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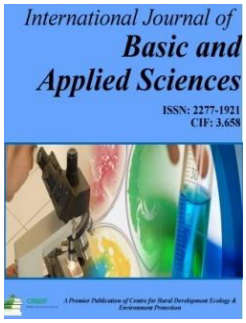

Full Length Research Paper

Antioxidant Activities of *Phaseolus vulgaris* Powder Added to Biscuits during Storage

Ali Monahi Nazal Al Shammari and Rasha Haji HassanAshkanani
Faculty of Home Economics, the Public Authority for Applied Education and Training, Kuwait.

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Corresponding Author:
Ali Monahi Nazal Al Shammari

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ABSTRACT

The main objective of the present study was carried to evaluate the possibility of Phaseolus vulgaris powder added to biscuits as natural antioxidants to help biscuits industry to minimize lipid oxidation and extend the shelf-life. Two concentrations of Phaseolus vulgaris powder (5 and 10%) were added to biscuits and stored at room temperature 25ºCfor 3 months. Total phenols and antioxidant activity % of Phaseolus vulgaris powder and fortified biscuits were determined. Also chemical composition and organoleptic properties were determined. Lipids extracted from fortified biscuits with Phaseolus Vulgaris powder during storage were assayed for antioxidant activity. Antioxidant activities of fortified biscuits were evaluated by the determination of moisture, acid, peroxide values and thiobarbituric acid(T.B.A)during 3 months storage. The present study showed that Phaseolus Vulgaris powder, increased the amount of total phenols and antioxidant activity % for all biscuits formula .Data indicated that the high amount of total phenols and antioxidant activity % in the formula containing 10% Phaseolus Vulgaris powder. Data showed that total phenol (%) as gallic acid in fortified biscuits made from wheat flour with 10%Phaseolus vulgaris powder was higher content than fortified biscuits made from wheat flour with 5% Phaseolus vulgaris powder (1.72vro.997).lipids extracted from biscuits samples without Phaseolus Vulgaris (control) reached a maximum Peroxide value of 10.3meq/kg after 3 month of storage. From the same results, Their corresponding inhibition rates were 43.7% and 50.5% from fortified biscuits 5% Phaseolus vulgaris powder and fortified biscuits 10% Phaseolus Vulgaris powder respectively after 3 month under storage conditions compared to biscuits (control). Fortified biscuits samples showed decreases in TBA values as compared to control.

Introduction

Phaseolus vulgaris L. (Family: Papilionaceae) is native of Tropical America and now widely cultivated throughout the tropics and temperate regions Salman et al. (2016). Different extracts of *Phaseolus vulgaris* have been evaluated for pharmacological activities and have shown analgesic,anti obesity,antibacterial, anticancer, antidiabetic, antifertilityanti-inflammatory anti-oxidant hepatoprotective, hypolipidemic. Hayatet al. (2014). It contained litholytic, trypsin and α-amylaseShi et al. (2017) . It contained anthocyanins, Onyilagha and Islam (2019), brassinosteroids, caffeic acid, catechic and gallic acid, coumestrol, daidzen, delphinidin, equol Hayatet al. (2014). ferulic acid, gallic acid and genistein, Diaz et al. (2016), Oomah et al. (2020) and Atchibri et al.(2020)

Biscuits are consumed throughout the world for their nutritional value and also can be stored for longer durations (Chavank et al. (2016). Biscuit manufacturing is a major processed food sector. Biscuits are available in a wide range of shapes, fillings, colors, and toppings and are hence accepted by consumers of all age groups. Biscuits, cookies, and crackers are enjoyed by consumers because of its favorites, flavor and appearance (Zydenbos et al. (2013). The present study was carried to evaluate the possibility of total phenols by using *Phaseolus Vulgaris* powder Added to biscuits as natural antioxidants to help biscuits industry to minimize lipid oxidation and extend the shelf-life.

Material and methods

Fortified biscuits

Ingredients of biscuits

Wheat flour Ttritucumvulgara (72% extraction).
*Phaseolus Vulgaris, s*ugar, salt, vanillin, baking powder were obtained from Mansoura local market.

Methods

Preparation of *Phaseolus Vulgaris* powder

Phaseolus Vulgaris were cleaned and washed with distilled water, soaked in boiling water for 15 min then cooled with distilled water and dried in an air oven dryer 40-50 ºC for 24 hr, finally milled in order to pass through 30 mesh sieves according to Mahagoub (2008).

Preparation of biscuits

Ingredient used for making control biscuit sample were included 100 g of wheat flour with baking powder 1.6g,

sunflower oil16.0gm, shortening 20g, salt 0.3g,Vanilla 1 gm,Milk 2.6 and sugar 40g according to the method described by Abdel-Magied (1991). The supplemented biscuits were prepared using the same formula expect for wheat flour (72%) with *Phaseolus Vulgaris* flour at 5and 10 % (table 1). All different biscuits formula were baked at 200C for 10: 15 min then cooled and packaged in poly ethylene bags and storage at room temperature 25ºC.The samples were analyzed periodically during the validity period (for 3 months), every month. The fat from the samples was extracted by biscuits, using the cold extraction with petroleum ether. It was determined as a chemical indicator.

Table (1): Biscuits formula from wheat flour, *Phaseolus Vulgaris* powder.

Samples Ingredients (%)	Control	1	2
Wheat flour(W.F)	100	95	90
*Phaseolus Vulgaris*powder	-	5	10

Organoleptic evaluation of biscuits

Biscuits samples were evaluated organoleptically by a panel of ten panelists for appearance, color, thickness, crispiness, shrinkage, taste and odor as the method described by **Smith (1972)**. The scores are shown in table (2).

Table (2): Organoleptic evaluation of the prepared biscuits

Characteristics	score
Appearance	10
Color	15
Thickness	15
Crispiness	15
Shrinkage	15
Taste	15
Odor	15
Total	100

Chemical analysis

The content of moisture, crude fiber, crude protein, crude fat, and ash were determined according to the method of A.O.A.C. (2000). Total carbohydrate (%):Total carbohydrate content was calculated by difference between 100 and the sum of ash, protein, crude fat and fiber content.

Determination of phenolic compounds

Phenolic compounds were determined by HPLC according to the method of (Goupy et al., 1999). At Central lab. of Food Technolpgy Research Institute Arric. Rec. cent. Egypt

Evaluation of antioxidant activity for fortified biscuits

Acid and peroxide values

Acid and peroxide values were determined in each oil sample according to the method described in A.O.A.C. (2000).

Thiobarbituric (TBA)

The test was performed according to the methods previously stated by some authors (Ottolenghi, 1959; Kikuzaki and Nakatani, 1993).

Statistical analysis

Statistical analyses were processed by IBM- PC computer using SPSS software program 2000. Mean and standard deviation (SD) were calculated for the majority of variables. Qualitative variables expressed as percentage were compared to different groups. T test was also used to know if there were any significant differences between two groups.

Results and discussion

The organoleptic properties of fortified biscuits made from wheat flour (72%) with Phaseolus Vulgaris powder:-

Biscuit supplemented by 10% *Phaseolus Vulgaris* powder were sensory evaluated with different parameters appearance, color, thickness, crispness, shrinkage, taste, odor and overall acceptability values and compared with biscuit from wheat flour 72% as presented in table (3).Data presented in table (3), showed that formula contained 10% *Phaseolus Vulgaris* powder showed the best acceptability, with ability value 99.6%.

Table (3): The organoleptic properties of fortified biscuits made from wheat flour and *Phaseolus Vulgaris* powder.

Samples of biscuits	Appearance (10)	Color (15)	Thickness (15)	Crispness (15)	Shrinkage (15)	Taste (15)	Odor (15)	Total (100)	Ability Value %
100% W.F (control)	9.0	14.1	13.5	12.3	12.3	14. 3	14.5	90	100.0
95% W.F +5% *Phaseolus Vulgaris*powder	8.9	13.6	13.2	12.3	12.2	13.2	13.9	87.3	97
90% W.F+ 10%*Phaseolus Vulgaris*powder	9.1	14.0	13.2	12.3	12.2	14.7	14.2	89.7	99.6

W.F = Wheat flour

Chemical composition of fortified biscuits made from wheat flour (72%extract.)with *Phaseolus Vulgaris* powder.

Data in table (4) showed the gross chemical composition of biscuits processed from wheat flour 72% and *Phaseolus Vulgaris* powder results indicated that moisture content ranged from 5.6 to5.7% in biscuits formulae. All biscuits formulae were higher in moisture content. Results also, indicated that protein content of control formula was higher than those of the other mixtures. This could be due to that the ratio of additives namely carrot powder contained low amount of protein in comparing with wheat flour. The ash content in biscuit formula was increased slightly comparing with biscuit mixture. Reviewing to the data in the same table, the fiber content in biscuit processed from wheat flour mixtures was higher than of control sample. The increasing of fiber related to the addition of *Phaseolus Vulgaris* powder, which covered a gradual increase in fiber content, generally, these results are in

agreement with those given by (Hussein, 2001). From the same table, the results revealed that biscuit from wheat flour 72% with 10% carrot powder extraction contain the highest amount value of carbohydrates (75.54%) on dry weight basis. These results are in agreement with those of Ghoniem, (2002).

Table (4): Chemical composition of fortified biscuits made from wheat flour(72%extract) with *Phaseolus Vulgaris* powder.

Samples of biscuits	*Constituents*					
	Moisture %	**C. Protein %**	**C.Fat %**	**Ash %**	**C.Fiber %**	**Carb. %**
Control with 100%W.F	5.7±0.17	10.53±0.8	12.33±0.22	2.5±0.49	0.13±0.34	70.38
95% W.F +5% *Phaseolus Vulgaris*powder	5.7±0.56	8.77±0.32	13.33±0.2 2	2.10±1.40	0.50±0.23	75.3
90% W.F+10% *Phaseolus Vulgaris* powder	5.7±0.67	8.51±0.43	13.00±0.43	2.41±1.43	0.54±0.22	75.54

W.F = Wheat flour *Each value is the mean ± SE.*

Total phenols and antioxidant activity content of *Phaseolus Vulgaris* powder used in biscuits formulation

Total phenols and antioxidant activity % content of *Phaseolus Vulgaris*, (on dry weight basis) was shown in table (5). It could be noticed from the results that *Phaseolus Vulgaris* had total phenols (50.87mg/gm) and antioxidant activity 89.59%, these result at accordance with Ally (2001).

Table (5): Total phenols (mg\gm) and antioxidant activity % content in *Phaseolus Vulgaris* powder (on dry weight basis).

Ingredients	Total phenols (mg\gm)	Antioxidant activity %
***Phaseolus Vulgaris* powder**	**50.87**	**89.59**

Phenolic acids of *Phaseolus Vulgaris* powder and fortified biscuits made from wheat flour (72%extract) with *Phaseolus Vulgaris* powder.

Polyphenolic compounds are very important fruit constituents, by virtue of their antioxidant activity by chelating redox- active metaions, inactivating lipid free radical chains and preventing hydroperoxide. The main phenolic acids identified in *Phaseolus Vulgaris* powder and fortified biscuits made from wheat flour(72%extract) with *Phaseolus Vulgaris* powderare presented in Table (6). The results showed that *Phaseolus Vulgaris* powder was higher contents of chlorogenic, catechol, benzoic, caffeic, vanillic and ellagic were (299.72.108.36, 80.24, 39.25, 17.97 and 17.7 mg/100g,) respectively. Data in Table (6) showed that total phenol (%) as gallic acid in fortified biscuits made from wheat flourwith 10%*Phaseolus Vulgaris* powder was higher content than fortified biscuits made from wheat flour with 5% *Phaseolus Vulgaris* powder (1.72vr0.997). Phenols are very important plant constituents because of their scavenging ability on free radicals due to their hydroxyl groups (Heim et al., 2002). Several studies showed good correlation between the phenols and antioxidant activity (Haung et al., 2005 and Silva et al., 2006). Gonzalez et al.(2011). Phenolic compounds and their antioxidant capacity in *Daucus* cultivars, *Phaseolus Vulgaris* contains phenolic constituents with a single aromatic ring (phenolic acids), mainly chlorogenic acid Arscott and Tanumihardjo(2010). *Phaseolus Vulgaris* contained mainly hydroxyl cinnamic acid derivatives, among them chlorogenic acid represented 42.2–61.8 % of total phenolics Zhang and Hamauzu2004. Chlorogenic acid is also accumulated in *Phaseolus Vulgaris* Simoeset al.,(2011), this compound accompanied by ferulic and dicaffeoylquinic acid reached 82 % of total phenolics in wounded root tissue Heredia and Cisneros (2009). Chlorogenic acid, caffeic acid, *p*-hydroxybenzoic acid, ferulic acid and other cinnamic acid isomers predominated in *Phaseolus Vulgaris* of different colors Sun et al.,(2009)and Alasalvar et al(2001).

Table (6):Phenolic acids of*Phaseolus Vulgaris*powder and fortified biscuits made from wheat flour(72%extract) with *Phaseolus Vulgaris* powder.

Samples	*Phaseolus Vulgaris*powder	Controlbiscuits	95% W.F +5% *Phaseolus Vulgaris* powder	90% W.F+10% *Phaseolus Vulgaris* powder
Gallic	15.77	----	0.997	1.72
Protocatechuic	6.44	1.99	1.63	0.54
Catechein	---	---	2.60	0.90
Catechol	108.36	---	----	0.36
Chlorogenic	299.72	66.52	65.89	20.41
Caffeic	29.35	----	1.29	0.13
Vanillic	17.97	175.58	172.64	154.18
Caffeine	7.71	---	----	33.61
Ferulic	15.65	---	----	1.32

Salicylic	----	---	----	2.23
Benzoic	80.24	----	14.09	---
Ellagic	17.70	13.95	6.68	2.71
Coumarin	---	---	0.55	0.51
Cinnamic	---	13.40	11.13	----

W.F = Wheat flour

Total phenols (mg\gm) and antioxidant activity% content in fortified biscuits made from wheat flour (72%) with *Phaseolus Vulgaris* powder

Total phenols and antioxidant activity % content in fortified biscuits made from wheat flour (72%) and *Phaseolus Vulgaris* powder tabulated in table (7), fortified biscuits made from wheat flour with 10%*Phaseolus Vulgaris* powder had the highest level of total phenols was (54.17mg/gm) and antioxidant activity% was (94.19%) .The results showed that ,total phenols and antioxidant activity% in fortified biscuits made from wheat flour with 5%*Phaseolus Vulgaris* powder were (44.12mg/gm)and(93.87%) respectively. On the other hand, the results showed that total phenols and antioxidant activity% in control biscuits were (33.37mg/gm) and 93.43% respectively. Generally, from data presented in table (7), the addition of *Phaseolus Vulgaris* powder, increased the amount of total phenols and antioxidant activity %for all biscuits formula Data indicated that the high amount of total phenols and antioxidant activity % in the formula containing 10% *Phaseolus Vulgaris* powder.

The role of *Phaseolus Vulgaris* as the precursors of vitamin A as well as excellent antioxidants has been commonly known for many years (Simon et al., 1989 and Simon 1990)**.**

Table (7): Total phenols(mg\gm) and antioxidant activity% content in fortified biscuits made from wheat flour (72% extraction)with *Phaseolus Vulgaris* powder (on dry weight basis)

Sample	Total phenols (mg\gm)	Antioxidant activity %
Control biscuits	33.37	93.43
95% W.F +5% *Phaseolus Vulgaris* powder	44.12	93.87
90% W.F +10 % *Phaseolus Vulgaris* powder	54.17	94.19

W.F = Wheat flour

Effect of storage on moisture content of prepared biscuits

From Table (8), it was noticed that moisture content was increased by increasing storage time in the all samples. lipids extracted from biscuits samples without the antioxidant (control) reached of 6.4after 3 month of storage, A difference results moisture content was observed between the control and fortified biscuits as 5% *Phaseolus Vulgaris* powder was(6.2) and fortified biscuits as10% *Phaseolus Vulgaris* powder was (6.0) respectively after 6 month under storage. The obtained data were in the line with the findings of (Hassanen 2005), who found that the moisture of biscuit increase when it was stored at the beginning of the month 5 for 6 month Abdel Azim (2007) found that the moisture of biscuit increase when it was stored at 3C.

Table(8): Effect of storage on moisture content of prepared biscuits

Biscuit samples	zero	1month	2month	3month
100% W.F (control)	5.7±0.17	5.8±0.3	5.8±0.1	6.4±0.2
95% W.F +5% Phaseolus Vulgaris powder	5.7+0.56	5.7±0.2	5.8±0.1	6.2±0.3
90% W.F+10% Phaseolus Vulgaris powder	5.7±0.67	5.6±0.1	5.6±0.1	6.0±0.1

W.F = Wheat flour Each value is the mean ± SE

Inhibitory effect of *Phaseolus Vulgaris* powder on the primary oxidation of lipids extracted from fortified biscuits as measured by using acid value during storage

The development of free fatty acid content in oils is usually considered to be one of the main parameters used in evaluating the quality oil (El-Sayd, 2015). From Table (9), it was noticed that acid value was increased by increasing storage time in the all samples. lipids extracted from biscuit samples without the antioxidant (control) reached of 11.1mg KOH/gm oil after 3 month of storage, A difference results in acid value was observed between the control and fortified biscuits as 5% *Phaseolus Vulgaris* powder was(3.4mg KOH/gm oil) and fortified biscuits as10% *Phaseolus Vulgaris* powder was (2.1mg KOH/gm oil) respectively after 3 month under storage. *Phaseolus Vulgaris* (Daucuscarota) is a good source of natural antioxidants, especially phenolic compounds (Chantaro et al., 2018).

Table (9): Inhibitory effect of *Phaseolus Vulgaris* powder on the primary oxidation of lipids extractedfrom fortified biscuits as measured by using acid value during storage

Biscuit samples	Zero	1month	2month	3month
100% W.F(control)	**0.98±2.2**	**1.1±0.89**	**1.7.2±6.12**	**11.1±1.98**
95% W.F +5% Phaseolus Vulgaris powder	**0.98±2.2**	**1.01 ±8.78**	**1.3±2.29**	**3.4±4.63**
90% W.F+10% Phaseolus	**0.98±2.2**	**1.01±11.2**	**1.1±5.12**	**2.1±2.12**

Vulgaris powder	
W.F = Wheat flour	Each value is the mean ± SE.

Inhibitory effect of *Phaseolus Vulgaris* powder on the primary oxidation of lipids extracted from fortified biscuits as measured by using peroxide value during storage.

Inhibitory effect of lipids extracted from fortified biscuits of *Phaseolus Vulgaris* powder on peroxide value. Peroxide value (PV) is a measure of the concentration of peroxides and hydroperoxides formed in the initial stages of lipid oxidation. As it is known, peroxide value is one of the most tests used for the measurement of primary oxidation in oils and fats. In this work, oxidation degree on lipids extracted from fortified biscuits samples was determined by measuring peroxide value during storage. The effect of *Phaseolus Vulgaris* powder during storage period on PV in lipids extracted from fortified biscuits samples is shown in(Table 10).The results revealed that PV increased linearly with storage time. lipids extracted from biscuits samples without the antioxidant (control) reached a maximum PV with10.3meq/kg after 3 month of storage. From the same table (10), Their corresponding inhibition rates were 43.7% and 50.5% from fortified biscuits 5% *Phaseolus Vulgaris* powder and fortified biscuits 10% *Phaseolus Vulgaris* powder respectively after 3 month under storage conditions compared to biscuits (control). However, the antioxidant effects of *Phaseolus Vulgaris* powder were better little research has systematically investigated their antioxidant content and antioxidant capacity, especially the relationship between specific antioxidants and total antioxidant capacity (Sun ,2019). Active oxygen and free radical species such as superoxide and hydroxyl radicals play an essential role in the immune system. They are produced as by-products in normal metabolism. However, an excess of free radicals is believed to cause many diseases and promote aging (Endo et al., 2016).

Table (10): Inhibitory effect of *Phaseolus Vulgaris* powder on the primary oxidation of lipids extractedfrom fortified biscuits as measured by using peroxide value during storage.

Biscuit samples	Zero	1month	2month	6month
100% W.F(control)	**1.4±0.2**	**1.8±0.1**	**2.2±0.1**	**10.3±0.5**
95% W.F +5% Phaseolus Vulgaris powder	**1.4±0.2**	**1.5±0.08**	**1.8±0.09**	**5.8±0.2**
90% W.F+ 10% Phaseolus Vulgaris powder	**1.4±0.2**	**1.5±0.2**	**1.6±0.2**	**5.1±0.2**

W.F = Wheat flour *Each value is the mean ± SE.*

Inhibitory effect of *Phaseolus Vulgaris* powder on the malondialdhyde formation of lipids extractedfrom fortified biscuits as measured by using TBA during storage

Malondialdehyde (MDA) is a degradation product generated from lipid peroxidation (oxidative degradation of polyunsaturated fatty acids in cell membrane). MDA has been extensively used as an index for lipid peroxidation and as a marker for oxidative stress (Kubow, 2012). The reaction of MDA with TBA has been widely adopted as a sensitive assay method for lipid peroxidation (Ohkawa et al., 1978). The data in (Table11) showed that lipids extracted from biscuits samples without the antioxidant (control) TBA reached 0.58after 3 month of storage. While lipids extracted from fortified biscuits samples is shown in (Table11), decreases in TBA values as compared to the control, fortified biscuits as 5% *Phaseolus Vulgaris* powder was (0.46) and fortified biscuits as10% *Phaseolus Vulgaris* powder was (0.38).*Phaseolus Vulgaris* powder could play an important role in improving antioxidant intake in the human diet.

Table (11): Inhibitory effect of *Phaseolus Vulgaris* powder on the malondialdhyde formation of lipids extracted from fortified biscuits as measured by using TBA during storage.

Biscuit samples	Zero	1month	2month	3month
100%W.F(control)	0.09±0.1	0.16±0.04	0.22±0.01	0.58±0.02
95%W.F+5% Phaseolus Vulgaris Powder	0.09±0.1	0.19±0.1	0.20±0.01	0.46±0.02
90%W.F+10% Phaseolus Vulgaris Powder	0.09±0.1	0.13±0.01	0.18±0.4	0.38±0.1

W.F = Wheat flour *Each value is the mean ± SE.*

Conclusion

The results obtained , we can underline the beneficial effect of using the natural antioxidant for stabilizing in time the biscuits produced and for their sensorial qualities.

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