



Effect of Storage on Sensory Acceptability and Oxidative Rancidity of Wheat Biscuits Fortified with *Asparagus racemosus* Root Powder

Priyanka Rani¹, Varsha Rani^{1*}, Renuka Jandu¹, A. Lavanya¹, Reena¹
and Josephine John¹

¹Department of Foods and Nutrition, Chaudhary Charan Singh Haryana Agricultural University, Hisar 125001, Haryana, India.

Authors' contributions

This research work and manuscript writing was done with an effective collaboration of all authors. Authors PR and VR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors PR, RJ and AL managed the analyses of the study. Authors JJ and Reena managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: *Asparagus racemosus* is locally known as *shatavari* in India and possess a strong antioxidant and galactogogue activities. This study was aimed to reduce the bitterness of *Asparagus racemosus* root powder (ARRP) and to analyse the effect of storage on acceptability and oxidative rancidity of ARRP fortified biscuits.

Study Design: Biscuits were developed substituting whole wheat flour with 5, 7.5 and 10 per cent of ARRP.

Methodology: ARR were blanched to eliminate the bitterness in developed powder. Developed biscuits were analyzed for sensory characteristics using 9-point hedonic scale by 25 semi-trained panelists. Biscuits were packed in plastic zipper bags and stored in an airtight plastic container at room temperature for 90 days. Effect of storage period on sensory acceptability, fat acidity and peroxide value of biscuits was observed.

*Corresponding author: Email: varshadangi@hau.ac.in;

Results: Blanching of *A. racemosus* roots at 80°C temperature for 3 minutes reduced the bitterness of developed powder considerably. Results showed that biscuits were found to be 'liked moderately' on 9-point hedonic scale. During storage period of 90 days, the scores of sensory characteristics were decreased gradually for colour, appearance, aroma, taste, texture and overall acceptability, however biscuits were found acceptable by panellist. The increase in oxidative rancidity with the advancement of storage period was observed in terms of fat acidity (mg of KOH/g) and peroxide value (meq of O₂/kg) in control as well as ARRP biscuits however, this increase was witnessed less in *A. racemosus* fortified biscuits than control.

Conclusion: ARRP upto 10 per cent can be successfully used in the development of products with increased shelf life along with galactogogue activity.

Keywords: *Asparagus racemosus*; sensory acceptability; biscuits; storage; oxidative rancidity.

1. INTRODUCTION

The medicinal and nutritional value of any conventional food item can be increased by incorporating functional ingredient such as *Asparagus racemosus* and thus a conventional food item can be tailored into a functional food. *A. racemosus* has been commonly known as *Shatavari* in India, a nature's boon to cure all the ailments of female reproduction system [1-3]. From nutritional point of view, *A. racemosus* root powder (ARRP) has been found rich in iron, potassium, vitamin A, folate and glutathione. It contained high amount of iron (38 mg/100 g) and very low contents of phytate and tannin, the well-known iron inhibitors. However, it lacks in iron absorption-promoting activity [4] and that may be achieved if it is incorporated with basic ingredients having high iron absorption promoting activity. *A. racemosus* possesses a strong antioxidant activity and hence acts as anti-ageing which protects cells against toxins such as free radicals. The possible antioxidant effects of crude extract and polysaccharides fraction of *A. racemosus* against membrane damage induced by the free radicals generated during γ -radiation were examined in rat liver mitochondria [5]. The antioxidant effect of polysaccharides fraction even at a very low concentration was more pronounced against lipid per oxidation, while that of crude extract was more effective in preventing protein oxidation. Further, the possibility of utilization of *A. racemosus* as a novel natural preservative in meat products and *burfi*, an Indian sweet was explored. Products containing *A. racemosus* in comparison to control indicated a significant effect on the lipid oxidative stability and storage quality without compromising the sensorial characteristics. It has a great potential as a natural preservative [6,7]. Unfortunately, *A. racemosus* dried roots are endowed with a bitter after taste which may be reduced though appropriate processing technique. A variety of products such as

functional bread, multigrain herbal biscuits, *burfi*, *cheela*, *laddoo* and herbal aonla *laddoo* have been developed using *A. Racemosus* powder in varying concentrations of 1 to 15 per cent [7-12].

The roots of *A. racemosus* have been extensively used in the treatment of female oriented ailments such as irregular menstruation cycle, anaemia, lactation failure and menopausal syndrome. *A. racemosus* has repeatedly been shown galactogogue activity in Ayurvedic literature and also has been confirmed through a number of animal as well as human trials. In a double-blind randomized clinical trial, the galactogogue property of *A. racemosus* was analysed in 60 lactating mothers by measuring the changes in their prolactin hormone level [13] and a significant increase in prolactin authenticated its galactogogue property.

Not only extended shelf-life of biscuit endorses large scale production rather its good taste and diverse nature make it more popular among people. Biscuits are convenient, ready to eat, liked and appreciated by everyone, inexpensive and have been considered as important snacks. Due to their wide acceptability, biscuit serves the best vehicle for food fortification [14-16]. Lactating women prefer soft diet especially during first six months of postpartum and therefore biscuits along with milk or tea become a good choice for them. Despite of its high medicinal value and rich antioxidants profile *A. racemosus* has been neglected for its use for human consumption. Hence, keeping in view the copious antioxidant and galactogogue activities of *A. Racemosus* efforts have been done to develop biscuits incorporated with ARRP for lactating women.

2. MATERIALS AND METHODS

This study was carried out in the Department of Foods and Nutrition, Chaudhary Charan Singh

Haryana Agricultural University (CCSHAU), Hisar. *A. racemosus* (*Shatavari*) roots were procured in a single lot from Medicinal, Aromatic and Underutilized Plants Section, Department of Genetics and Plant Breeding, CCSHAU, Hisar during the month of February, 2018.

2.1 Preparation of *A. racemosus* Root Powder

Fresh *shatavari* roots were washed with tap water, cleaned and rinsed again with distilled water and divided into two lots. One lot was dried under shade for 6 hrs. to evaporate the excessive moisture, dried in the hot air oven at $50\pm 5^{\circ}\text{C}$ for 6 hrs. The dried roots were grinded in electric grinder and sieved through 60 mesh sieve; stored in low density polyethylene (LDPE) bags till its further use for analyzing saponin content. The second lot of roots was blanched in water (1:5 w/v) at 80°C for 3 minutes followed by immediate wash with cool distilled water. After blanching roots were treated in the similar way to get the powder.

2.2 Chemical Analysis

Raw and blanched *A. racemosus* root powder was analysed for saponin and antioxidant activity. The samples for antioxidant activity were extracted by the method of Serrano et al. [17] and the extracts, on the basis of the scavenging activity of the stable DPPH free radical, was determined by the modified method given by Brand-Williams et al. [18] and Tadhani et al. [19]. Quantitative determination of saponin was carried out using the method reported by Ejikeme et al. [20] and Obadoni and Ochuko [21].

2.3 Preparation of *A. racemosus* Root Powder Biscuits

The basic ingredient for control biscuits was whole wheat flour (100 g) containing 10 per cent of protein which was replaced with 5, 7.5 and 10 per cent of ARRP in experimental biscuits. This level of incorporation of ARRP was finalized on the basis of its level of incorporation in various products quoted in previous studies [7-12] and also the standardization of biscuits before the commencement of actual study was done where level beyond 10 percent imparted a bitter after taste. Other ingredients used in the preparation were as fully hydrogenated vegetable fat, baking

powder, sugar, ammonia, whole buffalo milk and crushed corn flakes (Table 1). The flour was sieved with baking powder twice. Ghee was creamed until light fluffy and then sugar was mixed. The sieved flour was added to ghee and sugar mixture to prepare the dough. Small round balls were made, put into baking tray and pressed slightly. Biscuits were baked at 160°C for 25 minutes in a preheated oven.

2.4 Sensory Evaluation of Developed Biscuits

The developed biscuits were analyzed for their sensory characteristics with respect to color, appearance, aroma, texture, taste and overall acceptability using 9-point hedonic scale by 25 semi-trained panelists. Where, acceptability was expressed as liked extremely, liked very much, liked moderately, liked slightly, neither liked nor disliked, disliked slightly, disliked moderately, disliked very much and disliked extremely from 9 to 1 point, respectively. Biscuits were packed in the plastic zipper bag and stored in an airtight plastic container at room temperature for 90 days (August- October, 2018). Effect of storage period on sensory acceptability, fat acidity and peroxide value of biscuits was observed.

2.5 Evaluation of Oxidative Rancidity during Storage

The fat acidity was determined by the standard method of analysis AOAC [22]. Fat acidity was calculated as mg of potassium hydroxide required to neutralize free fatty acids of 100g of flour. Peroxide value of stored products was determined by the method of AOAC [22]. Five gram sample was taken in conical flask. Thirty ml acetic acid-chloroform mixture was added to the flask and swirled to dissolve. Then 0.5 ml saturated potassium iodide solution was added, kept for one minute with occasional shaking and 30 ml distilled water was added. This was slowly titrated against 0.01 N sodium thiosulphate with vigorous shaking until yellow colour almost disappeared. Then 0.05 ml starch solution was added and titration continued with shaking vigorously to release all iodine from chloroform layer until blue colour just disappeared. The blank was run in the similar way. Peroxide value was calculated as milli equivalent peroxide per 1000 g of biscuits.

Table 1. Formulation of control and experimental biscuits

Ingredients	Control	ARRP1	ARRP2	ARRP3
Whole wheat flour	100	95	92.5	90
A. racemosus root powder	-	5	7.5	10
Fat	40	40	40	40
Sugar	60	60	60	60
Baking powder	1/4 th tea spoon	1/4 th tea spoon	1/4 th tea spoon	1/4 th tea spoon
Ammonia	A pinch	A pinch	A pinch	A pinch
Whole buffalo milk	40 ml	40 ml	40 ml	40 ml
Corn flakes	10g	10g	10g	10g

2.6 Statistical Analysis

Data were statistically analyzed using SPSS software. Means and standard error of sensory scores were calculated using descriptive analysis. Two sample t-test was applied to compare the saponin and antioxidant content between raw and blanched *shatavari* root powder. Standard errors of means were used to state the difference within the sample. The effect of storage period and level of incorporation on sensory acceptability and oxidative rancidity of developed products was compared using two-way ANOVA test.

3. RESULTS AND DISCUSSION

3.1 Effect of Blanching on Saponins and Antioxidant Activity

Blanching of *A. racemosus* roots at 80°C temperature for 3 minutes reduced the content of saponins significantly and imparted a mild sweet taste in developed powder. Saponins have been considered the major bitterness contributing factor in *A. racemosus*. Blanching had also reduced the radical scavenging activity, though it retained in substantial amount in developed powder (Table 2). The fresh roots of *A. racemosus* were slightly sweet in taste due

the low concentration of saponins, whereas, its powder had bitter taste as the concentration of bitter saponins and other alkaloids become high in dried roots [23-25].

Though the exact mechanism of decreasing the bitterness upon blanching is not known, however, blanching has reduced the contents of saponins in *A. racemosus* roots (Table 1). Therefore, we hypothesized that the contents of alkaloids and other constituents contributing bitterness may also get reduced during blanching. A similar decrease by 24.42 per cent in saponins content in *A. racemosus* roots upon blanching was observed by Saini et al. [7]. *A. racemosus* has more than 50 organic chemical compounds of different groups such as steroidal saponins, glycosides, alkaloids, polysaccharides, mucilage, *racemosol* and *isoflavones* that possess a wide range of medicinal properties [26]. All the non-nutritive but healthy native phyto-chemicals of ARRPs such as alkaloid, steroidal saponin and terpenoid, except the flavonoids, were found present in substantial amounts in functional bread developed even with a low (3.5%) level of incorporation [9]. In the present study, the acceptable level of incorporation was almost three times higher (10 per cent) and thus the presence of all phyto-chemicals in developed biscuits may be postulated.

Table 2. Effect of blanching on saponins and antioxidant activity of *A. racemosus* root powder (% , on dry weight basis)

Treatment	Saponins	DPPH RSA
Raw	5.86±0.02	76.57±0.57
Blanched	5.43±0.02	72.26±0.59
t-value (p=0.05)	13.88**	5.21**

Values are Mean ±SE of three independent determinations

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

3.2 Sensory Evaluation of Developed Biscuits

The mean sensory scores given to control biscuits developed using 100 per cent whole wheat flour were ranged from 7.90 to 8.40 for color, appearance, aroma, texture, taste and overall acceptability and these biscuits were adjudged as 'liked very much'. With the increased level of fortification (5, 7.5 and 10 %) of *A. racemosus* (*shatavari*) root powder in biscuits, a slight decrease in the scores was observed however, this decrease was not significant. The scores given to overall acceptability of *A. racemosus* incorporated biscuits ranged from 7.81 to 7.98 and as a result, these biscuits were adjudged as 'liked moderately' (Table 3). In a previous study multi-grain herbal biscuits were developed by incorporating 2, 4 and 6 per cent of ARR. Aroma, texture, taste and color of biscuits developed using 4 per cent *A. racemosus* obtained highest score (8.00) with overall acceptability of 7.85. They observed that the taste of *A. racemosus* incorporated biscuits was preferred over control biscuits by the judges. They further observed that level beyond 4 per cent of ARR was not acceptable as it imparted a slightly bitter after taste [8]. Singh et al. [9] developed functional bread by substituting refined flour with *A. racemosus* root powder (1-6 %). They also observed that due to the slightly bitter taste of ARR it could not be possible to incorporate more than 3.5 per cent level in the development of functional bread. In the present study, *A. racemosus* roots were blanched at 80°C for 3 minutes, which improved the taste of dry root powder and that make it possible to incorporate upto 10 per cent level in the development of biscuits without giving an after taste of bitterness. During lactation, women are generally advised to consume *A. racemosus* (*Shatavari*) granules to improve milk production.

With the similar objective Malgi et al. [10] prepared *laddoo*, a traditional Indian sweet fortified with *A. racemosus* (*Shatavari*) root powder up to 15 per cent. They roasted ARR along with other ingredients as a part of the step involved in *laddoo* preparation that might have reduced the bitterness of ARR. In the present study, biscuits were chosen as a suitable vehicle to deliver the benefits of ARR to lactating mothers as these are convenient, ready to eat, inexpensive and easily acceptable by postpartum lactating women.

3.3 Effect of Storage Period on Sensory Acceptability

Stored products were studied for sensory characteristics at an interval of 15 days up to 90 days by a panel of 25 semi-trained judges using the nine-point hedonic scale. The results of the sensory evaluation of stored biscuits have been presented in Fig. 1. The mean scores for sensory parameters of control (whole wheat flour) and *A. racemosus* fortified (@ 5, 7.5 & 10%) biscuits declined gradually during the storage period. However, it was observed that this decline was more in control biscuits than *A. racemosus* fortified biscuits. It was further observed that in both types of biscuits the most affected sensory parameters were aroma and taste whereas the least affected parameters were color and appearance.

As per the mean sensory scores given to color, appearance, texture, taste, aroma and overall acceptability, control biscuits were adjudged as 'liked very much' at zero day which was slightly degraded to 'liked moderately' at the end of storage period (90th day). On the other hand, *A. racemosus* fortified biscuits were adjudged as 'liked moderately' at zero day and remained within the same category at the end of the storage period (90th day).

Table 3. Sensory acceptability of fresh sweet biscuits developed using *A. racemosus* root powder

Treatment	Color	Appearance	Aroma	Texture	Taste	OAA
Control (WF:100)	8.20±0.00	7.90±0.10	8.00±0.00	8.40±0.16	8.00±0.13	8.10±0.06
ARRP ₁	8.10±0.00	7.90±0.13	7.80±0.13	8.35±0.22	7.75±0.16	7.98±0.08
ARRP ₂	8.10±0.11	7.80±0.17	7.70±0.15	8.30±0.15	7.65±0.16	7.91±0.08
ARRP ₃	8.00±0.17	7.70±0.26	7.60±0.29	8.20±0.20	7.55±0.21	7.81±0.15
CD(p=0.05)	0.29	0.50	0.51	0.42	0.62	0.29

Values are mean ± SE of responses from a panel of 25 judges

Biscuits containing wheat- *A. racemosus* root powder 95:5(ARRP1), wheat-*A. racemosus* root powder 92.5:7.5(ARRP2) and wheat-*A. racemosus* root powder 90:10(ARRP3); WF= wheat flour

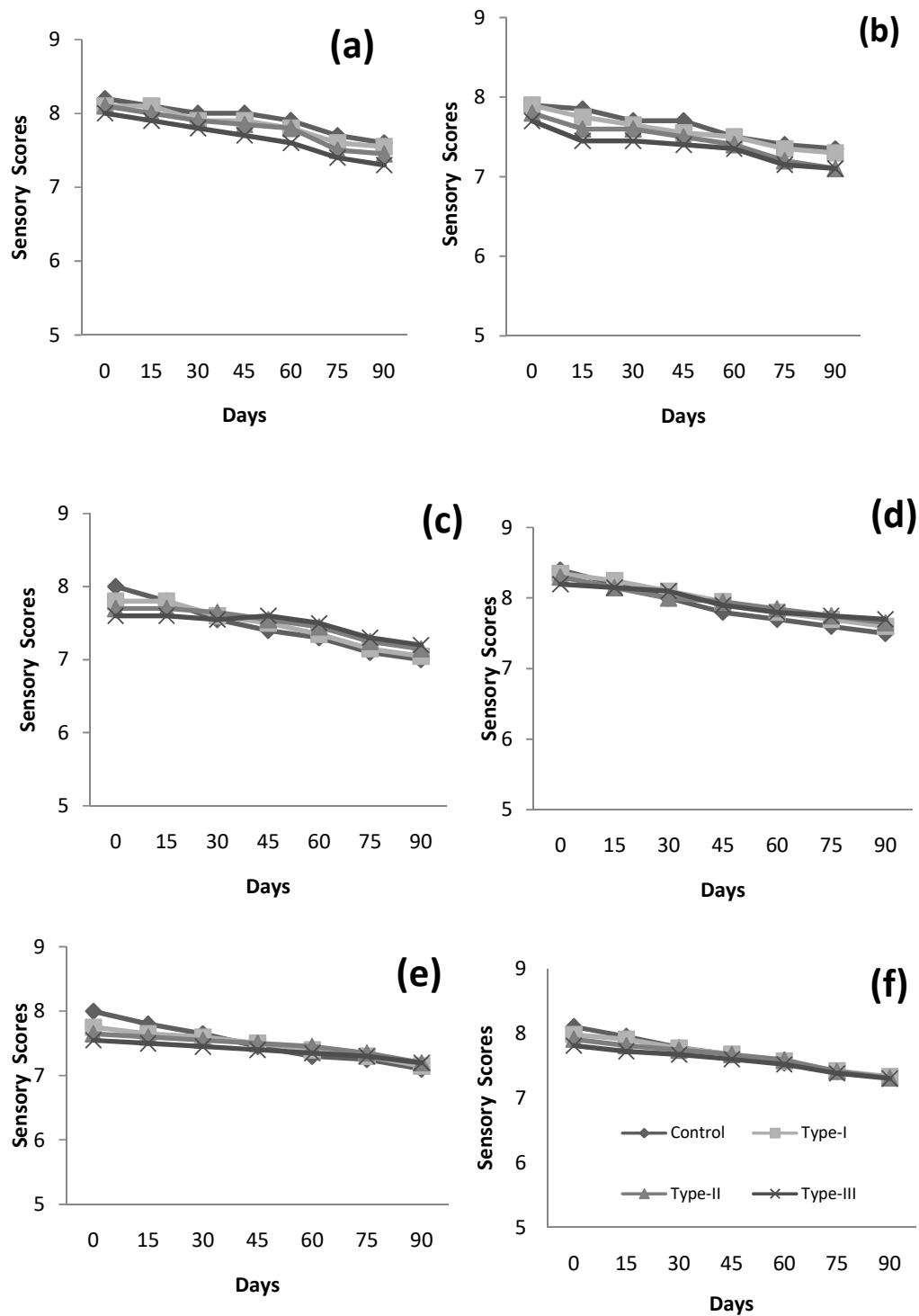


Fig. 1. Effect of storage period on sensory acceptability of biscuits developed using *A. racemosus* root powder (a) color (b) appearance (c) aroma (d) texture (e) taste (f) overall acceptability

Previous researches also reported similar findings regarding effect of storage on sensory scores of stored products, which were found in agreement of those obtained in present study [6,11,12,27, 28].

3.4 Effect of Storage Period and *A. racemosus* Fortification Level on Oxidative Rancidity

Fat acidity of fresh control biscuit at zero days was 0.35 mg KOH/g fat and in *A. racemosus* fortified biscuits, it ranged from 0.34 to 0.35 mg KOH/g fat. It was observed that the fat acidity increased gradually with increase in storage period, in all four types of biscuits (Table 4). However, it was noticed that this increase was higher in control biscuits than *A. racemosus* fortified biscuits. At the end of storage period (90 days), fat acidity in control biscuit was 0.75 mg KOH/g, while in fortified biscuits it ranged from 0.57 to 0.64 mg KOH/g. At the storage period of 75 and 90 days, the ARR_{P3} biscuits fortified with 10 per cent ARR_P had significantly lower fat acidity than control biscuits. The fat acidity of all types of biscuits including control was found within the acceptable level during the whole storage period.

3.5 Peroxide Value (PV)

A non-significant difference was observed in the peroxide value of different types of biscuits at zero day. Control and fortified biscuits had a PV of 0.30 and 0.22 to 0.28 meq/kg fat, respectively on zero day of storage. Significant (P=0.05) increase was observed in PV of biscuits with the progression of the storage period; however control biscuits had highest PV (5.94 meq/kg) at the end of the storage on 90 days (Table 5). The PV of fortified biscuits at 90 days ranged from 4.76 to 5.52 meq/kg fat which was found lowest in biscuits fortified with 10 per cent ARR_P. The PV of all the biscuits including control was also found within the acceptable level during the whole storage period.

During storage, fat oxidation has been one of the major reasons for the development of rancidity in food items. The rate of fat oxidation is influenced by storage conditions such as light exposure, moisture content, temperature and oxygen availability. Natural antioxidants play significant role in prevention of auto oxidation in fats, oils and fat containing food products and therefore, have gained considerable attention in recent years [29]. Jeyasanta et al. [30] reported that peroxide values in biscuit fat were within the acceptable limit of 10-20 meq/kg of fat throughout the storage period. The high acidity of extracted fat indicates rancidity and as per the PFA act, a fat acidity more than 1.5 per cent is not desirable. In the present study, the fat acidity in all the four types of biscuits ranged from 0.57 to 0.75 mg KOH/g. ARR_P fortified biscuits had significantly lower fat acidity and peroxide value than that of control biscuits, which may be attributed due to the presence of high level of antioxidants in *A. racemosus* root powder (Table 2). ARR_P possess a very strong antioxidant property and three compounds namely asparagine A, racemosol and racemofuran have been known to responsible for antioxidant activity of which racemofuran was identified as the actual principle for the antioxidant property (IC₅₀ 130µg) [31]. The Free radical scavenging activity in 100 g of ARR_P ranged between 70-80 per cent [32-38].

Reddy et al. [29] prepared biscuits by adding natural antioxidants present in ethanolic extracts of raisins (1%), drumstick leaves (2%) and aonla (1%) and studied the oxidative rancidity in biscuits up to 45 days of storage. Antioxidants from all three sources delayed the rate of peroxide formation, since PV of all samples contained natural antioxidants were lower than

Table 4. Effect of storage period on fat acidity (mg of KOH /g) of biscuits developed using *A. racemosus* root powder

Treatment	Storage period (days)						
	0	15	30	45	60	75	90
Control (WF:100)	0.35±0.27	0.39±0.10	0.45±0.04	0.51±0.19	0.58±0.06	0.66±0.18	0.75±0.16
ARR _{P1}	0.34±0.07	0.39±0.09	0.41±0.04	0.45±0.06	0.51±0.19	0.58±0.02	0.64±0.09
ARR _{P2}	0.35±0.16	0.37±0.08	0.38±0.10	0.43±0.07	0.48±0.09	0.54±0.29	0.60±0.11
ARR _{P3}	0.35±0.05	0.36±0.13	0.37±0.06	0.42±0.05	0.46±0.22	0.50±0.04	0.57±0.12
CD(p=0.05)	Period: 0.11		Supplementation level:0.12		Period × Supplementation level: 0.31		

Values are mean ± SE of three independent determinations

Biscuits containing wheat-*A. racemosus* root powder 95:5(ARR_{P1}), wheat-*A. racemosus* root powder 92.5:7.5(ARR_{P2}) and wheat-*A. racemosus* root powder 90:10(ARR_{P3}); WF= wheat flour

Table 5. Effect of storage period on peroxide value (meq/kg) of biscuits developed using *A. racemosus* root powder

Treatment	Storage period (days)						
	0	15	30	45	60	75	90
Control (WF:100)	0.30±0.01	1.09±0.02	1.76±0.01	2.62±0.05	3.13±0.03	4.52±0.03	5.94±0.03
ARRP ₁	0.28±0.01	0.98±0.01	1.58±0.33	2.45±0.02	2.96±0.02	4.15±0.02	5.52±0.04
ARRP ₂	0.25±0.01	0.86±0.01	1.34±0.01	2.19±0.02	2.82±0.02	3.74±0.02	5.18±0.02
ARRP ₃	0.22±0.02	0.70±0.02	1.19±0.01	2.10±0.01	2.76±0.02	3.38±0.01	4.76±0.03
CD(p=0.05)	Period: 0.10		Supplementation level: 0.08		Period × Supplementation level: 0.20		

Values are mean ± SE of three independent determinations

Biscuits containing wheat-*A. racemosus* root powder 95:5(ARRP1), wheat-*A. racemosus* root powder 92.5:7.5(ARRP2) and wheat-*A. racemosus* root powder 90:10(ARRP3); WF= wheat flour

that of control sample, during storage. Chitosan (a source of natural antioxidant) addition slowed the lipid oxidation of mayonnaises up to 63 days at 37; 47 and 57°C. The mayonnaise with chitosan with the bigger molecular weight showed better stability during accelerated storage at evaluated temperatures [38].

4. CONCLUSION

Blanching of *A. racemosus* roots at 80°C temperature for 3 minutes reduced the bitterness of developed powder considerably due to the reduced amount of saponins. The level of AARP was found acceptable up to 10 per cent in biscuit preparation without compromising the sensory attributes. Biscuits stored upto 90 days were adjudged as liked moderately by the panelists. ARRP fortified biscuits had significantly lower oxidative rancidity as measured by fat acidity and peroxide value than that of control biscuits, which may be attributed to high content of antioxidants in *A. racemosus* root powder. Hence, ARRP may be used in bakery products as a preservative to improve their shelf life along with medicinal benefits.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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