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RESEARCH ARTICLE

NUTRITIONAL QUALITY OF ROSELLE SEED FLOUR COOKIES

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ABSTRACT

The Roselle seeds were procured from the local market. The following pre-treatments where adopted for Roselle seeds, Un-sprouted Whole Roselle Seeds Flour (UWRSF); Un-sprouted Decorticated Roselle Seed flour (UDRSF); Sprouted Whole Roselle Seed Flour (SWRSF); Sprouted Decorticated Roselle Seed Flour (SDRSF). These treated Roselle seed flour then used for preparation of cookies. From the preliminary studies 15 % inclusion as the best treatment of Roselle seed flour for cookie production was finalized. The results for cookie nutritional values shows an improvement in proximate composition and reduction in total carbohydrate due to the enhancement in cookie protein value with a health benefit of reducing diabetes due to reduced spike in glucose when cookie is consumed. There was also an improvement in macro minerals magnesium, calcium, potassium and phosphorus critical for the sustenance of body functions and overall health. In addition, there was an improvement in amino and fatty acid profile of the cookie product suggesting it could serve as a health food.

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INTRODUCTION

Roselle (*Hibiscus sabdariffa Linn.*) is a tropical plant belonging to the Family *Malvaceae* and widely cultivated for its jute like fiber in India, the East Indies, Nigeria and to some extent in tropical America (Yayock, 1988). A woody sub-shrub growing 7-8 feet (2-2.5m) tall, acting as annual or perennial, takes about six months to mature. The mature plants are highly drought resistant but may require water during dry periods when soil moisture is depleted to the point where wilting occurs. Roselle requires a chalky, loamy and peat-rich soil with pH of 7.6-9.0; and grows best in weakly alkaline soil (Myfolia, 2016). The lipid profile indicated Roselle seed oils are good sources of phospholipids, the levels of which compare favourably with that of Soybean oil (1.5 to 2.5%) (Gunstone, 2002). This high level of phospholipids may contribute to the stability and antioxidant activity of the oils. Antioxidant compounds are gaining importance due to their dual role in food and pharmaceutical industries as lipid stabilizers (Ramadan and Morsel, 2004). Nutritionally important antioxidants such as tocopherols improve the stability of oil. In a study, Roselle seed oil (RSO) and Roselle seed extract (RSE) was mixed with Sunflower oil, respectively to monitor degradation rate and investigate antioxidant activity during accelerated storage. The antioxidant activity was found to stabilize Sunflower oil of various samples and in the order of RSE > RSO > Tocopherol > Sunflower oil (Nyam *et al*., 2012). The proximate composition of whole Roselle seeds indicated that, seeds contained relatively high fat and protein (20.97% and 29.61% respectively). The physico-chemical parameters of crude oil extracted from Roselle seeds by soaking at room temperature (cold extraction) indicated the oils had 1.4674 refractive index; 0.078 (at 420nm) yellow-greenish colour, 0.78% acidity, 198.82 saponification value, 97.62 (g of I_2/100g oil) iodine value; 1.52% unsaponifiable matter; 4.82 (Meq 0_2/Kg oil) peroxide value; 6.21p-anisidine value; and 15.85 totox number. Gas Liquid Chromatography technique has been developed for identification and quantitative determination of total unsaturated and saturated fatty acids. This technology showed that Roselle crude oil had 73.40% unsaturated and 26.57% saturated fatty acids respectively. Major fatty acid found was oleic acid (38.46%) followed by linoleic (33.25%) and Stearic (5.79%). Stability of crude Roselle seed oil against oxidation during the accelerated storage of oil indicated that the crude oil induction period to be 10 days at 65^0C. The relatively high fat content of the seeds and high protein content of resulted meal beside the relatively high oxidation stability of Roselle suggest that Roselle seeds could be a novel and economic source of healthy edible fat and for other food industry applications.

Roselle plants are mostly used in the processing industry for extraction of fiber. Roselle seed is the byproducts of the Roselle processing industry (Bamgboye and Adejumo, 2009).

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This unwanted byproduct can be recycled as value added food supplements, as it provides advantageous bio-active compounds, good source of edible oil and proteins (Bertagnolli *et al*., 2014; Nyam *et al*., 2012; Karma and Chavan, 2016). Roselle seeds can be ground into fine flour and used for enriching other cereals such as wheat in value added products. The wheat (*Triticum spp.*) is grown on more land area than any other commercial crop (USEA, 2016). World trade in wheat is greater than all other crops combined (Curtis *et al*., 2002). Blending Roselle seed flour with wheat flour in value added products will greatly popularize Roselle seeds, curbing it wastages especially in the tropics and also exposing its rich nutritional potentials for the overall health benefits to mankind. Protein fractions, proteins isolates or concentrates obtained from Roselle seeds might be an alternative source of low cost protein substitute in dietary supplement in ingredients for food industry. This may reduce the heavy dependence on conventional sources such as animal, fish and soybean proteins. At present, there are very few reports on harnessing the bio-nutritional potential of Roselle seeds in value added products (Nyam *et al.,* 2014). Adding cereals with complementary nutritive profiles, such as Roselle seeds, may yield a more complete enrich food source (Okafor *et al.,* 2002; Arshad *et al*., 2007; Bala *et al*., 2015; Wani *et al.,* 2015). Combining the nutritional value of wheat and Roselle seeds in *composite formulations* may yield good quality food products with excellent nutritive qualities. Hence, the aim of this study was to investigate the bio-nutritional viability of Roselle seeds for the benefit of human race with production of Roselle seed flour cookies.

MATERIALS AND METHODS

Raw Materials Collection

Roselle (*Hibiscus sabdariffa Linn.*) and Wheat (*Triticum aestivum spp.*) seeds were both sourced from vegetable markets in Ahmednagar, Maharashtra State, India and both were of local varieties. The various ingredients: Margarine, granulated sugar, salt, sodium bi carbonate and ammonium bicarbonate used for baking were all provided from the pilot bakery unit of the Department of Food Science and Technology, Mahatma Phule Agricultural University, Rahuri

Germination

The cleaned seeds were soaked for 6 hours to activate the process of germination, after which the seeds were washed and allowed to drain. The drained seeds were then spread on a damped cloth in a perforated container with water sprinkled occasionally in a dark room to activate germination for a another 12 hour period; then gently washed and spread sparsely to dry under fan at ambient temperature to preserve its nutritive value, packed in a HDPE bag and stored in a cool dry place until used.

Preliminary Studies

To ascertain the best treatment of Roselle seeds to be selected for composite *Roselle-Wheat Flour* formulation in cookie production for an enhanced nutrition and functional properties of the value added product, the following preliminary studies on pre-treatments where adopted for Roselle seeds.

- Un-sprouted Whole Roselle Seeds Flour (UWRSF), as *Control*
- Un-sprouted Decorticated Roselle Seed flour (UDRSF)
- Sprouted Whole Roselle Seed Flour (SWRSF)
- Sprouted Decorticated Roselle Seed Flour (SDRSF)

The pre-treatments were prepared accordingly: both cleaned raw and germinated (sprouted) seeds were divided into two portion each, the first portion was grind whole with a laboratory scale hammer mill and the resulting powder sieved through a 60 mesh screen filter until a fine whole seed powder was obtained; the second portion was coarse grind with sieving intermittently to separate out the bran to obtain a decorticated flour then further grind to a fine mix. The resulting whole and decorticated cleaned (raw and sprouted) seed flour fractions obtained were packed separately in a HDPE bag and stored in a cool dry place until used.

Cookie Preparation

The *molded cookie* was adopted for this study owing to its simplicity Wikipedia (2016). The Cookie was prepared according to proposed method with modification; using basic ingredients (Wani *et al.,* 2015) to simplify critical investigations.

Ingredients formulation

Ingredients and composition for composite flour for cookies

Ingredients (at 100 g basis)	Control	Flour Replacement			
		10%	15%	20%	25%
Flour	100	10:90	15:85	20:80	25:75
Sugar	40	40	40	40	40
Margarine	50	50	50	50	50
Sodium bicarbonate	0.5	0.5	0.5	0.5	0.5
Ammonium bicarbonate	0.5	0.5	0.5	0.5	0.5
Water	20	20	20	20	20
Salt	1	1	1	1	1

Proximate and inorganic mineral composition

The proximate analysis of Roselle seed flour treatments and cookies were determined according to AACC (2000). The moisture content was determined by AACC method 08-01, crude protein by Kjeldahl's AACC method 44-15A, lipids content by AACC method 46-13, dry ashing by AACC method 30-25 and gravimetric method AACC 30-25 were used respectively. Total carbohydrates was estimated by difference and calculated as 100% - [% (Moisture + Fat + Ash + Protein)].

Inorganic Mineral Analysis

For exploring the potentials of Roselle seeds, their mineral composition was determined by using the following methods. Macro elements: Calcium (Ca) and magnesium (Mg) where both determined using complexometric titration with potenciometric indicator method as described by El Mahi *et al.* (1987); potassium (K) was determined by using method described by Knudsen and Peterson (1982); and phosphorus (P) was determined by the method described by Jackson (1973).

Amino acid profile analysis

This particular parameter was outsourced to SGS India Pvt. Ltd., Agricultural Services Laboratory, Thane Maharashtra State, due to lack of facilities in the university to run the sample amino acid profile analysis.

In house methods: SO-IN-MUL-TE-027 and SO-IN-MUL-TE-039 Job Number: CG16-009612 SGS India Pvt. Ltd (2016).

Fatty acid profile analysis

Fatty acid profile analysis of Roselle seed and prepared product oil extracts where analyzed by gas-liquid chromatography using flame ionization detector. The GC was outsourced.

Cost of production of cookies

The expenses on raw materials, fixed cost of the equipment, labour charges and other items were taken into account for calculating cost of production of Roselle seed flour at prevailing rates during experimental period; it was worked out using standard economic procedure (Lal *et al.*, 1980).

Statistical analysis

All experiments were carried out in such a way that the degree of freedom remains more than 12 with suitable replications and treatments. Results obtained in the present study were analyzed by Completely Randomized Design (CRD) design as given by Panse and Sukhatme (1967).

RESULTS AND DISCUSSION

Proximate and inorganic mineral composition of cookies

The result of physical and sensory evaluation, colourimetry and texture analysis coincidentally in this study preferentially adjudged 15 % inclusion as the optimum level for un-sprouted decorticated and sprouted decorticated flour and also Roselle seed oil replacing margarine as the best in the cookie treatments.

Amino acid profile of Roselle seed flour and cookie products

Roselle seeds have been reported to be rich in protein (Ismail *et al.,* 2008; El-Adawy and Khalil 1994; Al-Wandawi *et al.,* 1984). Protein plays a crucial role in almost all biological processes and amino acids are the building blocks of it, and Roselle seeds contains essentially eighteen (18) of amino acids (Emmy *et al.* (2008a) and Tim, 2014). Table 3 shows amino acid profiles of Roselle seed flour (sprouted and un-spouted) and cookie products made from same. The estimated values of Roselle seed from the test sample falls within the overall ranges reported by Mohammed *et al.* (2007), Rao (1996), El-Adawy and Khalil (1994) and FAO/WHO (1975). The analysis for Roselle seed flour Table 3 showed that glutamic acid (21.08, 20.34 g/16gN) was the most abundant followed by arginine (10.89, 10.17 g/16gN), aspartic acid (11.33, 12.89 g/16gN), leucine (6.97, 6.95 g/16gN), proline (5.44, 5.29 g/16gN), serine (5.09, 5.44 g/16gN), it was observed that sprouted decorticated Roselle seed flour (SDRSF) had more level of amino acid contents than the whole seed flour. These results suggested that proteins in Roselle seeds are concentrated in the cotyledon. Also, Table 3 showed that there was an appreciable increase in the overall amino acid content in the cookie products when compared to control sample after inclusion of 15 % Roselle seed flour in the composite flour blend.

Fatty acid profile of oil extracts from Roselle seeds and cookie

The fatty acid profile of oil extracted from Roselle seed and cookie products (Table 4) which showed substantial and significant ($p<0.05$) lipid contents. The ratio of saturated to unsaturated fatty acids in whole Roselle seed and sprouted seed oil were approximately 1:3.

Table 1. Proximate analysis (% db) of cookies incorporated with de-corticated Roselle seed flour

Treatments	Moisture	Protein	Lipid	Ash	Crude Fiber	Total Carbohydrate
Wheat Flour (Control)	2.30	12.54	31.87	1.55	0.50	53.54
UDRSF (15-85 %)	2.99	21.44	32.48	1.85	1.23	43.0
SDRSF (15-85 %)	2.00	21.13	34.51	1.98	1.10	41.28
RSO (15-35%)	2.20	12.19	29.71	1.65	0.35	53.90
S.E.(±)	0.12	2.09	0.08	0.09	0.06	0.10
CD at 5%	0.35	6.30	0.24	0.26	0.19	0.31
CV (%)	8.48	21.52	0.43	8.45	13.61	0.37

Each value is an average of six determinations on a dry weight basis (db). UDRSF = Un-sprouted Decorticated Roselle Seed flour; SDRSF = Sprouted Decorticated Roselle Seed Flour; RSO = Roselle seed oil.

Table 2: Effect of inclusion of Roselle seed flour on mineral composition of cookie (mg/100g)

Treatments	Calcium (Ca)	Magnesium (Mg)	Phosphorus (P)	Potassium (K)
Control (100 % Wheat)	29.97	118.86	278.30	337.50
UDRSF 15-85 %	86.97	169.97	328.67	548.38
SDRSF 15-85 %	87.13	171.86	331.49	553.76
RSO 15-35 %	28.74	114.64	284.54	302.50
S.E.(±)	1.61	1.82	7.86	3.84
CD at 5%	4.84	5.49	23.68	11.58
CV (%)	4.78	2.19	4.45	1.53

Each value is an average of six determinations on a dry weight basis (db). UDRSF = Un-sprouted Decorticated Roselle Seed flour; SDRSF = Sprouted Decorticated Roselle Seed Flour; RSO = Roselle seed oil.

The nutritional and mineral analysis of cookies presented in Table 1 and 2 showed that inclusion of 15 % Roselle seed flour significantly ($p<0.05$) improved the proximate and mineral composition of the cookie products when compared to control.

Mohiuddin and Zaidi (1975) reported same; cookie RSO with margarine replaced by 15 % also showed improved ratio approximately 1:1 (1: 0.96), followed by SDRSF (1: 0.74) and UDRSF (1: 0.73) with control (1: 0.69) having the least (Table 4).

Table 3. Amino acid profile of Roselle seed flour and cookie (g/16gN)

Parameter	Wheat (Control)	Roselle Seed Flour		Cookie	
		SDRSF	UWRSF	UDRSF 15-85%	SDRSF 15-85 %
Phenylalanine	4.32	4.62	4.63	4.33	4.27
Valine	4.17	4.39	4.53	3.79	4.14
Threonine	2.88	3.34	3.52	2.98	2.98
Tryptophan	1.87	1.75	0.70	4.19	1.42
Isoleucine	2.88	2.87	2.87	2.84	2.85
Methionine	0.72	0.54	0.15	0.68	0.65
Histidine	2.30	2.48	3.02	2.44	2.33
Leucine	6.33	6.97	6.95	5.95	6.47
Lysine	2.73	4.97	4.93	3.11	3.49
Arginine	3.88	10.89	10.17	5.41	5.43
cysteine	6.62	2.58	2.37	7.04	6.60
Glycine	4.17	4.81	5.89	4.47	4.40
Glutamic acid	30.07	21.08	20.34	26.37	27.68
Proline	11.94	5.44	5.29	9.34	9.96
Tyrosine	1.15	2.64	2.17	1.22	1.29
Alanine	3.45	4.20	4.13	3.52	3.75
Aspartic acid	5.77	11.33	12.89	7.31	7.50
Serine	4.75	5.09	5.44	5.01	4.79

*Results of single determinations. SDRSF = Sprouted Decorticated Roselle Seed flour; UWRSF = Un-sprouted whole Roselle seed flour. UDRSF = Un-sprouted Decorticated Roselle Seed flour.

Table 4. Fatty acid profile of Roselle seeds and cookie oil extract (%).

Fatty Acids (g/100g)	Roselle Seed Oil		Oil Extract from Cookies			
	Raw Seed Oil	Sprouted Seed Oil	Control 100 %	SDRSF 15-85%	UDRSF 15-85 %	RSO 15-35 %
Lauric acid (C12:0)	0.01	0.01	0.21	0.15	0.16	1.14
Myristic acid (C14:0)	0.20	0.20	1.07	0.87	0.93	0.85
Palmitic acid (C16:0)	21.45	21.41	50.88	49.36	49.78	44.12
Stearic acid (C18:0)	3.94	3.87	5.52	6.13	5.87	5.25
Arachidic acid (C20:0)	0.59	0.56	0.39	0.49	0.45	0.45
Eicosenoic acid (C20:0)	0.01	0.10	0.18	0.16	0.17	0.15
Behenic acid (C22:0)	0.25	0.25	0.08	0.11	0.10	0.13
Lignoceric acid (C24:0)	0.14	0.15	0.08	0.11	0.10	0.11
Total Saturated Fatty Acid	26.2	26.55	58.41	57.38	57.56	52.2
Palmitoleic acid (C16:1)	0.44	0.43	0.12	0.13	0.14	0.21
Oleic acid (C18:2n6c)	31.22	31.99	31.71	32.64	32.08	33.23
Linoleic acid (C18:2n6c)	41.18	40.07	7.72	9.23	9.57	16.32
Gamma linolenic acid (C18:2n6)	0.01	0.01	0.03	0.04	0.04	0.05
Alpha linolenic acid (C18:3n3)	0.27	0.52	0.16	0.20	0.22	0.26
Cis-11,14-eicosadienoic acid (C20:2)	0.01	0.01	0.07	0.08	0.08	0.09
Total Unsaturated Fatty Acid	73.13	73.03	39.81	42.32	42.13	50.16
Lipid Dietary Profile Ratio	1: 2.79	1: 2.75	1: 0.69	1: 0.74	1: 0.73	1: 0.96

*Results of single determinations. UDRSF = Un-sprouted Decorticated Roselle Seed flour; SDRSF = Sprouted Decorticated Roselle Seed Flour; RSO = Roselle seed oil.

Table 5. Cost of cookie production with Roselle seed flour

S/N	Recipe		Rate	Cost (Rs)
1	85 g	Wheat Flour	Rs. 32/Kg	2.72
2	15 g	Roselle Seed Flour	Rs. 50/Kg	0.75
3	40 g	Sugar	Rs. 40/Kg	1.60
4	50 g	Margarine	Rs. 55/Kg	2.75
5	18 mL	Water	Rs. 20/Lit	0.36
6	0.5 g	Sodium bicarbonate	Rs. 35/50 g	0.35
7	0.5 g	Ammonium bicarbonate	Rs. 30/50 g	0.30
8	1 g	Salt	Rs. 17/ Kg	0.02
Total	210 g		8.85	
9	Total raw material	210 g		
10	Total Weight of Cookies Product	144.66 g		
11	Losses	65.34 g		
12	10 % Price Changes			0.89
13	40 % Overhead cost:	Energy: Labour, electricity, equipment, Packaging materials and miscellaneous		3.54
14	Total weight of cookies produced	144.66 g	Total Cost	13.28
15	Cost of producing 1 Kg of Cookie			91.80
16	20 % Profit margin			18.36
17	Selling Price of Cookies (INR/Kg)			110.16

The results for raw Roselle seeds oil corroborate with Mohiuddin and Zaidi (1975); Tounkara *et al.* (2011) for both raw seed and sprouted seed oil. The major fatty acid found for raw and sprouted seed oil were linoleic acid (41.18; 40.07%) followed by oleic acid (31.22; 31.99 %), palmitic acid (21.45; 21.41 %) and stearic acid (3.94; 3.87 %) respectively; the results obtained in this research findings are in close harmony with El-Adawy and Khalil (1994); Emmy *et al.* (2008b); and Cissouma *et al.* (2013). The Roselle seed oil can be classified as belonging to linoleic-oleic category as the most abundant unsaturated fatty acids present, suggesting it beneficial use in lowering blood pressure and serum cholesterol (Savage, 2001; Enujiugba and Akanbi, 2008; Tounkara *et al.,* 2011 and Cissouma *et al*., 2013).

Cookie production and cost assessment

The results of preliminary investigations on flour of Roselle seed pre-treatments suggested sprouted decorticated and un-sprouted decorticated flour had ranked higher in nutritional and functional properties thus further studies alongside replacing margarine with Roselle seed oil in cookie production to adjudge the best level of inclusion was studied. The costs of the cookie production are outlined in the Table 5, the parameters were estimated at 100 percent basis and finally expressed as cost per Kilogram (Kg) weight of the final cookie products: for control sample (100% wheat flour), both sprouted (SD) and un-sprouted decorticated (UD) Roselle seed flour and Roselle seed oil, at 15 % inclusion. The results with substitution of margarine at 15 % inclusion with Roselle seed oil (RSO) in cookie formulation gave higher cost compare with margarine alone (Rs. 117.58/kg and Rs. 110.16/kg, respectively). The higher cost for Roselle seed oil could be the high cost of Roselle seed oil compared to the margarine in cookie formulation (Table 5). Hence the need to commercialize the oil extraction of Roselle seed oil for industrial application is necessary.

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