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Biological evaluation of biscuits prepared from citrus peels as a lowering cholesterol

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ABSTRACT
The present investigation was carried out to study the protective effects of some functional foods in reducing cardiovascular risks. Thus we prepare biscuits formulas containing the different citrus peel powder. Biscuit formulas were evaluated the biological evaluation on rats to study their effect on lipid profile, which are the main factors affecting atherosclerosis diseases. Eight groups were fed on a different diet and formulated biscuit as follows; basal diet (normal control), hypercholesterolemic (cholesterol and cholic acid 3:1) diet and biscuit containing abo-sora orange, balady orange and mandarin peels at level 5 and 10%. The results showed that a significant reduction in serum total lipids, triglycerides, total cholesterol, HDL- cholesterol and decreased of LDL-cholesterol at the end of the feeding period. The best effect was found in the groups fed on abo-sora orange peel 10%, followed by the balady orange and mandarin peel. Therefore it can be concluded and recommended that biscuit product from orange abo-sora and balady and also mandarin can be used as a new trained of functional food which has a healthy role in addition to the nutritional value for the consumers. They keep them healthy and protect them from diseases, namely cardiovascular and atherosclerosis.

***Keywords**:* Citrus, Functional formulas, biscuits, lipid profile, cholesterol

Introduction

Biscuit is the most widely consumed preferable bakery products because of their availability easy to eat convenience, with regarding its long shelf life (Vijaykumar *et al.,* 2013). Biscuits, among all bakery products, are more significant since they are vastly used as snacks by children and adult (Dhankar, 2013) in addition to health and natural functions, taking into account the economic cost effective manner (Mosoodi and Bashir, 2012). For that, keeping a great quality of biscuit is the more important economic target.

Citrus is one of the most important crops in the world that represent large amount of waste (about 50% of hall fruit) 8. Therefore, the functionality of peel components, flavonoids, pectin and carotenoids should be studied (Li *et al.,* 2007).

Obtained data from previous study indicated that, the citrus peel is our interest because of its high content and sheep source of bioactive components. The citrus by-products had a high amount of bioactive compounds varied from Vit. C, ß-carotene and total phenolics content. The effect of variety and drying methods on bioactive compounds amount of peels were investigated previously (Marwa *et al.,* 2015 and Azza *et al.,* 2016). Valencia orange had the highest amount of bioactive compounds and antioxidant activities. The microwave was the best method for drying the citrus by-products with regarding to bioactive compounds and its antioxidant activities (Marwa *et al.,* 2015 and Azza *et al.,* 2016).

It is noteworthy to clarify that citrus peel: the waste by-product of the citrus factories is reckoned as valuable functional food. So, citrus peels may provide a health benefit beyond the traditional nutrients they contain, as well as prevent diet-related diseases, e.g. metabolic syndrome, type II diabetes, coronary heart disease, obesity, hypertension, certain types of cancer, gastrointestinal diseases and osteoporosis (Donalson, 2004 and Youssef, 2007).

Citrus is one of the largest and most popular fruit crops commercially grown across the globe. It is not only important in terms of economy but is also popular for its nutritional benefits to human and farm animals. Citrus is available in several varieties, all with attractive colors. It is consumed either fresh or in processed form (Sharma *et al.*, 2018).

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Risk of chronic diseases can be reduced by frequent consumption of fruits and vegetables. A common component of food products are dietary fiber which consists of a variety of non starch polysaccharides such as cellulose, hemicelluloses, pectin، b-glucans, gums and lignin (Elleuch *et al.,* 2011) and due to their beneficial effects on food nutritional properties are consumed as foods. Consumption of dietary fiber plays a significant role in the prevention, reduction and treatment of chronic diseases such as bowel, gastrointestinal disorders, obesity، diabetes, cardiovascular disease, cancer and also promotes physiological functions such as lowering blood triglycerides and glucose control (Figuerola *et al.,* 2005).

Thus the aim of this work is to study the protective effects of some functional foods prepared from some citrus peel which contain important dietary fiber, phytochemicals and antioxidants compounds against diseases; namely cardiovascular disease and atherosclerosis risks.

Materials and Methods

Materials

Citrus peel (mandarin, balady orange and abo-sora orange) obtained from American company for foods, 6 of October City Egypt. Also, wheat flour 72% extraction and other ingredients to prepare biscuits were obtained from local markets.

All used chemicals and reagents were (Sigma, Aldrich &Fluka) purchased from Sigma, Aldrich &Fluka Chemical Co. (St. Ouis, Mo, 63103 USA). All other chemicals and reagents used were of analytical grade.

Experimental animals

Adult albino male rats (48 rats) weight 110-130 g was obtained from the farm of the National Organization for Drug Control and Research, Giza, Egypt.

Biological evaluation

Basal diet consist of 15% casein, 10% corn oil, 4% salt mixture, 1% vitamins mixture, 5 % cellulose and 65% starch. Salt and vitamin in according to AOAC, (2010).

Methods

Preparation of biscuits formulas from different citrus peel

Dry ingredients were mixed together and then combined with others according to the recipe presented in Table (1). The dough was mixed for 10 min to obtain a homogeneous consistency and then placed into the fridge at 6 °C over a period of 30 min. The dough was sheeted to a thickness of about 3 mm using a rolling machine. The sheeted dough was cut into round shape using a 45 mm diameter cutter and baked on an aluminium tray in an electric oven at 180 ºC for 6 minutes. The supplemented biscuits with orange peel powders were prepared using the same formula except for replacing the wheat flour was added at four levels 5 and 10% orange peel powder in the same way as the control sample (biscuits without orange peel) in the biscuit was cooled for 30 minutes, packed in polyethene bags stored under desiccation (Krystyjan *et al.,* 2015).

Table 1: Ingredients of biscuit formulas from different citrus peels.

Ingredients	Control biscuits	Biscuits formula fortified citrus at	
		5%	10%
Wheat flour	100	95.0	90.0
Margarine	45.0	45.0	45.0
Sugar powder	30.0	30.0	30.0
Egg	75.0	75.0	75.0
Vanillin	2.0	2.0	2.0
Baking powder	1.0	1.0	1.0
Citrus peels	---	5.0	10.0

Biological experimental

Control negative: Rats were fed on basal diet
Control positive: Rats fed on basal diet containing mixture of cholesterol and Cholic acid 3:1
Group I: Rats fed on basal diet containing 5% mandarin, cholesterol and cholic acid 3:1
Group II: Rats fed on basal diet containing 10% mandarin, cholesterol and cholic acid 3: 1
Group III: Rats fed on 5% balady orange, cholesterol and cholic acid 3:1
Group IV: Rats were fed on basal diet containing 10% balady orange, cholesterol and cholic acid 3:1
Group V: Rats fed on basal diet containing 5% abo-sora orange, cholesterol and cholic acid 3:1
Group VI: Rats were fed on basal diet containing 10% Babo-sora orange, cholesterol and cholic acid 3:1

Rats were weighed and blood samples were obtained at time 0, 15 and 30 days according to Shermer (1967).

Serum was separated then to run the serum biochemical analysis, at the end of experiment, rats were sacrificed.

Biochemical analyses

At the end of biological experiential serum was separated and it was used for the following biochemical analyses:

Total lipids were determined according to the method of Zoliner and Kirsch (1962). Triglycerides were determined according to the method of Fossati and Prencipe (1982). HDL-Cholesterol was determined according to the method of Lopez-caballero (1977). LDL-cholesterol was determined according to Salamatullah *et al.,* (1983).

Statistical analysis

The obtained data were exposed to analysis of variance. Duncan's multiple range tests at ($P \leq 0.05$) level was used to compare between means. The analysis was carried out using the PRO ANOVA procedure of Statistical Analysis System (SAS, 2004).

Results and Discussion

Effect of biscuits from different citrus on body weight

Data in Table (2) showed that feeding on biscuits containing different ratio (5 and 10 %) from Mandarin, balady orange and abo-sora orange peel. The results showed that the highest increase was found for groups feeding on bicuits containing ratio 10% from balady and abo-sora orange. Data showed the highest one 10% abo-sora orange followed by10% balady. Similar findings were reported by Wolf (2010) who stated that the citrus peel (orange peel) is a very good source of pectin, which can help diminish an overactive appetite, which leads to unwanted weight gain. Horner (2010) reported that mandarin peels powder achieved weight loss, which is in good agreement with our data. On the basis of such findings in the present investigation fortified biscuits with 10% citrus peels powders could be recommended for caloric reduced diets for obese, over-weight persons and diabetic and cholesterol persons.

A number of studies have recognized the presence of polyphenols, vitamins, minerals, dietary fibers, essential oils and carotenoids content which makes citrus a health-benefit promoting fruit. To this regard several examples about the use of citrus fruits as therapeutic remedies can be cited: oranges to cure scurvy (Magiorkinis *et al.,* 2011) orange, lime, and lemon juices as remedies for the prevention of kidney stones formation (Pak, 2004), grapefruits as agents able to lower blood pressure and to interfere with calcium channel blockers (Sica, 2006), citrus flavonoids as effective in vivo agents able to modulate hepatic lipid metabolism (Cha *et al.,* 2001), orange juice to prevent and modulate inflammatory processes (Assis *et al.,* 2013), kumquat peel polyphenolics as effective antioxidant agents (Sadek *et al.,* 2009), grapefruit juice having anti-genotoxic effects (Alvarez-Gonzales *et al.,* 2010) and several others.

Table 2: Body weight gain (g) for rats fed biscuits containing 5% and 10%, mandarin, balady, abo-sora orange peel for 30 days.

Groups	Feeding period (days)			Body weight gain
	Initial time	After 15 days	After 30 days	
Control negative	119.58	180.60	203.21	83.63
Control positive	118.38	130.75	144.75	26.38
Balady orange 5%	119.55	148.07	172.76	53.21
Balady orange 10%	111.67	149.98	187.90	76.23
Abo-sora orange 5%	120.27	145.02	176.66	56.39
Abo-sora orange 10%	125.03	186.71	205.04	80.01
Mandarin 5%	114.78	146.78	157.53	42.75
Mandarin 10%	117.87	136.83	160.88	43.01

Effect of biscuits from different citrus peel on total lipids

The effects biscuits from mandarin, balady and abo-sora orange peel on Lipids pattern parameters were studied using 6 rats in each group. Data in Table (3) revealed significant differences in total lipids. The results for the arithmetic means showed a very highly significant decrease among all groups comparing to the control positive group. The same trend was found for serum total lipids in rats fed on biscuit produced containing 10% from the citrus.

Citrus flavonoids as polyphenolic compounds have emerged as promising therapeutic agents for the treatment of obesity, insulin resistance, and dyslipidemia (Nakajima *et al.,* 2014). Flavonoids such as naringin, hesperidin, and nobiletin have been experimentally proven to be able to lower lipid levels with insulin-sensitizing and anti-inflammatory properties primarily through inhibition of hepatic fatty acid synthesis and induction of increased fatty acid oxidation (Alam *et al.,* 2014).

Table 3: Total lipids serum (mg/dl) of rats feeding biscuits containing 5% and 10% from mandarin, balady, abo-sora orange peel for 30 days.

Groups	Period (days)		
	Initial time	After 15 days	After 30 days
Control negative	389.50±12.8	443.8±10.3	370.87 ± 11.2^{d}
Control positive	353.18±17.73	521.45±18.9	556.73 ± 15^{a}
Balady orange 5%	353.18±17.73	497.43±16.3	422.45 ± 12^{ab}
Balady orange 10%	353.18±17.73	494.04±15.2	413.45 ± 12^{c}
Abo-sora orange 5%	353.18±17.73	493.91±14.5	422.45 ± 12^{ab}
Abo-sora orange 10%	353.18±17.73	494.52±14.6	410.6 ± 11^{c}
Mandarin 5%	359.50±12.8	460.8±11.5	432.61 ± 14.6^{b}
Mandarin 10%	383.18±17.73	497.43±16.3	420.45 ± 12^{ab}

Values are mean and SD (n = 3); where: Mean values in the same with the letter are significantly different at 0.05 levels.

Effect of biscuits from different citrus peel on triglycerides

The results in a table (4) showed that the effects of feeding rats on the basal diet had contained 5 and 10% biscuits prepared from each mandarin, balady orange and abo-sora orange for 30 days. A highly significant reduction was found on triglycerides in groups' rat fed on 10% abo-sora which contained 97.63 mg/dl equal control negative (96.91 mg/dl), followed by balady orange and mandarin were 110.25 and 140.25 mg/dl compared to control positive groups was 188.16 mg/dl. These results confirmed by Parmar, and Kar (2007) who found that when diet fed animal containing a citrus extract a significant decrease in total cholesterol, TG, LDL, VLDL, and glucose level in the serum.

Effect of biscuits from different citrus peel on total cholesterol

Table (5) showed that the reduction in total cholesterol levels in all groups) compared to the control positive group. A highly significant decrease was found in the group abo-sora was100.66mg/dl nearly the control negative group followed by balady and mandarin groups fed al 10% form each were 102.66 and 110.44 mg/dl than control positive group was 280.26 mg/dl, respectively. These results decreased may be due to the cholesterol-lowering properties of the viscous fiber pectin from citrus may depend on its physico-chemical properties (viscosity, molecular weight

(MW) and degree of esterification (DE)), but these are not typically described in publications, nor required by European Food Safety Authority (EFSA) with respect to its generic pectin cholesterol-lowering claim (Brouns *et al.,* 2012).

Citrus peel is low in calories, sugar, and fats; and is free from cholesterol (Bobroff, 2002). Furthermore, the antihyperglycaemic activity of methanol extract of citrus peel in streptozotocin-induced diabetic rats was reported by Kundusen *et al*, (2011).

Table 4: Triglycerides serum (mg/dl) of rats feeding biscuits containing 5% and 10% from mandarin, balady, abo-sora orange peel for 30 days.

Groups	Period (days)		
	Initial time	**After 15 days**	**After 30 days**
Control negative	83.76±7.5	106.09±12.1	96.91±10.89^{c}
Control positive	83.76±6.5	175.51±13.8	188.16±11.3^{a}
Balady orange 5%	83.76±5.5	134.23±12.3	129.05±11.1^{ab}
Balady orange 10%	83.76±7.5	130.64±12.2	110.25±13.21^{ab}
Abo-sora orange 5%	83.76±7.5	130.64±12.2	121.09±11.1^{ab}
Abo-sora orange 10%	83.91±671	102.640±12.11	97.630±10.45^{c}
Mandarin 5%	83.76±6.52	145.51±13.8	140.16±12.3^{b}
Mandarin 10%	83.91±7.1	145.445±12.21	130.25±13.99^{ab}

Values are mean and SD (n = 3); where: Mean values in the same with the letter are significantly different at 0.05 levels.

Table 5: Total cholesterol serum (mg/dl) of rats feeding biscuits containing 5% and 10% from mandarin, balady, abo-sora orange peel for 30 days.

Groups	Period (days)		
	Initial time	**After 15 days**	**After 30 days**
Control negative	62.58±6.37	180.15±16.11	90.31±6.31^{c}
Control positive	62.58±5.37	230.31±17.81	280.26±27.62^{a}
Balady orange 5%	62.56±6.37	196.30±18.53	104.22±10.52^{b}
Balady orange 10%	62.58±5.37	193.74±16.62	102.66±17.10^{b}
Abo-sora orange 5%	62.58±4.37	195.57±14.95	103.50±12.21^{b}
Abo-sora orange 10%	62.58±6.37	190.21±15.11	100.66±10.80^{b}
Mandarin 5%	62.56±4.37	240.57±21.43	109.21±10.52^{b}
Mandarin 10%	62.56±5.37	227.67±23.52	110.44±10.71^{b}

Values are mean and SD (n = 3); where: Mean values in the same with the letter are significantly different at 0.05 levels.

Effect of biscuits from different citrus peel on HDL

Serum HDL –Cholesterol in Table (6) showed a significant increase in HDL -Cholesterol in tested groups at the end of the feeding period. Also, a significant increased in HDL-Cholesterol among all groups fed on 5 and 10% products comparing to the positive control group and non-significant changes were found in all group (containing 5-10%).

Citrus peel could be considered to be a potential source of pectin which is composed of white, spongy and cellulosic tissue (Terpstra *et al.,* 2002). Frequent consumption of dietary fiber is associated with low risk of life-threatening chronic diseases such as bowel, gastrointestinal disorders, obesity, diabetes, cardiovascular disease, cancer and also promoting physiological functions including reduction in blood cholesterol level and glucose attenuation, in addition, it was improved the HDL and LDL in serum (Figuerola *et al.,* 2005). The effectiveness of citrus peel in lowering the plasma liver cholesterol, serum triglyceride level, serum total cholesterol, liver total lipids, and liver cholesterol (Terpstra *et al.,* 2002) is proven by many epidemiological studies.

Effect of biscuits from different citrus peel on LDL

High significant increases were observed in LDL cholesterol in control positive rats which were feeding on a basal diet containing cholesterol and cholic acid 3:1 for 30 days and the results are recorded in Table (7). Data also feeding rats on 10% mandarin balady orange and abo-sora orange diet leads to gradually decreases LDL cholesterol at the end of the feeding period. The results for the arithmetic mean showed a highly significant decrease for all groups comparing to the positive control

groups, on the other hand, non-significant changes were found in groups fed on mandarin and balady orange.

LDL-C is known to be directly and independently associated to cardiovascular conditions (CVD) and LDLC-induced reactive oxygen species (ROS) and increased NADPH oxidase activity are major causative factors in endothelial perturbation and in the pathogenesis of (Adhyaru and Jacobson, 2015).

Table 6: HDL serum (mg/dl) of rats feeding biscuits containing 5% and 10% from mandarin, balady, abo-sora orange peel for 30 days.

Groups	Period (days)		
	Initial time	After 15 days	After 30 days
Control negative	41.74±3.05	73.65±7.35	80.10±8.78[a]
Control positive	41.57±4.05	44.10±5.91	44.67±4.72[c]
Balady orange 5%	41.57±3.05	84.66±8.90	61.56±6.76[b]
Balady orange 10%	41.74±3.05	84.15±7.37	64.15±6.72 [b]
Abo-sora orange 5%	41.57±4.05	84.66±8.90	61.56±5.76 [b]
Abo-sora orange 10%	41.74±4.05	75.66±7.36	69.66±7.81 [b]
Mandarin 5%	41.57±4.05	90.32±9.84	55.23±5.86[ab]
Mandarin 10%	41.74±4.05	89.32±8.38	62.15±6.79 [b]

Values are mean and SD (n = 3); where: Mean values in the same with the letter are significantly different at 0.05 levels.

Table 7: LDL serum (mg/dl) of rats feeding biscuits containing 5% and 10% from mandarin, balady, abo-sora orange peel for 30 days.

Groups	Period (days)		
	Initial time	After 15 days	After 30 days
Control negative	26.82±2.32	29.55±3.29	25.0 ±2.56[c]
Control positive	21.01±2.32	185±16.87	185.53±16.86[a]
Balady orange 5%	21.01±2.32	45.64±5.59	114.05±15.21[b]
Balady orange 10%	21.01±2.32	48.27±4.35	105.25±14.11[ab]
Abo-sora orange 5%	26.82±2.32	40.07±3.2	108.15±10.16 [ab]
Abo-sora orange 10%	23.82±2.32	41.5±4.3	103.88±10.1[ab]
Mandarin 5%	21.01±2.32	39.89±6.29	118.09±15.71[b]
Mandarin 10%	21.01±2.32	39.1±5.04	105.55±14.21[ab]

Values are mean and SD (n = 3); where: Mean values in the same with the letter are significantly different at 0.05 levels.

Conclusion

Therefore it can be concluded that biscuit product from orange abo-sora, balady peel and mandolin peel can be used as a new treatment functional food which has a healthy role in addition to the nutritional value for the consumers. They keep them healthy and protect them from diseases, namely cardiovascular and atherosclerosis.

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