

Biochemical and Biological Study of Biscuit Fortified With Apple Powder

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ABSTRACT

The nutritional value, sensory evaluation and the hypolipidemic effect of biscuits fortified with 15% of apple fruits powder were evaluated. The Apple powder was characterized by lower moisture, protein, carbohydrate and some minerals (iron, zinc and sodium) and higher content of fat, fiber, ash, pectin and calcium compared to wheat flour. Sensory evaluation showed that apple biscuit had non-significant decrease in shapeliness, crust color, texture, softness and non-significant increase in crumb color, aroma and eating quality but significant decrease in texture compared to ordinary biscuit. Biological study revealed that consumptions of apple biscuits to hyperlipidemic rats showed improvement in weight, weight gain and feed efficiency ratio and appeared within normal. Apple group showed non-significant increase in serum lipid profile and also non-significant increase in liver cholesterol and total lipids compared to control ve- group. Moreover, it showed non-significant difference in TC/HDLc and significant increase in LDLc /HDLc compared to control ve- group. In generally, inclusion of apple powder as an ingredient in bakery products as biscuit could improve the nutritional properties of these products and also had hypolipidemic effect as apple powder is rich source of dietary fiber.

Keywords: Wheat flour, apple powder, biscuits, sensory evaluation, hyperlipidemia, rats

Introduction

Bakery products include biscuit, muffin, cake, bread, pastries and pies that contain significant amount of flours which are mixed with various other ingredients and ultimately undergo dry-heating process in a baking oven. However, majority of bakery products are high in carbohydrate, fat and calorie, but low in fiber content (Mishra and Chandra 2012). Biscuits are one of the most liked bakery product by the urban as well as rural consumers with good shelf life. Biscuit is one of the most commonly accepted snack foods amongst children and adult and considered as one of the good supplementary food for distributing to the undernourished children through developmental agencies. Because of the high consumption of biscuits, they can potentially be used as carriers of dietary fiber (Lebesi and Tzia, 2011 and Jauharah *et al.*, 2014). High dietary fiber diets are associated with the prevention, reduction and treatment of some diseases, such as coronary heart diseases and large intestine cancer (Figueroa *et al.*, 2005 and Gupta 2006). Furthermore, increased consumption of dietary fiber improves serum lipid concentrations, lowers blood pressure, improves blood glucose in diabetes, promotes regularity, aids in weight loss, and appears to improve immune function (Anderson *et al.*, 2009).

Presence of fiber content in biscuit improves its nutritional quality and acceptability and improvements are achieved by changing the ratio of raw materials whole grains other than wheat or by changing the ratio of fiber content in basic recipes (Kohajdová *et al.*, 2011). Apple fiber is made from apple pulp (apple pomace) by drying and making it to powder, without any refining or bleaching and is composed mainly of carbohydrates and dietary fiber, small amounts of protein, fat and ash. Apple fiber is also a good source of phytochemicals primarily phenolic acids and flavonoids which correlated with antioxidant capacities (Sudha *et al.*, 2007, Cetkovic *et al.*, 2008 and Diñeiro- García *et al.*, 2009).

Because of increasing demand for healthy, natural and functional biscuits, the objective of this study was:

- 1) To evaluate the effect of substituting wheat flour with 15% of apples powder on the nutritional value of the produced apple biscuit.
- 2) To study sensory evaluation of apple biscuits.
- 3) To study the hypolipidemic effect of apple biscuits (fortified with 15% of apples powder) in hyperlipidemic experimental rats.

Materials and Methods

Raw materials

Apples, wheat flour 72% extraction (*Triticum aestivum*) and other ingredients for biscuit preparation were purchased from a local market in Saudi Arabia.

Apple powder preparation

Apples washed in tap water for removing of non-edible parts and pressing of juice. The residue (pomace) was dried at 40°C for 8 h. A grinder mill and sieves were used to obtain powder particle of the size of 160–270.

Chemical composition of wheat flour and apple powder

Moisture (oven dry method), fat (Soxhlet extraction), protein (Kjeldahl method), and ash content were determined according to AOAC (2007). Total dietary fiber (TDF) and pectin content were measured by the enzymatic/gravimetric method according to Sun-Waterhouse *et al.*, (2010); Kohajdova *et al.*, (2009), respectively. Carbohydrate (CHO) content was determined by difference method. Furthermore, percentages of iron, calcium, zinc and sodium were also determined using the atomic absorption spectrophotometer and flame photometer according to AOAC, (2007).

Preparation of ordinary and apple biscuits

Two pastes were made from 32g of sugar, 19g of milk powder, 15g of butter, 25g egg, 1.5 vanillas, 0.30 g of sodium bicarbonate, 0.65 ammonium bicarbonate and 0.5 salts. Then 100 g of flour was added to the first past and blend of wheat flour 85% and apple powder 15% was added to the other past and mix with 30 ml water in dough mixer where shafts rotate the dough simultaneously in two different directions to decrease the amount of balling. The dough was sheeted to 3.5mm thickness and molded into 45mm thickness and then baked in a bakery oven at 200°C for 10min as shown in table1. Biscuits samples were cooled for 30 min and packed in polypropylene pouches and sealed for chemical, sensory analysis and biological experiment. The method was adapted from the American Association of Cereal Chemists (AACC, 2000).

Proximate composition of ordinary biscuit and apple biscuit

All experiments were performed in triplicate. Moisture, fat, protein, ash, carbohydrate (CHO), crude fiber and ash content were evaluated (AOAC, 2007 and Serrem *et al.*, 2011). The minerals contents as iron, calcium, zinc and sodium of wheat and apple biscuits were determined according to the methods described in AOAC (2007).

Sensory evaluation of biscuits

Biscuits were evaluated by assigning a score for shapeling (10), crust color (10), crumb color (10), brightness of crumb (10), texture (10), softness (10), crust character (10), aroma (15) and eating quality (15). That was carried out by the help of ten semi trained judges (BIS, 1971).

Biological experiment

The experimental protocol was approved by the Animal Ethics Committee of the Animals Center in Research center in Prince Sultan military medical city. The basal diet contained 20% casein, 63.2% sucrose, 10% corn oil, 2% agar, 0.8% vitamin mixture and 4% salt mixture. After adaptation period (seven days), rats were randomly assigned to 4 groups (7 rats each). Control -ve group received basal diet only. The other groups fed on hyperlipidemia C diet consisted of the basal diet supplemented with 1.5% cholesterol, 0.5% cholic acid and 0.05% thiouracil in place of an equal amount of sucrose. Control +ve group received hyperlipidemic diet only. Ordinary and apple groups received hyperlipidemic diet with 20% of ordinary biscuits and apple biscuits with consideration the composition of biscuits. Weekly body weight and daily food intake were measured. At the end of the experimental period (45 days), all the animals were scarified by cervical decapitation. Blood and liver samples for each rat were collected.

Moreover, serum total cholesterol (TC), triglycerides high-density lipoprotein cholesterol (HDLc), low-density lipoprotein cholesterol (LDLc) and very low-density lipoprotein cholesterol (VLDLc) were estimated by the methods described by Thomas (1992), Stein (1987), Finley *et al.*, (1978), Friedewald *et al.*, (1972) and Bauer (1982), respectively. Liver was homogenized and extracted to estimate liver cholesterol and total lipids according to Richmond (1973) and Folch *et al.*, (1957), respectively. Final weight, body weight gain, food intake, and Feed efficiency ratio (FER) was calculated at the end of the experiment (Chapman *et al.*, 1950). Atherogenic indices were calculated from TC / HDLc and LDLc/ HDLc (Castelli and Levitar, 1977).

Statistical analysis: The obtained data were statistically analyzed and the significant difference between groups was evaluated by t-test as explained by Snedecor and Cochran (1982).

Results and Discussion

Table 1 summarized the proximate composition of fine wheat flour and apple powder applied in this study. The apple powder was characterized by lower moisture, protein and carbohydrate and higher content of fat, fiber, ash and pectin compared to wheat flour. Pectin was not detected in wheat flour. These results were agreed with Kamaljit *et al.*, (2011), Reis *et al.*, (2012) and Ktenioudaki *et al.*, (2013). The composition of apple pomace with respect to its fiber content viz sugar, cellulose, hemicelluloses, pectin and roughage appears to have the best proposition for incorporation in the bakery industry for production of high fiber baked foods. The crude fiber content of apple pomace is approximately 14-30% of the dry weights. Apple fiber is higher in total dietary fibers than wheat and oat bran so it has good water holding capacity in certain food products. Apple pomace from the straight pressing, the primary by-product of the apple juice industry, is rich in cell wall material and is an interesting source of pectins (Massiot and Renard, 1997).

Table 1: Proximate composition of wheat flour and apple fiber powder

Variables Samples	Moisture %	Fat %	Protein %	Fiber %	Ash %	CHO %	Pectin
Wheat flour	12.51±1.33a	1.39±0.11b	12.13±2.66a	2.89±0.21b	0.77±0.01b	70.31±3.66a	-
Apple powder	5.55±0.42b	2.75±0.17a	7.53±0.38b	48.23±0.21a	1.77±0.07a	34.17±2.14b	19.55±1.24a

Mean values in each column having different superscript (a, b, c, d) are significant

Table 2 showed that high amounts of iron, zinc and sodium and low amount of calcium were found in wheat flour compared to apple powder. Apple variety, maturation stage, soil conditions, fertilization, irrigation and temperature effect on content of apples. The obtained values for Ca, Fe, Na and Zn found in the apple ash were similar to those reported by Gorinstein *et al.*, (2001). Fortification of biscuit with apple powder resulted non significantly increased protein, and ash, content while fat, and carbohydrate content was non significantly decreased as compared to ordinary biscuit. Apple biscuit showed significant increase in fiber and significant decrease of moisture as shown in Table 4. Apple biscuit had significant increase in content of iron and non significant increase in content of calcium, zinc and sodium compared to ordinary biscuit as shown in Table 5. The results agreed with previous research that apple powder is a rich source of carbohydrates, total dietary, fiber including cellulose, hemicellulose, lignin, pectin, and galacturonic acid, and minerals such as calcium, magnesium, zinc, iron, and copper. Apples also offer some advantages over cereal brans and legume hulls. It lacks phytic acid which renders minerals like zinc unavailable (Gupta, 2006, Kohajdová *et al.*, 2009, O'Shea *et al.*, 2012 and Reis *et al.*, 2012).

Table 2: Mineral content of wheat flour and apple fiber powder

Variables Samples	Iron (mg/kg)	Calcium (mg/kg)	Zinc (mg/kg)	Sodium(mg/kg)
Wheat flour	23.77±2.11a	173.61±11.33b	38.36±3.61a	49.13±3.05a
Apple powder	4.41±5.61b	310.31±46.33a	1.33±0.05b	10.62±1.33b

Mean values in each column having different superscript (a, b, c, d) are significant

Table 3: Ingredients of ordinary biscuit and apple biscuit

Ingredient	Wheat flour	sucrose	Butter	Milk	Egg	Vanillin	NaHCO ₃	NH ₄ HCO ₃	Salt	Water	Apple powder
Ordinary biscuit	100	32	15	19	25	1.5	0.30	0.65	0.5	30 ml	-
Apple biscuit	85	32	15	19	25	1.5	0.30	0.65	0.5	30	15

Table 4: Nutritive values of ordinary and apple biscuits

Variables Samples	Protein %	Fat %	Ash %	CHO %	Fiber %	Moisture%
Ordinary biscuit	14.55±1.76a	19.66±1.95a	1.02±0.14a	45.66±4.25a	8.71±1.11b	10.41±2.14a
Apple biscuit	13.05±1.26a	19.45±0.99a	1.55±0.15a	44.92±5.33a	12.48±2.14a	8.55±0.41b

Mean values in each column having different superscript (a, b, c, d) are significant.

Table 5: Mineral content of ordinary and apple biscuits

Variables Samples	Iron (mg)	Calcium(mg)	Zinc (mg)	Sodium(mg)
Ordinary biscuit	75.99±5.41b	569.11±45.50ab	125.66±10.72ab	196.55±19.61ab
Apple biscuit	97.41±6.45a	603.11±33.66a	139.71±11.21a	201.41±15.96a

Mean values in each column having different superscript (a, b, c, d) are significant

Table 3 illustrated the ingredients of ordinary and apple biscuits. These ingredients were in agreed with Srivastava *et al.*, 2012 and Sudha *et al.*, (2007) who recorded that biscuits have different nutritionally rich ingredients as wheat flour, sugar, salt, fat and water. The Sensory evaluation revealed that apple biscuit had non significant decrease in shapeliness, crust color, texture, softness and non significant increase in crumb color,

aroma and eating quality but significant decrease in texture compared to ordinary biscuit as illustrated in Table 6. The obtained results were agreed with results of Sudha *et al.*, (2007) who recorded that addition of apple powder provides fruity taste attributes such as fruit aroma and taste, thus allowing reducing the level of sugar added and also avoiding the use of many other flavoring ingredients. Addition apple powder exhibited significantly higher firmness values and the same effect was observed after incorporation of dietary fiber rich by products from different sources into the bakery products (Ajila *et al.*, 2008 and Abdul-Hamid and Luan, 2000). Further it was observed that addition of apple powder resulted in markedly darker colour of cookies (Garau *et al.*, 2007). However, Kohajdová *et al.*, (2014) reported that no significant differences were observed in the overall acceptance of ordinary biscuits and biscuits with 5 mass % of apple powders. Furthermore, it was found that higher amounts (10 mass % and 15 mass %) of apple powders significantly reduce the overall acceptance of biscuits.

Table 6: Sensory properties of ordinary and apple biscuit.

Variables Samples	Shapeliness (10)	Crust color (10)	Crumb color (10)	Brightness (10)	Texture (10)	Softness (10)	Crust character (10)	Aroma (15)	Eating quality (15)
Ordinary biscuit	9.22± 0.96a	9.50± 1.03a	9.11± 1.32a	9.44± 0.25a	9.66± 0.96a	9.56± 1.31a	0.50± 0.88a	13.77± 1.25a	13.88± 1.41a
Apple biscuit	8.55± 1.05a	9.41± 1.30a	9.22± 1.38a	8.11± 0.48b	8.59± 1.25b	8.66± 1.34a	8.77± 1.21a	14.70± 1.61a	14.11± 1.55a

Mean values in each column having different superscript (a, b, c, d) are significant.

Hyperlipidemic control +ve and ordinary rat groups showed significant increase in final weight, weight gain and FER and that appeared obviously in control +ve group compared to control –ve group while apple group showed improvement in these parameters and appeared within normal. Food intake appeared non significant compared to control –ve group as illustrated in Table 7. Bakery products are sometimes used as a vehicle for incorporation of different nutritionally rich ingredients. Addition of dietary fiber to bakery products increases fiber intake and decreases of the caloric density of baked goods. Apple powder is also good source of pectin compounds (Uchoa *et al.*, 2009 and Hussein *et al.*, 2011). Soluble fiber develops high viscosity, which is frequently associated with the effects of reducing gastric emptiness, thus promoting a greater fulfillment sensation. Therefore, in our study, diet consumption did not increase with the increase of apple flour concentration (Plaami, 1997). Moreover, the incorporation of enriched fiber flours with significant values of antioxidants is a way to improve the nutritional value of these snacks (Ainsworth *et al.*, 2007 and Stojceska *et al.*, 2008). Pectin is fermented in the intestines, producing short-chain fatty acids that prevent the growth of harmful bacteria. Food intake of apple group was similar to control –ve because soluble fiber develops high viscosity which could reduce gastric emptiness so promoting a greater fulfillment sensation (Marlett *et al.*, 2002 and Donalson 2004).

Table 7: Body weight, food intake and FER in hyperlipidemic rat groups

Variables Groups	Initial weight	Final weight	Weight gain	Food intake	FER
Control -ve	103.44± 5.67a	193.71± 12.23c	90.27± 5.11c	14.85± 1.22b	0.135± 0.002c
Control +ve	104.55± 4.33a	270.41± 11.08a	165.86± 6.75a	17.77± 2.14a	0.207± 0.001a
Ordinary	105.33± 5.60a	255.11± 10.70ab	149.78± 9.66b	16.78± 2.11ab	0.198± 0.004b
Apple	103.77± 4.96a	198.88± 9.41c	95.11± 3.60c	15.13± 1.70b	0.139± 0.003c

Mean values in each column having different superscript (a, b, c, d) are significant.

The feeding of hyperlipidemic diet to rats all over the experimental periods (Control+ve group) showed highly significant increase in serum TC, TG, LDLc and VLDLc and significant decrease in HDLc when compared to control -ve group. Consumptions of ordinary biscuit showed significant increase in serum TC, TG, LDLc and VLDLc when compared to control -ve group and significantly decrease in compared to control +ve group while HDLc was in non significant decrease in compared to control-ve group. Consumptions of apple biscuits showed non significant increase in serum TC, TG, LDLc, VLDLc and HDLc compared to control –ve (Table 8). Several investigations have shown that rat receiving cholesterol- enriched diet showed sever hyperlipidemia hypercholesterolemia, elevated serum total cholesterol, LDLc and VLDLc and decreased high density lipoprotein levels compared to the control group of rats fed on a normal diet (Bennani-Kabchi *et al.*, 2000 and Augusti *et al.*, 2001).

Control +ve group showed highly significant increase in atherogenic indices and also ordinary group showed increased in atherogenic indices compared to control –ve and significant decrease than control +ve. Apple group showed non significant difference in TC/HDLc and significant increase in LDLc /HDLc compared to control –

ve as shown in Table 9. Liver cholesterol and total lipids were significantly increased in control +ve group and also increased in ordinary biscuit group compared to control –ve group. Apple rat group had non significant increase in liver cholesterol and total lipids compared to control–ve group as illustrated in Table 10. Fruits are a natural source of dietary fiber, trace elements and antioxidant compounds, and that diets rich in fruits positively influenced plasma lipid levels and antioxidant capacities in experiments with laboratory animals

Table 8: Serum lipid profiles in hyperlipidemic rat groups

Variables Groups	TC (mg/dl)	TG(mg/dl)	HDLc (mg/dl)	LDLc (mg/dl)	VLDLc(mg/dl)
Control -ve	87.41±8.81cd	95.77±9.71cd	37.99±3.66ab	30.27±2.99d	19.15±1.17cd
Control+ve	186.31±18.22a	174.33±16.66a	28.66±3.22c	122.79±11.33a	34.86±4.10a
Ordinary	135.77±11.66b	14.03±15.27b	33.55±3.11bc	74.16±7.11b	28.06±3.01ab
Apple	101.61±9.65c	109.66±8.80c	39.66±3.20a	40.02±5.11c	21.93±2.11c

Mean values in each column having different superscript (a, b, c, d) are significant

Table 9: Atherogenic indices in hyperlipidemic rat groups

Variables Groups	TC/HDLc	LDLc /HDLc
Control -ve	2.30±0.57c	0.79±0.01d
Control+ve	6.50±1.21a	4.28±0.41a
Ordinary	4.04±0.55b	2.21±0.31b
Apple	2.56±0.33c	1.01±0.22c

Mean values in each column having different superscript (a, b, c, d) are significant

(Gorinstein *et al.*, 2002). Liver is the primary organ involved in cholesterol and lipid metabolism, transport and excretion, it is logical to assess the hepatic total lipids and cholesterol status in hyperlipidemia condition. The dietary use of 5% unprocessed pomace caused an increase in cecal short-chain fatty acid production and a decrease in blood triacylglycerols, leading to a drop in serum atherogenic index. The variations in the atherogenic index indicate flavonoids as the key pomace component in relation to blood lipid profile benefits (Kosmala *et al.*, 2011). Pectins are examples of soluble fibers as in apple that can dissolve in water, slows the movement of food through the body and maintain healthy cholesterol. Dietary fiber in apple may give protection against cardiovascular diseases, diabetes and obesity (ADA 2008 and Pasha *et al.*, 2008). Apple powder is also a good source of phytochemicals primarily phenolic acids such as chlorogenic, protocatechuic, caffeic acid and flavonoids (flavanols and flavonols) that have been associates with many health-enhancing benefits as cancer cell proliferation, lipid oxidation decrease and cholesterol lowering (Reis *et al.*, 2012, O'Shea *et al.*, 2012 and Plaza *et al.*, 2014).

These data suggest that apple powder has chemical and nutritional importance and biscuits fortified with 15% apple powder can be successfully incorporated in biscuit conferring hpolipidemic properties to the final products.

Results indicate that apple powder, a by-product from apple processing, could be considered as an alternative dietary fiber for cookies and other bakery products that could give protection from chronic disease.

Table 10: Liver cholesterol and total lipids in hyperlipidemic rat groups

Variables Groups	Cholesterol	Total lipids
Control -ve	6.33±1.22b	36.38±3.41c
Control+ve	9.66±1.45a	49.86±4.29a
Ordinary	7.33±1.29ab	42.93±4.44ab
Apple	6.77±1.11b	39.78±3.60bc

Mean values in each column having different superscript (a, b, c, d) are significant

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