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Fortification of Macaroni with Pomegranate Peels as Dietary Fiber and Natural Antioxidant for the Treatment of Obesity and High Cholesterol in Rats

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Abstract: Pomegranate (*Punicagranatum*) is rich with medicinal properties and extensively used in traditional medicines. Dried pomegranate peels were grinding to fine powder and it was added to produce macaroni as dietary fiber and natural antioxidant at different levels to give four blends. The first, second, third and fourth blends had added at levels 10, 15, 20 and 25% of peel powder which was compared with macaroni control that contained 100% semolina wheat considerable as control. Chemical, physical and organoleptic properties were determined in different macaroni blends. The results observed the pomegranate peel consisted of rich amounts from crude fiber, fiber fractions, and also, total phenolic acids and total flavonoids compounds. The sensory characteristics of macaroni made from blends at different levels from pomegranate peel give the acceptability results. At the end of the biological experiment of duration for four weeks all the measurements for the analysis of blood lipids in rats fed with macaroni blends and compare the results from two negative and positive control groups. The results reported that when the pomegranate peel increased in macaroni the cholesterol was lowered in rats' hypercholesterolemia. From the resultant it could be concluded that the uses peel pomegranate powder as dietary fiber and natural antioxidant to produce macaroni blends at different levels gives the best results for sensory characteristics till 20% peel pomegranate and lowering cholesterol in rats' hypercholesterolemia.

Key words: Pomegranate peel - natural antioxidant - biological experiment – parameters of blood lipids

INTRODUCTION

The pomegranate (Punicagranatum) is a nutrition source and it high contained phytochemical compounds and its extracts are utilized as vegetarian components, herbal medicines and dietary supplements (Elfalleh et al., 2011). Pomegranate is a great significance source of bioactive ingredients that are known to possess enormous antioxidant activity. Thus, pomegranate juice is influential in the protection of atherosclerosis, low-density lipoprotein oxidation, platelet aggregation and heart diseases due to its contained natural antioxidant (Singh et al., 2002 andAdhami and Mukhtar, 2006Also, it has anti microorganisms and antimutagenic properties in additional useful influence on oral and heart diseases. Moreover, this pomegranate peels have been found to have pronounced had contained high amount from antioxidant activity (Nagi and Jayprakasha, 2003).

Pomegranate fruits peel is an inedible part after processing pomegranate juice. Pomegranate peel had contained rich amounts of antioxidant compounds (Li et al., 2006). Reddy et al. (2007) and Al-Zoreky (2009) reported that the antioxidant and antibacterial characteristics of pomegranate peel have been less attention as a natural antioxidant in meat (Devatkal and Naveena, 2010Pomegranate peel extract had contained natural antioxidant and antimutagenic characteristics and there could be used as bio-preservative in food process and nutraceuticals (Naveena et al., 2008). In addition to their nutritional value, pomegranate peels were used since ancient times as anti-thelmintic, anti-tracheobronchitis, for healing wounds, ulcers, bruises, stomatitis, diarrhea, vaginitis, and against excessive bleeding Ross (2003). In recent years, more medicinal values of pomegranate peel have been investigated such as abortifacient, analgesic, antiameobic, antibacterial, anticonvulsant, antifungal, antimalarial, anti-mutagenic, antiviral, antispasmodic, diuretic, hypoglycemic, hypothermic, and antioxidant activities Seeram et al. (2006). The major class of pomegranate phytochemicals is the polyphenols that are predominant in the fruit and includes flavonoids, condensed tannins and hydrolysable tannins (HTs) (ellagi-tannins and gallo-tannins) Seeram et al. (2005). These tannins are highly susceptible to both enzymatic and non-enzymatic hydrolysis. Additional of pomegranate peel powder which include organic acid and polyphenolic, volatile oils, and alkaloids Fischer et al. (2011). The ellagi-tannins present in the pomegranate peel had contained about 92% from total antioxidant of pomegranate peel fruit Gil et al. (2000). Thus, the health useful of pomegranate peel is accredited for the pharmacological activities shown by bioactive phytochemical like polyphenolic. Natural antioxidants as a reduction of the lipid oxidation, and also antioxidant is prevent oxidation and have the ability for against scavenging of free radicals in tissues and thus the natural antioxidant are benefits health Barlow (1990).

The target of this study was achieved to estimate the effect of macaroni blends made from peel pomegranate powder from pomegranate fruit at different levels as dietary fiber and natural antioxidant on sensory evaluation of different macaroni blends and also, the effect of different macaroni formulae on serum total lipids, total cholesterol and triglycerides in hypercholesterolemia rats.

MATERIALS AND METHODS

Methods:

Preparation of peel pomegranate powders:

Mature pomegranate fruits were washed and cut manually to separate the peel (rind) and arils. Powder from pomegranate peel was prepared by drying in an air circulatory tray drier (WT-bimderTuttlingen / Germany) at 60 ºC for 48 h. The dried pomegranate peel was powdered in a kitchen grinder and sieved using a 60 mesh sieve, and packed into high density polyethylene bags and stored at – 4 ºC in refrigerator until used according to Singh and Sethi (2003) and Devatkal and Naveena (2010).

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Chemical analysis of raw materials:

Moisture, protein content, ash, crude fiber, lipids content and total carbohydrates were determined in pomegranate peel according to AOAC (2010). Total dietary fibers, soluble and insoluble dietary fibers were determined in raw materials according to Prosky et al. (1988).

Determination of minerals content:

Macro elements (calcium and magnesium) and Microelements (iron, zinc, manganese, selenium, aluminum and copper) of pomegranate peel were determined according to the method of the AOAC. (2010), using Atomic Absorption Spectrophotometer (Perkin Elmer, Model 3300, Germany). Phosphorus was determined by spectrophotometer according to the AOAC. (2010), while sodium and potassium contents were determined by Flame Photometer (CORNING 400, serial No. 4889.UK).

Determination of total phenolic compounds:

Total phenolic compounds was carried out according to existing protocols in the laboratory as described by Rajauria et al. (2013) using the Folin Ciocalteu assay. Results were expressed in mg gallic acid equivalents per gram of dry weight (mg GAE/g) extrapolated from a calibration curve of gallic acid (0-500 µg/mL).

Determination of total flavonoids content:

The aluminium chloride assay described by Jaiswal et al. (2012) was used to quantify the total flavonoids compounds of each herb. Results were expressed in mg quercetin equivalents per gram (mg QE/g) dry weight, through a calibration curve of quercetin (0-100 µg/mL).

Macaroni preparation processing:

The ingredients preparation processing macaroni are reported in Table (1). The ingredients were mixed in Hobert mixture at high speed until uniformly (10 min.) and the required amount of water was added. Macaroni was processed using a Demaco (De Francise Machine Corporation) Semi commercial scale Laboratory extruder, according to the method described by Dexter et al. (1990). The macaroni was dried at 60oC for 24h and the relative humidity was 75 to 85% according to Dexter et al. (1990).

Table (1): The ingredients macaroni blends (on dry weight bases/100g).

Blends	Semolina wheat	Peel pomegranate
Blend 1	100	-
Blend 2	90	10
Blend 3	85	15
Blend 4	80	20
Blend 5	75	25

Determination of starch:

Starch was determined by the method of dispersal in CaCl2, followed by iodine spectrophotometry (Idris, 2001). The blue color intensity was measured at 610 nm using WPA S101 spectrophotometer.

Estimation of amylose content:

A rapid colorimetric method described by William et al. (1975) was used for estimating the amylose content.The volume was diluted to 50ml and the absorbance of blue colour was measured at 625 nm after 5 minutes.

Determination of amylopectin:

The amylopectin content of starch was calculated by difference. Amylose content was calculated as percentage (%) from the starch. Then amylose percent was subtracted from 100 to obtain amylopectin content of starch (Idris ,2001) .

Sensory evaluation:

Samples of the variable macaroni were cooked to optimum cooking time (10minute for macaroni) and with optimum boiling water (1 liter/100gm sample). Then rinsed and drained off. A panel of ten panelists were chosen to judge the quality of the pasta (external appearance, firmness, stickiness, color, aroma, taste and general acceptability)-using the scoring test of 5 points described by Bahnassey and khan,(1986).

Sensory evaluation of macaroni blends:

The macaroni at different blends after cooking were organoleptically evaluated for their taste, odor, stickiness, color and appearance according to Dexter et al. (1990) by twenty experienced panelists.

Nutritional experiments:

Male adult rats (42 rats) weight ranging 140-150g were purchased from Central Animal House in Jeddah, Saudi Arabia. Animals..Rats were housed in individual cages with screen bottoms and fed ad libitum on a basal diet for one-week for acclimatization, which containing casein (20 %), corn oil (8%), corn starch (31%), sucrose (32%),mg cellulose (4%), salt mixture (4%) and vitamin mixture (1%) according to the method Pell et al. (1992 After feeding on basal diet for eight days, rats were divided into two groups. The first group consists of 6 rats were fed on the basal diet and considered as a negative control group. The second main group (36 rats) was fasted overnight and injected with streptozootocin (was dissolved in 0.1M citric acid buffer and adjusted at pH 4.5) into the leg muscle (5mg /100g body weight) to induce diabetic rats according to Madar (1983).After 48 h of injection the second main group was divided into six subgroups (6 rats for each). The first subgroup was fed on basal diet and considered as a positive control. From the second to six subgroups (6 rats for each) were fed on 20% from macaroni after cooking prepared from five blends contained obviously ingredients. Each rat was weighted every two days and the gain body weight was calculated.

At the end of experimental period (four weeks), the blood samples were taken with drawn from the orbital plexus and centrifuged at 3000 rpm to obtain the sera. After that, the sera were kept on a deep freezer at -20oC until their analyses.

Serum glucose, total cholesterol and triglycerides were determined according to knight et al. (1972), Fossati and Prencipe (1982) and Tietz (1986), respectively. High- and low-density lipoprotein- cholesterol in serum was determined according to Burstein (1970) and Fruchart (1982).

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).

RESULTS AND DISCESSION

Nutrition compounds of pomegranate peel powder:

The gross chemical components; namely crude protein, crude fat, ash content, crude fibers and carbohydrates, content of pomegranate fruits peel powder are represented as in Table (2). From the resultant, it could be noticed that the moisture content of produced pomegranate fruits peel powder was found to

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be 13.7 %. In addition, crude protein, crude fat, ash, crude fibers and carbohydrates contents for pomegranate fruits peel powder were 3.10, 1.73, 3.30, 11.22 and 80.50 %, on dry weight basis; respectively. Thereupon, the pomegranate fruits peel powder is considered a good source of crude fibers, ash and carbohydrates. Therefore, pomegranate fruit peels powder should be utilized in fortification of food stuffs. These results were agreement according to the resultant with those found by Fadavi et al. (2006) and Kingsly et al. (2006).

Table (2) showed that the pomegranate fruits peel powder had the highest amounted from total dietary fiber; soluble and insoluble dietary fibers were 56.23, 43.54 and 12.69%, respectively. These results are agreement with Figuerola et al. (2005) who found the total dietary fibers are great significance to functional properties. Generally, those fibers to be utilized as a nutritional component should have contained soluble dietary fiber to insoluble dietary fiber ratio close to 1/2.

Total phenolic acid and flavonoids compounds were determined in pomegranate fruits peel powder and the results are found in the same table. The results could be noticed the pomegranate fruits peel powder had higher amounted from total phenolic acid and flavonoids compounds were 58.63 mg/g GAE and 47.32 mg/gQ E, respectively. Pomegranate had contained rich amounts in natural antioxidant of polyphenolic class which includes tannins, anthocynins phenolics and flavonoids compounds (Ricci et al., 2006 and De Nigris et al., 2007). Pomegranate is useful for cases of high fever, chronic diarrhea and expelling intestinal worms especially tapeworms and treatment of hemorrhoids, as it is beneficial to cold and treatment of skin disease, scabies and a mix powder peel with honey and used daily in the form of paint.

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Table (2): Gross chemical composition of pomegranate peel (on dry weight basis)

Chemical components	Pomegranate peel powder
Moisture	13.7
Protein	3.10
Fat	1.73
Crude fibers	11.22
Ash content	3.30
Total carbohydrates	80.65
Total phenolic mg/100g	27.92
Total dietary fiber	56.23
Soluble dietary fiber	43.54
Insoluble dietary fiber	12.69
Total phenolic GAE mg/g	58.63
Total flavonoids Q E mg/g	47.32

Minerals content of pomegranate peel:

The nutritional quality of pomegranate peel powder with regards their minerals content was evaluated and the obtained results are recorded as in Table (3). The obtained results Table (3), showed the pomegranate fruits peel powder had contained at all test minerals, with the exception of Mg which was not detected in them. The fruits peel powder had contained the most determined minerals at adequate concentration and the predominant minerals in it were found to be Ca, K, P and Na at level of 338.5, 146.4, 117.9 and 66.4 mg/100g dry matter; respectively. In addition, the pomegranate peel powder had contained a considerable content of Fe, Zn and Cu at level of 5.93, 1.01 and 0.60 mg/100g dry matter; respectively. In general, it could be concluded that pomegranate fruits peel were characterized with their richness with the most determined nutritious minerals and they are consisted of a perfect source of macro and micro elements. Therefore, they have to be used in food application. Iron is important to control of infection and cell immunity (Beard, 2001). The decrease of iron has been described as the nutritional shortage due to anemia (Trowbridge and Martorell, 2002). Zinc is an absolutely necessary micronutrient for human growth and immune functions (Black, 2003). Manganese is plays a significant role in physiological processes as a constituent of some enzymes and an activator of other enzymes Nielsen (1999).

Table (3): Minerals content (mg/100g dry matter) of pomegranate peel

Minerals content	Pomegranate peel powder	RDA* (mg/day)	
		Children	Adults
Ca	338.5	800	800-1200
Mg	--	--	--
K	146.4	--	--
Na	66.43	808	800-1200
P	117.9	10	10-15
Fe	5.93	10	12-15
Zn	1.01	--	---
Mn	0.80	--	---
Cu	0.60	--	---

RDA*: Recommended dietary all allowances from minerals reported by Food and Nutrition Board, (1989).

Starch, amylose and amylopectin content of macaroni before and after cooking:

Starch, amylose and amylopectin content were determined in macaroni before and after cooking and the findings are tabulated in Table (4). The macaroni before cooking were decreased in starch, amylase and amylopectin when the pomegranate peel powder was increased in the different blends. Whilst, after cooking the macaroni blends at 10, 15, 20 and 25% pomegranate peel powder were decreased in starch by 9.37, 15.0, 20.0 and 25.0%, respectively. Moreover amylase and amylopectin were reduced by 14.44, 19.20, 23.95 and 28.7% in amylase and amylopectin was decreased by 10.50, 15.46, 20.44 and 25.41%, respectively. These decreases may be due to the blend macaroni had contained different levels from the pomegranate peel powder which contained high amounts from dietary fiber and natural antioxidants. Pasta is prepared mostly from durum wheat semolina (*Triticum turgidum* subsp. durum) with a small rise in the consumption of wholegrain/whole meal and bran containing pasta occurring over the last decade (Prückler et al., 2014). Regular pasta, which is made from semolina, is not an ideal source of dietary fiber as most have been removed during the milling of the grain. There is good evidence that regular consumption of wholegrain cereals offers a reduced risk of certain diseases like type 2 diabetes and cardiovascular disease (Zong et al., 2014). Despite this knowledge, the daily intake of dietary fiber falls well short of daily recommendations, with more than 90% of the population of the USA, for example, not meeting target levels (Jones, 2014).

Table (4): Starch, amylose and amylopectin content of macaroni before and after cooking

Blends	Starch %		Amylose %		Amylopectin %	
	Before cooking	After cooking	Before cooking	After cooking	Before cooking	After cooking
Control	65.00±0.56^{a}	48.00±0.42^{a}	22.50±0.14^{a}	16.83±0.13^{a}	42.57±0.41^{a}	31.17±0.30^{a}
Blend 1	59.50±0.54^{ab}	43.50±0.41^{ab}	19.80±0.18^{ab}	14.40±0.13^{ab}	37.80±0.45^{ab}	27.90±0.31^{ab}

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Blend 2	55.23±0.49ab	40.80±0.38^{ab}	18.70±0.12^{ab}	13.60±0.18^{ab}	35.70±0.39^{ab}	26.35±0.28^{ab}
Blend 3	52.00±0.57^{b}	38.40±0.35^{b}	17.60±0.19^{b}	12.80±0.15^{b}	33.60±0.35^{b}	24.80±0.27^{b}
Blend 4	48.75±0.41^{c}	36.00±0.29^{c}	16.50±0.11^{c}	12.00±0.12^{c}	31.50±0.28^{c}	23.25±0.16^{c}

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

Sensory evaluation for different blends of macaroni:

Pomegranate peel powder was added to prepare macaroni at different levels with semolina flour. The macaroni products were sensory evaluated after cooking and the findings are tabulated in Table (5). The sensory properties of the blends 1, 2 and 3 were high general acceptability by 4.18, 3.88 and 3.58, respectively and nearly control macaroni was 4.62.Moreover, it is observed that the sensory properties were decreased when the amount of pomegranate peel powder increasing. These findings may be due to the result of firmness, color and stickiness was reduced with increasing pomegranate peel powder amounts this cause influencing positively the overall quality of the macaroni blends. Whilst, the results from blends macaroni (1 and 2) were slightly decreased during external appearance followed by blends macaroni (3 and 4) may be due to pomegranate peel powder had contained high amounts of total dietary fiber 56.23%. Dietary fibers are highly water-binding and caused competing with starch for water absorption and the resulting in a greater endothermic temperatures value (Singh et al., 2003).

Table (5): Sensory properties of different macaroni

Blends	External appearance	Firmness	Stickiness	Color	Aroma	Taste	General acceptability
Control	5.00±0.02^{a}	4.70±0.04^{a}	4.30±0.07^{a}	4.70±0.05^{a}	4.50±0.04^{a}	4.50±0.06^{a}	4.62±0.06^{a}
Blend 1	4.55±0.02^{ab}	4.20±0.05^{b}	4.10±0.05a	4.00±0.04^{b}	4.25±0.05^{ab}	4.00±0.05^{b}	4.18±0.04^{b}
Blend 2	4.25±0.04^{ab}	3.75±0.02^{bc}	3.80±0.03^{ab}	3.75±0.03^{bc}	4.00±0.04^{b}	3.75±0.02^{bc}	3.88±0.05^{bc}
Blend 3	4.00±0.01^{b}	3.25±0.03^{bc}	3.50±0.04^{b}	3.50±0.04^{c}	3.75±0.06^{c}	3.50±0.03^{c}	3.58±0.03^{c}
Blend 4	3.50±0.03^{c}	3.00±0.02^{c}	3.00±0.02^{c}	3.00±0.02^{d}	3.50±0.04d	3.20±0.04^{d}	3.20±0.01^{d}

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

Effect macaroni from pomegranate peel powder on body weight in rats:

Table (6) found the influence of macaroni from pomegranate peel powder at different level on the initial, finial, gain body weight; total food intake and feed efficiency ratio of hyperglycemic rats and the results at the end of experimental (30 days) are found in Table (6). Moreover, it could be indicated the control negative was the highest in gain body weight and feed efficiency ratio by 82.08g and 13.18% than control positive was 6.58g and 1.92%, respectively. Moreover, the hyperglycemic rats fed on basal diet contained on 20% from the macaroni in all blends were slightly gradually increased in gain body by 51.32, 55.10, 60.47, 65.58 and 70.25g, respectively. Also, the same results from feed efficiency ratio were increased gradually in all blends. This slightly gradually increased in gain body weight and feed efficiency ratio in hyperglycemic rats may be due to the different macaroni blends containing pomegranate peel powder maybe due to the presence of dietary fiber there by leading to poor food intake. Dietary fiber had contained rich amounts from carbohydrates and lignin non digestible that has beneficial physiological effects in humans (Slavin, 2005).

Table 6: Effect of different diets on body weight in the rats

Groups	Initial BW (g)	Final BW (g)	BW gain (g)	Total Food intake (g)	Feed efficiency ratio
Control ve- G1	141.92 ±11.93	224.00 ±16.95	82.08 ±5.59	622.80 ±20.39	13.18 ±0.81
Control G2 ve+	147.98 ±12.16	154.57 ±12.06	6.58 ±0.83	362.47 ±9.30	1.92 ±0.02
Control macaroni	150.78 ±12.82	202.10 ±12.59	51.32 ±4.87	568.95 ±7.83	9.02 ±0.07
Blend 1	146.45 ±12.47	201.55 ±13.43	55.10 ±3.94	573.23 ±15.54	9.61 ±0.91
Blend 2	145.23 ±12.95	205.70 ±13.42	60.47 ±5.68	590.52 ±16.26	10.24 ±0.42
Blend 3	142.3 ±11.44	208.18 ±16.65	65.85 ±4.67	611.92 ±15.73	10.76 ±0.61
Blend 4	145.38 ±10.38	215.63 ±14.29	70.25 ±3.44	620.35 ±15.69	11.32 ±0.54

Values are mean and SD (n = 3)

Effect of macaroni blends after cooking on hyperglycemic in rats:

The parameters from serum hypercholesterolemia rats as triglycerides, total cholesterol, cholesterol fractions and blood sugar were determined in hyperglycemic rats and the findings are showed in Table (7). Furthermore, the rat fed on blend (5)was significant decreased in lipids profile and blood sugar followed by blends (4 and 3)fed on macaroni contained 20 and 15% pomegranate peel powder showed that lowering lipid profile and blood sugar compared with positive control fed on basal diet. These results confirmed with Colonna et al. (1990) who found that the soluble dietary fibers are decreasing sugars from the food and also lowering blood glucose ratio may be due to reduced amylolysis and specifically at the gastrointestinal level, through delayed gastric emptying.

In this concern, pomegranate fruits peel can be used as functional ingredient as a good source of crude fibers and natural antioxidants which provide numerous health benefits such as decreased serum LDL-Cholesterol level and the insulin response, reduced hyperlipidemia and hypertension, contribute to gastrointestinal health and the prevention of colon cancer (Lansky and Newman, 2007 and Viuda-Martos *et al*., 2010 a,b).

Table 7: Effect macaroni blends on lipid profile and blood sugar:

Groups	Triglyceride (mg/dl)	Total cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Blood sugar (mg/dl)
Control negative	108.66 ±3.88^{d}	116.25 ±3.61^{e}	74.36 ±1.47^{a}	21.61 ±2.32^{e}	90.25 ±2.35^{e}
Control positive	191.75 ±2.83^{a}	207.21 ±4.03^{a}	25.55 ±1.69^{d}	127.52 ±2.43^{a}	198.23 ±3.61^{a}
Control macaroni	188.50 ±4.52^{a}	193.63 ±3.63^{b}	27.15 ±1.05^{d}	120.90 ±3.30^{a}	190.47 ±3.11^{a}
Blend 1	168.40 ±5.19^{b}	171.53 ±2.90^{bc}	31.08 ±2.08^{cd}	89.01 ±0.82^{b}	170.1 ±2.39^{b}
Blend 2	141.27 ±3.57^{bc}	150.38 ±3.52^{c}	41.53 ±3.25^{c}	66.28 ±3.56^{c}	155.6 ±2.97^{c}
Blend 3	121.53 ±2.18^{c}	132.46 ±4.13^{d}	58.58 ±4.13^{b}	49.08 ±4.46^{d}	130.3 ±2.57^{d}
Blend 4	113.88 ±5.02^{d}	123.17 ±2.57^{e}	65.92 ±2.71^{a}	29.40 ±2.91^{e}	106.7 ±1.96^{e}

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

Conclusion:

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The obviously results concluded the macaroni fortified with pomegranate peel powder at different levels had revealed some useful information on their food industries. Pomegranate peel powder was contained rich amounts from total dietary fiber and natural antioxidants that are beneficial for health. The pomegranate peel powder components have made better the cooking of macaroni acceptability, improved nutritional value and desirable texture qualities for health consumers and hypoglycemic.

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