic carbohydrates (Sarabhai and Prabhasankar 2015). Flours with high water absorption have more hydrophilic constituents, such as polysaccharides (Yellavila et al. 2015). Mir et al. (2015) reported lesser WAC of WCF than the results reported here. The discrepancy could be due to and the method used. However, similar results were found by Singh et al. (2011) and attributed WAC of WCF to its lower protein and lipid fraction. This suggests that higher WAC of food material is advantageous for in baked products where hydration improves dough handling, a preferred characteristic. Okpala et al. (2013) reported high WAC of the studied composite flours and suggested it to be useful for bakery products as this could prevent staling by reducing moisture loss.

Oil absorption capacity (OAC) is the ability of the flour to absorb oil, which is important as oil acts as a flavor retainer and improves mouth feel. OAC of WF (94.72%) is significantly higher than that of WCF (80.56%). Higher OAC of WF could be attributed to low hydrophobic proteins which show superior binding of lipids (David et al. 2015).

Table 2 Physical and functional properties of wheat and water chestnut flour

Sample	BD(L) (g/ml)	BD(P) (g/ml)	WAC (%)	OAC (%)	SP (g/g)	S (g/g)	Color		
							*L**	*a**	*b**
WF	0.47 ± 0.01^{a}	0.71 ± 0.01^{a}	66.44 ± 0.05^{a}	94.72 ± 0.05^{a}	7.5 ± 0.05^{a}	0.07 ± 0.05^{a}	91.42 ± 0.05^{a}	−0.91 ± 0.01^{a}	15.89 ± 0.05^{a}
WCF	0.52 ± 0.01^{b}	0.79 ± 0.01^{b}	102.8 ± 0.05^{b}	80.56 ± 0.05^{b}	7.95 ± 0.05^{a}	0.17 ± 0.05^{b}	85.49 ± 0.05^{b}	0.19 ± 0.01^{b}	12.99 ± 0.05^{b}

BD(L) loose bulk density, *BD(P)* packed bulk density, *WAC* water absorption capacity, *OAC* oil absorption capacity, *SP* swelling power, *S* solubility

Values in the same column with different lower letters are significantly different ($P < 0.05$)

Table 3 Pasting properties of wheat and water chestnut flour

Samples	PV (cP)	TV (cP)	BV (cP)	FV (cP)	SB (cP)	PT (min)	P_{Temp}(°C)
WF	2598 ± 0.1^{a}	1652 ± 0.05^{a}	946 ± 0.05^{a}	2933 ± 0.01^{a}	1281 ± 0.05^{a}	6.4 ± 0.05^{a}	69.35 ± 0.05^{a}
WCF	2457 ± 0.05^{b}	1259 ± 0.05^{b}	1198 ± 0.05^{b}	2907 ± 0.01^{b}	1648 ± 0.07^{b}	5.13 ± 0.05^{b}	77.40 ± 0.07^{b}

PV peak viscosity, *TV* trough viscosity, *BV* breakdown viscosity, *FV* final viscosity, *SB* setback viscosity, *PT* peak time, P_{Temp} pasting temperature

Values in the same column with different lower letters are significantly different ($P < 0.05$)

The swelling power of wheat flour (7.5%) was found to be lower than WCF (7.95%). Swelling capacity is related to protein and starch content of the flour. High protein content in flour may limit the access of starch granule to water and thus swelling power of starch is restricted (David et al. 2015). High swelling power of WCF could be attributed to its low protein and high carbohydrate content. The extent of swelling of the flour also depends on the temperature, availability of water, type of starch, extent of starch damage and other carbohydrates (such as pectins, hemicelluloses and cellulose) and protein.

Solubility of WCF (0.17 g/g) was found to be higher than WF (0.07 g/g). Factors that may influence starch solubility were source, swelling power, inter-associative forces within the amorphous and crystalline domains, and presence of other components such as phosphorous. Mir et al. (2015) attributed higher WSI of WCF to the presence of greater extent of depolymerised starch.

Color values (*L**, *a**, *b**) of WF and WCF are depicted in Table 2. *L** value of the WF sample (91.42) was found to be higher than the WCF samples (85.49). Similar results were reported by Ahmed et al. (2016) for WCF. Color values (*a** and *b**) of WF (−0.91 and 15.89) and WCF (0.19 and 12.99) varied significantly.

Pasting properties

Pasting properties of WCF and WF are presented in (Table 3). Significant differences were observed between WF and WCF with regard to their pasting characteristics. Peak viscosity (PV) is an indicator of ease with which the starch granules are disintegrated and often correlated with final product quality. PV of WF (2598 cP) was higher than the PV of WCF (2457 cP) which indicated greater structural rigidity of starch granules of WF than that of WCF. However, PV of WF and WCF in the present study were not in agreement with the values of 1805 and 2235 cP for WF and WCF as reported by Yadav et al. (2014). Trough viscosity is the minimum viscosity value in the constant temperature phase of the RVA profile and measures the ability of paste to withstand breakdown during cooling. Trough viscosity for WF and WCF were 1652 and 1259 cP respectively. Breakdown (BD), a measure of degree of disintegration was higher for WCF (1198 cP) and lower for WF (946 cP). Starch granules with high swelling power easily reach to maximum viscosity and the granules are likely to be easily broken down due to weaker inter molecular forces (Correia and Beirao-da-Costa 2012). Singh and Singh (2010) reported breakdown values inversely proportional to protein content in common wheat and durum wheat varieties. A similar trend was observed in wheat (higher protein and lower breakdown) and water chestnut flour (lower protein and higher breakdown). Setback, a measure of retrogradation or re-ordering of starch molecules, was higher for WCF (1648 cP) than WF (1281 cP). This depicted that wheat starch retrograded less as compared to water chestnut starch. This can be explained on the basis that high-amylose (linear) starches re-associate more readily than high amylopectin (branched) starches resulting in greater retrogradation. Final viscosity of WF (2933 cP) was found to be higher than the WCF (2907 cP). Pasting temperature is the temperature at which irreversible swelling of the starch granules occur, leading to the formulation of a viscous paste in an aqueous solution. The pasting temperature of WCF (77.40 °C) was higher than the WF (69.35 °C). It may be pointed out here that pasting properties are mostly determined by starch content however, the interference of other constituents such as fibers, hemicelluloses proteins etc. cannot be ruled out.

Physical and textural properties of cookies

Physical and textural properties of WCF and WF blended cookies are depicted in Table 4. Cookies having higher spread ratios are considered most desirable. Spread ratio of cookies decreased with increase in the level of blending with WCF. Similar results were reported in cookies made from wheat-chickpea (Singh et al. 1991) and wheat-water chestnut flour blends (Singh et al. 2011). Composite flours may form aggregates with increased numbers of hydrophilic sites that compete for limited free water in cookies dough thereby increasing dough viscosity and limiting cookie spread (McWatters 1978). WCF starch showed higher amylose content than wheat starch (Tran et al. 2013); hence greater hydrophilic nature of WC starches limits the available water that decreases the cookie spread.

Table 4 Physical and textural properties of cookies made from different blends of wheat and waterchestnut flour

Blends (WF:WCF)	Diameter (mm)	Thickness (mm)	Weight (g)	Spread ratio	Hardness (g)	Fracturability (mm)	Color		
							L^*	a^*	b^*
100:0	62.67 ± 0.2^a	7.73 ± 0.7^a	10.77 ± 0.2^a	8.11 ± 0.7^a	811 ± 0.3^a	6.2 ± 0.5^a	47.54 ± 0.2^a	7.49 ± 0.2^a	38.26 ± 0.2^a
80:20	62.54 ± 0.5^a	7.80 ± 0.7^a	10.83 ± 0.1^a	8.02 ± 0.5^a	847 ± 0.6^b	6.9 ± 0.7^b	48.46 ± 0.5^a	7.41 ± 0.5^b	37.23 ± 0.4^b
60:40	62.35 ± 0.3^b	7.96 ± 0.5^b	10.98 ± 0.2^b	7.83 ± 0.5^b	885 ± 0.2^c	7.4 ± 0.6^c	48.97 ± 0.2^b	6.83 ± 0.3^c	37.17 ± 0.7^c
40:60	62.23 ± 0.5^b	8.14 ± 0.5^c	11.10 ± 0.6^b	7.64 ± 0.7^c	924 ± 0.5^d	7.8 ± 0.5^d	49.72 ± 0.6^b	6.21 ± 0.1^d	36.87 ± 0.3^d
20:80	62.10 ± 0.2^c	8.24 ± 0.5^d	11.36 ± 0.3^c	7.54 ± 0.5^d	965 ± 0.9^e	8.5 ± 0.6^e	50.08 ± 0.4^c	6.13 ± 0.4^e	36.58 ± 0.3^e
0:100	61.83 ± 0.6^d	8.37 ± 0.5^d	11.63 ± 0.3^d	7.39 ± 0.7^e	992 ± 0.5^f	8.7 ± 0.5^f	53.51 ± 0.5^d	4.56 ± 0.3^f	35.51 ± 0.2^f

WF refined wheat flour, *WCF* water chestnut flour

Values in the same column with different lower letters are significantly different ($P < 0.05$)

Cookies made with 100% WCF that had least spread ratio (7.39) also had the highest thickness (8.37 mm). Similar results were observed in cookies produced from fermented sorghum flour (Okpala et al. 2013) and cassava/soyabean/mango composite flours (Chinma and Gernah 2007). Chinma and Gernah (2007) attributed it to the hydrophilic nature of the flour, thus leading to an increase in thickness of the cookies. On the contrary, Singh et al. (2011) reported a decrease in both thickness and diameter of cookies prepared from WF and WCF. Increase in weight of cookies with the increase in proportion of WCF in the blends could be due to higher bulk density of WCF.

Replacement of refined wheat flour with WCF in cookie formulations resulted in a significant change in the textual quality of the cookies (Table 4). Force required to break the cookies increased progressively with the increase in levels of WCF. Replacement of wheat flour with WCF at 0, 20, 40, 60, 80 and 100% levels increased the hardness of cookie to 811, 847, 885, 924, 965 and 992 g, respectively. Fracturibility increased from 6.2 mm at 0% to 6.9, 7.4, 7.8, 8.5 and 8.7 mm at 20, 40, 60, 80 and 100% level of blending with WCF respectively. Increase in hardness and fracturability with increase in replacement level of WF with WCF may be due to lesser fat content of WCF than that of wheat. Similar results were also reported by Singh et al. (2011). High fat content increases brittleness due to migration of moisture from the centre to the surface that causes breakage.

Color

L^* value (lightness) of cookie surface was 47.54, 7.49 and 38.26 for wheat flour against 53.51, 4.56 and 35.51 respectively for cookies from WCF. Maillard browning and caramelization of sugar is considered to produce brown pigments during baking. These browning reactions are influenced by many factors such as water activity, pH, temperature, sugars, type and ratio of amino compounds. Lighter color of WC cookies can be due to lesser amount of proteins leading to lesser formation of maillard compounds.

Water activity during storage

Water activity (a_w) of cookies is an important parameter that determines shelf life of cookies and was significantly affected during storage period. Water activity (a_w) of cookies made from blended flour during storage period of 90 days is presented in Fig. 1. Water activity of samples decreased significantly from 0.415 to 0.311 with increase in level of blending from 0 to 100% with WCF respectively. Water activity of all cookies was unfavourable for growth of molds (0.80) bacteria (0.90) or yeast (0.85–0.88). Secchi et al. (2011) also reported a decrease in a_w of

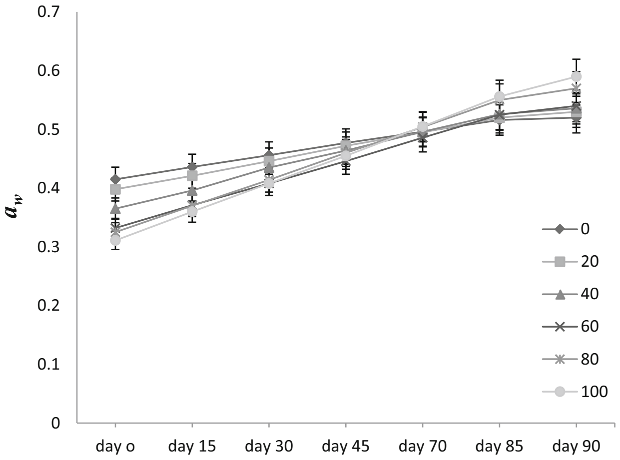

Fig. 1 Water activity (a_w) of cookies made from different flour blends during storage

cookies with incorporation of ovine whey powder and attributed this decrease to water binding capacity of proteins. High level of amylose content in WCF may significantly improve its water binding capacity and hence decrease a_w of cookies with increase in level of blending. An increase in a_w was seen during the storage of cookies (Fig. 1). Similar results were reported earlier in okra-based cookies (Park et al. 2015) and in amaretti cookies (Secchi et al. 2011). During storage greater increase was seen in a_w of water chestnut cookies than in wheat cookies. This might be due to greater content of sugars in WCF. Greater amount of sugars although may reduce water activity initially (as was seen in WC cookies) but during storage recrystallization of sugars takes place that leads to an increase in water activity. During storage of high sugar content cookies, increase in a_w has been attributed to recrystallization of sugars (Piga et al. 2005).

Antioxidant analysis of flour and cookies

DPPH• scavenging activity

DPPH• scavenging activity of WF, WCF and cookies is shown in Table 5. WC flour showed higher DPPH• scavenging activity and thus an increase in its proportion in blends led to a significant increase in the DPPH• scavenging activity of flour blends. Polyphenolic compounds present in water chestnut can be responsible for higher DPPH• scavenging activity of WCF. Baking resulted in an increase in the DPPH• scavenging activity of cookies in comparison to flour. Increase in DPPH• scavenging activity due to baking has already been reported (Jan et al. 2015) and has been attributed to melanoidins formed during processing (Baba 2015; Nisar et al. 2015). However, baking resulted in greater increase in scavenging activity of WC cookies (33.8%) than WF cookies (25%). Interpretation of color data however suggest lesser formation of melanoidins in WC cookies. This suggested greater stability of WC antioxidants during baking than wheat flour antioxidants. However, it should not be ruled out that antioxidant activity may not only be affected by temperature but also exhibits synergism (Brewer 2011).

FRAP

FRAP values showed a similar trend as seen in DPPH• scavenging activity. FRAP value of WCF and WF blends increased with the incorporation of WCF (Table 5). Baking

Table 5 Effect of baking on antioxidant properties of wheat and water chestnut flour cookies

	Blends (WF:WCF)	DPPH (% inhibition)	FRAP (g AA/1000 g)	TPC (gGAE/1000 g)	TFC (g QE/1000 g)
Flour	100:0	41.27 ± 0.1^{a}	0.54 ± 0.01^{a}	3.23 ± 0.05^{a}	0.11 ± 0.01^{a}
	80:20	43.27 ± 0.07^{b}	0.83 ± 0.1^{b}	3.35 ± 0.05^{a}	0.45 ± 0.03^{b}
	60:40	49.59 ± 0.05^{c}	1.13 ± 0.2^{c}	3.49 ± 0.07^{b}	0.82 ± 0.01^{c}
	40:60	55.61 ± 0.05^{d}	1.41 ± 0.06^{d}	3.76 ± 0.07^{c}	1.19 ± 0.01^{d}
	20:80	58.39 ± 0.05^{e}	1.68 ± 0.18^{e}	3.97 ± 0.05^{d}	1.55 ± 0.03^{e}
	0:100	61.57 ± 0.05^{f}	1.91 ± 0.01^{f}	4.25 ± 0.07^{e}	1.92 ± 0.02^{f}
Cookies	100:0	51.74 ± 0.07^{c}	$0.67 \pm .011^{g}$	1.57 ± 0.05^{f}	0.041 ± 0.01^{g}
	80:20	54.85 ± 0.07^{e}	1.05 ± 0.40^{h}	1.72 ± 0.05^{f}	0.19 ± 0.02^{h}
	60:40	63.82 ± 0.05^{g}	1.45 ± 0.04^{i}	2.13 ± 0.05^{g}	0.39 ± 0.01^{i}
	40:60	71.76 ± 0.01^{h}	1.81 ± 0.05^{j}	2.81 ± 0.07^{h}	0.65 ± 0.02^{j}
	20:80	76.71 ± 0.05^{i}	2.20 ± 0.06^{k}	3.03 ± 0.03^{a}	0.94 ± 0.01^{k}
	0:100	82.39 ± 0.07^{j}	$2.55 \pm 0.01\ ^{l}$	3.37 ± 0.05^{c}	1.25 ± 0.02^{l}

WF refined wheat flour, *WCF* water chestnut flour

Values in the same column with different lower letters are significantly different ($P < 0.05$)

increased the FRAP values of flour. An increase in the FRAP values after baking was previously reported in buckwheat (Jan et al. 2015) amaranth and quinoa flours (Chlopicka et al. 2012). However, greater increase was seen in WC cookies. Jan et al. (2015) also reported a greater increase in FRAP value of buckwheat cookies than those of wheat flour. Water chestnut like buckwheat is reported to contain high levels of quercetin that show antioxidant activity even at 150 °C (Elhamirad and Zamanipoor 2012) which might be responsible for higher activity of water chestnut cookies.

Total phenolic content (TPC)

TPC of WCF, WF and cookies made from there blends is shown in Table 5. WCF showed higher TPC content (4.25 gGAE/1000 g) than WF (3.23 gGAE/1000 g). Wheat polyphenols are principally concentrated in bran fraction and mostly refined wheat flour is used for production of cookies, which is free from bran fractions of wheat and hence lower polyphenolic content (Yu et al. 2013). Baking decreased the TPC content in both WC and wheat flour during baking. A decrease in polyphenolic content of wheat flour during baking was also reported by Yu et al. (2013) and is attributed to thermal unstability of polyphenols (Brewer 2011). A decrease of ≈30–40% (Yu et al. 2013), ≈75% (Chlopicka et al. 2012) and ≈45% (Alvarez-Jubete et al. 2010) in TPC was reported in different varieties of wheat during baking. In this study, a decrease of ≈51 and 36% was seen in TPC of wheat and WC cookies. The difference in decrease in percentage of TPC content of wheat may be due to varied phenolic profiling. Phenolic content of wheat flour showed a greater decrease during baking than WCF. Cookies made from 100% WCF (20.70%) showed lesser decrease in TPC than found in 100% WF cookies (51.39%) that suggests WC polyphenols show higher thermal stability than wheat polyphenols. It is worth mentioning here that WC has previously been reported to contain both the parent phenolic acid derivatives viz. hydrocinammic acid and hydroxybenzoic acid derivatives in the form of ferulic acid, *p*-coumaric acid and gallic acid respectively (Yu et al. 2013) unlike wheat flour that contains principally ferulic acid (Yu et al. 2013).

Total flavanoid content (TFC)

TFC of WF and WCF as well as their cookies is shown in Table 5. WCF had significantly higher flavanoid (1.92 g QE/1000 g) content than wheat flour (0.11 g QE/1000 g). TFC of both WF cookies (0.041 g QE/1000 g) and WCF cookies (1.25 g QE/1000 g) decreased significantly during baking. However, WF cookies showed a greater decrease in TFC content than WCF cookies. This can be attributed to the type of flavonoids present in WCF. One of the major flavonoids present in WCF was quercetin (Chiang et al. 2008). Elhamirad and Zamanipoor (2012) have reported Quercetin to have higher values of standard coefficient (Q_s), a measure of antioxidant activity at higher temperatures (140, 160, 180 °C). Thus, it can be suggested that an improved retention of antioxidant activity in water chestnut at higher temperatures can be due to hydroxylation of quercetin (B-ring). Elhamirad and Zamanipoor (2012) gave similar explanation for thermal stability of flavonoids in sheep tallow olien frying. Better retention of antioxidant activity due to hydroxylation of flavonoids is indirectly favored by the fact that WC flour has greater percentage of flavonoids than wheat flour.

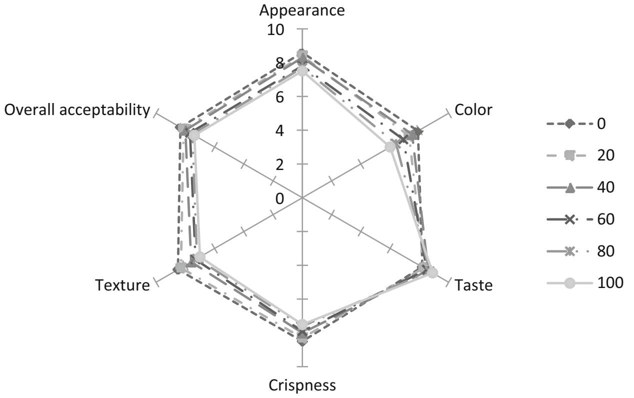

Fig. 2 Sensory characteristics of cookies made from different blends of wheat and water chestnut flour

Sensory evaluation

The effect of WCF incorporation on the sensory characteristics of cookies is presented in Fig. 2. Cookies made with 100% WF (control) were rated the highest score for appearance and color; however, appearance of 100% WCF cookies was statistically insignificant from those made from wheat flour blended with 20, 40 and 60% of WCF. Color variation in cookies varied significantly as level of blending with WCF reached 40% or higher. This can be due to varied protein content of WCF that affected generation of melanoidins and hence color of cookies. The taste/flavour sensory attribute is more important than appearance. As per the panelists, WCF on baking gave a pleasing nutty taste on account of which 100% WCF cookies received the highest scores for taste hence influencing scores for overall acceptability. Similar scores for taste were reported by Singh et al. (2011) and (Sarabhai and Prabhasankar 2015). Addition of WCF significantly decreased the crispiness of cookies from 8.5 to 7.9. On the basis of overall acceptability scores of WC cookie from different blends, there is a lot of scope to incorporate WCF in bakery products on industrial scale.

Conclusion

Water chestnut flour is a good option for production of low fat, gluten free flour with high mineral (P, K, Mg, Zn, Cu) and antioxidant content. Pasting properties of water chestnut flour especially low peak viscosity suggest its use in different product formulations such as weaning foods. Wheat flour can be completely replaced by water chestnut flour for production of gluten free cookies with improved taste and flavor. However, some modification such as incorporation of hydrocolloids or skimmed milk powder to improve texture and color of cookies is recommended. Water chestnut flour shows better retention of antioxidant properties than wheat flour during baking. Further research regarding antioxidant survival during processing and product development using water chestnut flour is strongly recommended.

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