

**Biochemical and Biological
Impact of Lemon and
Kumquat(Citrus Limon
and Citrus Japonica)
on Hypercholeserolemic
Rats**

Dr. Dalia R. Hassan
Home Economic Department,
Faculty of Specific Education,
Fayoum– University



المجلة العلمية المحكمة لدراسات وبحوث التربية النوعية

المجلد الثامن - العدد الرابع - مسلسل العدد (١٨) - أكتوبر ٢٠٢٢

رقم الإيداع بدار الكتب ٢٤٢٧٤ لسنة ٢٠١٦

ISSN-Print: 2356-8690 ISSN-Online: 2356-8690

موقع المجلة عبر بنك المعرفة المصري <https://jsezu.journals.ekb.eg>

JSROSE@foe.zu.edu.eg

البريد الإلكتروني للمجلة E-mail

Biochemical and Biological Impact of Lemon and Kumquat(Citrus Limon and Citrus Japonica) on Hypercholesterolemic Rats

Dr. Dalia R. Hassan

Home Economic Department, Faculty of Specific Education,
Fayoum– University
dr.dal.refaat@gmail.com

Abstract:

Hypercholesterolemia is the major risk factor for cardiovascular diseases, thus, this current study held to explore the positive effects of kumquat and Limon (peel and whole) adiposity and biochemical parameters in rats with hypercholesterolemia, as well as assessed the sensory properties of baked cookies containing 5% doses of kumquat and lemon(both peel and whole) . Thirty male albino rats were administered a basal diet as (control negative -ve) group of 5 rats each ; group (2) administered on hypercholesterolemic diet and the remaining groups(3th,4th,5th,6th) administered diet containing 5% kumquat and5% lemon (both peel and whole) with hypercholesterolemic diet for four weeks. Serum cholesterol and triglycerides, LDL,VLDL,HDL/LDL ,Moreover, ALT,AST, creatinine and uric acid were significantly decreased in groups supplemented with kumquat and Limon (peel and whole) at 5% . Therefore, kumquat and limon may consist of a valid strategy of phenolic compound which can be used in controlling hypercholesterolemia and its harmful consequences and enhance renal function in hypercholesterolemic rats. Also, sensory evaluation results revealed that cookies prepared with kumquat and Limon (peel and whole) at 5% were accepted by panelists with different ratings.

Key words: Citrus fruits, Limon, Kumquat, Dyslipidemia, Adiposity.

Introduction:

Cardiovascular disorders (CVD) that increase the production of reactive oxygen species (ROS) in the blood and tissues are most commonly caused by hypercholesterolemia (El Tantawy, 2015 and Baldissera *et al.*, 2017). Foods high in phytochemicals are regarded as a way to preserve excellent health in addition to providing nutrients for healthy body operation. Because orange peel is an industrial byproduct of the fruit processing industry, special focus was given to illuminating its health-promoting properties. Citrus fruits (high concentrations of phytochemicals and bioactive substances) are responsible for their health advantages (Toth *et al.*, 2016).

When consumed in moderation, the poly methoxylated flavones present in citrus peels may decrease total and LDL cholesterol without

having the negative side effects associated with conventional cholesterol medications. Findings by **Dayse et al. (2022)** suggest that kinkan orange may be utilised as an adjuvant in lipid problems and may be a diet that protects the heart.

Citrus fruits L. (*family Rutaceae*), according to **Giglio et al. (2016)**, protect against free-radical damage and increase high density lipoprotein cholesterol (HDL-C). Originally from China, kinkan orange (*Fortunella japonica*) is a good source of dietary fibre as well as a variety of bioactive substances, including as ascorbic acid, terpenoids, carotenoids, flavonoids, and essential oils (**Lim, 2012**).

These plants are extremely valuable as medicines since they contain a variety of chemical compounds that have physiological effects on people. Alkaloids, flavonoids, glycosides, saponins, resins, oleoresins, sesquiterpenes, phenolic compounds, and oils are only a few of the chemical components found in medicinal plants (**Devanooru et al., 2015**).

Hesperidin, a flavonoid found in lemon peel and pulp, lowers triglycerides and cholesterol. In a study conducted in collaboration with the US Department of Agriculture, compounds that isolated from lemon peels showed promise in animal studies as a potent natural alternative for lowering LDL cholesterol. These compounds lack the potential side effects of conventional cholesterol-lowering medications, such as liver disease and muscle weakness. Lemon peel is also a source of pectin, a natural fibre that lowers cholesterol levels (**Sohn et al., 2016 and Pultrin et al., 2017**).

The smallest member of the Rutaceae family of real citrus fruits is the kumquat (*Fortunella margarita Swingle*). Fruit is consumed with the peel or added to drinks since the flesh is sour. The minerals and phytochemicals found in kumquats are likewise very high quality. Kumquats work well in a variety of items (**Wang et al., 2012; and Peng et al., 2013**).

Given this, the current experiment was created to examine the value of lemon and kumquat (peeled and whole) fruits as preventative measures against various lifestyle-related diseases including hypercholesterolemia.

Materials and Methods:

Materials

Kumquat fruits and lemon (*citrus limon and citrus japonica*) were purchased from Agriculture research center, Cairo, Egypt.

Cholesterol, bile salts and casein were purchased from El-gomhoriya Pharm. and Chem. Ind. Comp., Cairo, Egypt.

RATS: The Ministry of Health and Population, Helwan, Cairo, Egypt, provided thirty male adult albino Sprague Dawley strain rats

(140±10g each). Wire cages with controlled environments were used to house the rats. As a week-long adaptation phase, rats were provided a regular meal. Rats were given their diet in specialised feeding containers to prevent food spillage. Additionally, glass tubes extending through the wire cages from an upside-down bottle held on one side of the cage were used to deliver water to the rats. Ad-labium food and drink were given and monitored every day.

Methods

The *citrus limon* and *citrus japonica* were cleaned, peeled, and dried in an oven at 50°C for four days until completely dry. The same procedure was used with entire fruits as well. Powders were created by grinding the dried fruit and peels. Glass jars containing the powdered citrus peel and fruit were maintained in the refrigerator at 4°C for examination. (IZLI *et al.*, 2018) .

Hypercholesterolemic induction:

By combining 2.5% cholesterol and 2% animal saturated fat with the typical baseline diet, a high cholesterol diet for the induction of hyperlipidemia was created. Carefully installed in the cage, the food was fed for 4 weeks (Pandya *et al.*, 2006).

Determination of total phenolic and flavonoids:

The Folin-Ciocalteu micro-method was used to quantify the total amount of phenol in the sample (Saeedeh and Asna, 2007). The approach of using the total flavonoid content as method by (Ordon *et al.*, 2006).

Design of the experiment

Albino adult male Sprague Dawley strain rats were randomly assigned to one of two main groups: the negative control group (first group) (n=5), which received a basal diet; and the hypercholesterolemic group (n=25), which received a basal diet (Reeves *et al.*, 1993) and was divided into five subgroups: the second group (positive group), as well as the third, fourth, fifth, and sixth groups, which received peels and whole fruits at a level of 5% for both kumquat and limon substituted from corn starch (*citrus limon* and *citrus japonica*) .

Rats were fasted overnight and given anesthesia before the experiment was over. Each group's retro-orbital plexus was used to draw blood samples, which were then placed in sterile, dry tubes with labels. Heparin, an anticoagulant, was present in the tubes. Plasma was extracted from blood by centrifuging it at 3500 rpm for 15 min. The plasma was then carefully stored in tubes at -20°C for the completion of the biochemical analysis. The liver and kidney were weighed in addition to measuring the weight increase and feed intake of the rat groups in order to estimate the feed efficiency ratio (FER).

Biochemical assay:

Serum glucose was determined using kits supplied by Bio-diagnostic laboratory reagents and products according to the method of **Trinder (1969)**, triglycerides in serum and Serum total cholesterol were determined according to (**Fossati and Prencipe, 1982**).

According to **Wieland and Seidel, (1981)**, high density lipoprotein cholesterol (HDL-C) was measured, and low density lipoprotein cholesterol (LDL-c) was measured using the method of (**Lee and Nieman, 1996**). , The blood uric acid was determined using **Caraway's technique (1955)**, and the serum creatinine level was determined using the method of (**Bohmer, 1971**). **Reitman and Franke (1957)** recommended measuring serum alkaline phosphatase (ALP), whereas **Roy, (1970)** recommended measuring aspartate aminotransferase and alanine aminotransferase (AST and ALT).

Sensory evaluation:

Preparation and sensory evaluation of cupcakes:

According to **Dubat (2010)**, the dry components for cupcakes were eggs, flour, baking powder, dried skim milk, and vanilla. These ingredients were stirred together, and then sugar and salt were added to a medium mixing bowl. For all mixing operations, a handheld electric mixer was utilized. After creaming the margarine for 1 minute, sugar and salt were added, and the mixture was then continued for another minute. A third of the dry mixture and water were added, and all of this was combined for 45 seconds before being added to the creamed mixture. This process was done again. The metal cup was filled with the batter. The cooked cupcakes were allowed to cool completely at room temperature before being sealed in aluminum foil bags and kept chilled. Likewise concurrently made available to the panelists. Ten members of the panel were asked to rate several samples for color, taste, aroma, texture, and overall quality on a 9-point hedonic scale (**Moretti et al., 2004**).

Statistical analysis:

The replicated results were presented as mean standard deviation according to **McCormick and Jesus (2017)** The least significant difference and a one-way analysis of variance (ANOVA) were used. At ($P < 0.05$), significance was accepted.

Results and discussion:

According to **Mithun et al. (2022)**, the high concentration of polyphenolic content in fruits and vegetables increases antioxidant activities and shields cell constituents from oxidative damage and a number of degenerative conditions linked to oxidative stress, including cardiovascular conditions, diabetes mellitus, and neurodegenerative

conditions (Ghasemi *et al.*, 2009), Thus from table (1) The total phenolic contents and flavonoid varied among the two citrus species tested (*limon and japonica*). The limon (W and P) having the higher total phenol content while japonica (W and P) had the higher total flavonoid content. However, none of the readings were higher than previously reported levels (Ademosun and Oboh 2012). Additionally, it was noted that the flavonoid concentration of certain Brazilian citrus species was higher in the fruit peels than the liquids (Pereira *et al.* 2017).

The total flavonoids, however, were shown to be lower than those listed by Wang *et al.* (2008). Dried kumquat peel in our result contained 0.013 QE g⁻¹ flavonoids. Another study by Wang *et al.*, (2008) reported that total flavonoids in dried kumquat peels were 41.0 mg/g, db (rutin equivalents). This difference between the obtained results and the previous results (Sadek *et al.*, 2009 and Ramful *et al.*, 2011) might be due to different environmental conditions.

Dried whole kumquat had the highest F/P ratio compared to the peel, table(2) assessed the phenolic, flavonoid acid rich of kumquat, one of the plant foods, for its beneficial effect on lowering plasma lipid profile in (Table 3,4,) as maintained by another study by (maria *et al.*, 2022).

Table (1) The total phenolic and total flavonoid in citrus japonica and Limon (P and W) samples

samples	T phenolic mgGAE/g-1	T Flavonoids mg QE / g-1
lemon p 5%	0.26	0.078
kumq p 5%	0.28	0.041
lemon W 5%	0.64	0.063
kumq W 5%	0.49	0.053

Gallic acid equivalent (GAE)

Quercetin equivalent (QE)

Table (٢) biological evaluation of hypercholesterolemic rats (+ve) positive group compared to the (-ve) negative control group, exhibited a substantial increase in feed intake, body weight gain percentage, and feed efficiency ratio at (P<0.05), feeding 5% of limon and japonica (peel and whole) on feed intake, feed efficiency ratio and body weight gain of hyperlipidemic rats are also shown in Table (1).

Concerning the feed intake, it was highly significant increase as 15.83±1.01g/day for negative control and 19.02±1.90 g / day for positive control group. However, the feed intake for groups 3, 4 5 and 6 significantly decreased at (P<0.05) as compared to positive control group. For body weight gain, the mean levels significantly decreased at (p< 0.05) gradually for groups 3, 4, 5 and 6. There are no significant changes between 3 and 4 group and the negative control group. For FER, the all group was decreased significantly when compared with the positive group, this decreases were all statistically significant (P<0.05).

These outcomes were consistent with (Dayse *et al.*, 2022) who discovered that hypercholesterolemic diets had greater caloric densities than control diets, increasing body weight ($P < 0.0001$) and relative liver weight, which was supported by increased adiposity. Additionally, our findings supported earlier research that suggested a diet high in 1% cholesterol causes weight growth (Abreu *et al.*, 2014).

The results of the recent investigations were consistent with those of Abdelbaky *et al.* (2009), who examined the impact of lemon peel powder on biochemical parameters in hypercholesterolemic rats and reported a significant decrease in the ratio of body weight gain in the citrus peel-supplemented group of rats compared to the control group. The increase in liver weight of the hyperlipidemic control group is the cause of the treated rats' weight loss. This could be explained by the control group's high rate of hepatic cholesterol production. There was no significant difference among negative control (2.33 ± 0.12) and groups 3, 4, 5 and 6 in liver weight. Also, However, the kidney weight for negative control group was 0.57 ± 0.022 g, there was no significant difference between negative control group (-) and groups 3, 4, 5 and 6. These results are in accordance with those of (Abdelhaliem and sheha, 2018) who reported that lemon peels protected the organs as liver and kidney from toxins and damage.

We found that supplementation with 5% kumquat orange did not affect the caloric density of diets and reflected in the similar results of limon peel in food and energy intake, body weight, liver relative weight and adiposity index in control groups (Dayse *et al.*, 2022).

Table (2) biological evaluations of rats fed on citrus Limon and Japonica (peels and whole) at 5% (Mean \pm S.D)

groups	feed intake g/d	BWG g/weeks	FER %	liver W g%	kidney w g/%
negative control	15.83 ± 1.01^b	46.18 ± 3.31^b	0.104 ± 0.006^b	2.33 ± 0.12^b	0.57 ± 0.022^b
positive control	19.02 ± 1.90^a	62.41 ± 2.07^a	0.123 ± 0.016^a	3.38 ± 0.15^a	0.74 ± 0.35^a
lemon p 5%	14.24 ± 1.20^b	44.6 ± 2.07^b	0.108 ± 0.006^b	2.5 ± 0.10^b	0.60 ± 0.017^b
kumq p5%	13.99 ± 1.03^b	42.1 ± 2.2^b	0.107 ± 0.007^b	2.4 ± 0.07^b	0.60 ± 0.013^b
lemon W 5%	12.2 ± 0.23^{cb}	36.2 ± 1.87^b	0.106 ± 0.005^b	2.6 ± 0.14^b	0.63 ± 0.022^b
kumq W 5%	11.09 ± 1.10^{bc}	33.4 ± 2.3^{bc}	0.107 ± 0.008^b	2.36 ± 0.05^b	0.64 ± 0.05^b

The value in each column with different superscript are significant at ($p < 0.05$).

Table (3) revealed the effect of adding 5% levels of kumquat and limon (W and P) on serum lipid profile in hypercholesterolemic rats. Hypercholesterolemic groups administrated with 5% kumquat (whole)

represented the highly significant at ($P < 0.05$) improvement percentage in serum (CHO, TG, LDL and VLDL) compared to positive control group. While high density lipoprotein (HDL) was elevated significantly at ($P < 0.05$) with 5% kumquat (whole), followed by 5% limon (whole), 5% kumquat (peel), 5% limon (peel) group respectively compared to positive group. These results agree with those of other researchers (**Lien et al., 2009** and **jing-li et al., 2013**), who suggested that the flavonoids may once again be a key mediator of the effectiveness. Rats' serum cholesterol and triglyceride concentrations have already been shown to decrease with increasing phenolic compound intake. Moreover, Citrus peel was investigated as a regulator of lipoprotein metabolism in rats against diet-induced fatty liver by (**Park et al., 2011**). Additionally, **Mollace et al. (2011)** noted noteworthy.

Citrus peel bioactive molecules activate receptor cells so that extra LDL and triglycerides are incorporated into liver and adipose tissue instead of circulating in the circulatory system and forming hard plaque. This hypothesis was affirmed by **Wang et al. ,(2011)**, who summarized that hesperidin (bioflavonoid found in a variety of nutritional supplements that have various beneficial effects on blood vessel disorders) has potential to prevent fatty degeneration of liver thus control hepatic lipid metabolism

According to **Choi, (2005)** and **Sadek et al. (2009)**, dietary fibre and phenolic compounds, which replace cholesterol and slow down the hydrolysis of cholesterol esters, may also be primarily responsible for these outcomes by lowering the absorption of dietary and biliary cholesterol in the small intestine (**Jesch and Carr, 2017**). Bile acids would become unavailable as surfactants in the small intestine due to the binding to dietary fibre and phenolic compounds. This would interfere with lipid emulsification, the formation of mixed micelles, the complete digestion of lipids, and their absorption, lowering levels of circulating triglycerides and the bioavailability of lipophilic nutrients (**Capuano, 2017**).

Mulvihill et al. (2009) found that LDL Receptor- Null Mice had decreased hepatic carnitine palmitoyl-transferase 1 α (CTP-1 α), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 α), and fatty acid oxidation in response to a western diet high in fat and sodium and low in fruits and vegetables. The flavonoid produced from citrus raised PGC-1 α gene expression, which in turn increased mitochondrial DNA, facilitated fatty acid oxidation, and reduced triglyceride buildup in the liver. Citrus flavonoids' strong inhibition of hepatic apo B secretion has been attributed as the mechanism by (**Lin et al. 2011**).

Table (3) Effect of citrus limon and japonica (peels and whole) on lipids profile of the hypercholesterolemic rats (Mean±S.D)

groups	TC mg/dl	TG mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl
negative control	92 ±3.73 ^d	74.4 ±4.9 ^c	45.4 ±2.8 ^a	31.8±3.4 ^d	14.8±1.9 ^c
positive control	198.5± 9.9 ^a	163.8 ± 5.07 ^a	16.4±1.5 ^c	149.3 ±6.8 ^a	32.8±2.8 ^a
lemon p 5%	140.36 ±11.4 ^b	102.2 ±3.6 ^b	34.4 ±3.8 ^b	85.2 ±5.2 ^b	20.4±2.1 ^b
kumq p5%	137.56 ± 6.5 ^b	94.2 ±4.9 ^b	36.8 ±3.5 ^b	81.96± 7.8 ^b	18.8±1.8 ^b
lemon W 5%	119 ±5.07 ^c	90.1±5.00 ^b	37.6±3.7 ^b	63.4 ±6.1 ^c	18.0±1.7 ^b
kumq W 5%	116 ±4.00 ^c	87.4 ±3.88 ^b	35.7 ±3.99 ^b	62.8±5.9 ^c	17.5±1.8 ^b

The value in each column with different superscript are significant at (p<0.05).

The effects of 5% levels of kumquat and Limon (whole, and peel) on the TC/HDL ratio and LDL/HDL ratio in hypercholesterolemic rats were demonstrated by the data in table (4). Generally, all treatments enhanced the HTR and LHR to a level expected of healthy rats. In the hypercholesterolemic groups, the diet supplemented with 5% whole kumquat and lemon was found to be the most effective therapy for lowering HTR and LHR significantly at (P<0.05). These results corroborated those of **Abdel Haliem and Sheha (2018)**, who noted the similar tendency.

It has been established that the phenolic and flavonoid chemicals have hypocholesterolemic effects via controlling hepatic gene expression involved in lipid metabolism (**Jung et al., 2012**). As a result, phenolic substances as well as the flavonoids in table (2) may contribute to the lipid-lowering properties of the whole kumquat. The risk of coronary heart disease was negatively correlated with the HDL: TC (HTR %) ratio (**Rajendra and Estari, 2013**).

Atherogenic indices are effective predictors of the likelihood of getting heart disease; calculated using the formula $\log (TG/HDL-C)$, as a marker of plasma atherogenicity based on a positive association with the lipoprotein particle size, cholesterol esterification rates, and remnant lipoproteinemia (**Quispe, 2015**). The data obtained have revealed that both the peel and the whole kumquat have anti-atherogenic properties. According to this study, the whole kumquat has hypocholesterolemic properties, which may once more be mediated by flavonoids and phenolic substances (**Jung et al., 2012**).

Table (4) Atherogenic index (LDL/HDL, TC/HDL) of rats fed on citrus limon and japonica (peels and whole) at 5% (Mean \pm S.D)

groups	LDL/HDL	TC/HDL
negative control	0.70 \pm 0.01 ^c	2.03 \pm 0.07 ^c
positive control	9.11 \pm 0.90 ^a	12.10 \pm 1.21 ^a
lemon p 5%	2.47 \pm 0.09 ^b	9.08 \pm 0.19 ^b
kumq p 5%	2.23 \pm 0.08 ^b	3.73 \pm 0.33 ^b
lemon W 5%	1.68 \pm 0.05 ^b	3.16 \pm 0.13 ^b
kumq W 5%	1.76 \pm 0.06 ^b	3.25 \pm 0.29 ^b

The value in each column with different superscript are significant at (p<0.05).

In hypercholesterolemic rats, the current study demonstrated the impact of kumquat and limon on liver and kidney functions in table (5). We discovered that, as previously reported, hyperlipidemia was accompanied by an increase in the quantity of liver damage caused by a significant increase in serum ALT (Abreu *et al.*, 2014 and Ben Gara *et al.*, 2017). Kumquat and limon intake in hypercholesterolemic condition was represented by decreased blood AST, ALT, urea, and creatinine levels significantly at (P<0.05), suggesting a potential protective impact on liver function. These results suggested that kumquat phenolic compounds may be effective liver protectants against hypercholesterolemic toxicity, primarily by reducing oxidative stress and enhancing metabolic profile

In comparison to the negative control group, all groups had greater values; the creatinine levels of all groups displayed a high significant difference when compared with negative control group. Also, the uric acid level in the negative control group was 1.31 \pm 0.06 mg/dL and there were no significant differences in the plasma uric acid of groups 2, 3, and 4 as compared with negative group. The outcomes are consistent with those of Abdelhaliem and Sheha (2018), who claimed that supplementing with lemon peel at low dosages over an extended period of time can enhance kidney functioning.

Table (5) Effect of kumquat and limon (peel and whole) at 5% on serum kidney and liver parameters of the hypercholesterolemia rats (Mean \pm S.D)

groups	uric acid mg/dl	Creatinine mg/dl	AST U/L	ALT U/L
negative control	1.31 \pm 0.06 ^c	0.67 \pm 0.05 ^c	52.24 \pm 2.9 ^c	36.62 \pm 2.6 ^c
positive control	3.19 \pm 0.48 ^a	1.99 \pm 0.03 ^a	77.46 \pm 3.8 ^a	55.48 \pm 2.04 ^a
lemon p 5%	2.28 \pm 0.12 ^b	1.27 \pm 0.41 ^b	67.14 \pm 2.9 ^b	44.84 \pm 2.13 ^b
kumq p 5%	2.29 \pm 0.04 ^b	1.26 \pm 0.04 ^b	59.59 \pm 1.9 ^c	44.32 \pm 2.6 ^b
lemon W 5%	2.2 \pm 0.07 ^b	1.3 \pm 0.08 ^b	55.96 \pm 3.7 ^c	45.24 \pm 2.9 ^b
kumq W 5%	2.35 \pm 0.15 ^b	1.25 \pm 0.09 ^b	56.54 \pm 2.5 ^c	43.02 \pm 2.6 ^b

The value in each column with different superscript are significant at (p<0.05).

AST: aspartate aminotransferase ALT: alanine aminotransferase

Sensory evaluation:

From table (6) data revealed that colour , appearance, taste , texture and overall quality of cupcake with limon(peel) at 5% had nearest score to the control cake with wheat flour ,followed by kumquat (peel) at 5% , lemon W 5% and kumquat W at 5% levels.

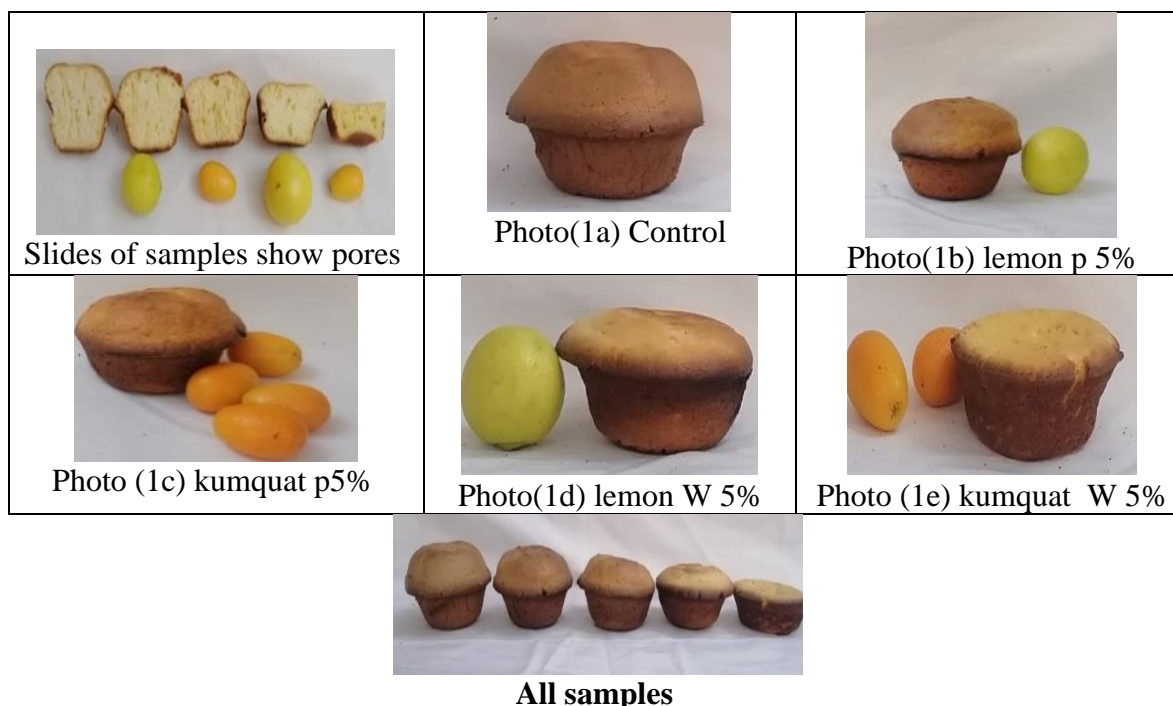
After the enrichment, the cupcakes took on a more bitter taste, which means that the presence of these ingredients might have influenced the taste of the final products due to the presence of bitter-tasting compounds typically found in the fibrous component of the lemon peel. Thus, results indicated that the incorporation of lemon processing by-products allowed the production of functional enriched cupcakes with improved antioxidant properties (Valeria *et al.*, 2021).

Table (6): sensory evaluation (colour, taste, texture, appearance and overall quality) of cake of kumquat and limon (peel and whole) at 5% (Mean \pm S.D)

Cake samples	Color	Appearance	Texture	Taste	Overall Quality
control	9.8 \pm 0.44	9.1 \pm 0.33	9.6 \pm 0.48	9.5 \pm 0.52	9.5 \pm 0.54
lemon p 5%	9.1 \pm 0.29	8.7 \pm 0.5	8.5 \pm 0.50	8.9 \pm 0.33	8.8 \pm 0.53
kumq p5%	8.4 \pm 0.52	7.9 \pm 0.60	7.2 \pm 0.67	7.7 \pm 0.50	7.8 \pm 0.56
Lemon W 5%	7.9 \pm 0.60	7.6 \pm 0.48	7.6 \pm 0.50	7.7 \pm 0.59	7.7 \pm 0.54
kumq W 5%	7.5 \pm 0.66	6.9 \pm 0.33	6.5 \pm 0.47	7.8 \pm 0.53	7.2 \pm 0.67

The low volume of cupcake affects the texture of cake negatively. Our findings of sensory attributes of cake are in accordance with findings of Mrabet *et al.* (2016) who reported sensory attributes of muffins enriched with fruit fiber. According to them enrichment of fiber in muffin is acceptable upto a limit after that fiber enriched product got lower marks by panelist. But average results showed that all the treatment had good scores, between 6 and 8 on a 9-point scale.

So, with regard to the effect of addition lemon and kumquat on the sensorial properties it could be concluded that addition of lemon and kumquat at the level of 5% have an improvement of cupcakes quality and were also accepted by the panelists. These data were nearly in accordance with those given by Abozeid *et al.*, (2011), who stated that 10% of orange peel and pulp could be incorporated as an ingredient in making biscuits and cakes , as they are a suitable source of dietary fiber with associated bioactive compounds (Flavonids, Carotenoids etc.).



Conclusion

In rats, given a hypercholesterolemic diet, dietary supplementation with 5% of kumquat and lemon (peel and whole) improved lipid profiles and exhibited a protective impact against oxidative damage and renal function. Our findings point to kumquat and lemon as a possible meal for cardiovascular protection and as an adjuvant in lipid disorders.

Enriched cupcakes showed higher polyphenol content and antioxidant activity than the control one, with suitable acceptability evaluated by the consumers through the preference test.

It may be concluded that lemon peel could be incorporated in the formulation of cupcakes increasing their bioactive compounds content without affecting their overall appearance, resulting in acceptable products and with improved functional and Nutraceutical properties. In this view, innovative uses of food wastes, rich in phenolic, can lead to an increase in the production of food with suitable nutritional characteristics and to satisfy customers' expectations of healthy.

References:

- Abdelbaky, M.S., Hanaa F. El mehiry, and Ali, N.K.M., (2009):** .Effect of some citrus peels on hypercholesterolemic rats .The 1st international and 4th Arab annual scientific conference on: academic accreditation for higher specific education institutions and programs in Egypt and Arab world “Reality and Expectation”, Faculty of Specific Education Mansoura University, Egypt .1626-1639.

- Abdelhaliem H. S. and Sheha H. G. (2018):** Biological Effect of Lemon Peels Powder on Hyperlipidemic Rats. J. Food and Dairy Sci., Vol. 9, (9), 321 - 325 ,
- Abozeid , W.M., Salama, F. and Moawad, R. (2011):**Utilization of Fat Replacer in the Production of Reduced Cakes and Cookies . Austr. J.of Basic and App.Sci., 5,(12), 2833-2840,
- Abreu, I.C, Guerra, J.F, Pereira, R.R, Silva, M, Lima ,W.G, Silva, M.E and Pedrosa ,M.L. (2014):** Hypercholesterolemic diet induces hepatic steatosis and alterations in mRNA expression of NADPH oxidase in rat livers. Arq. Bras. Endocrin .Meta. 58, 251-259.
- Ademosun, A.O. and Oboh, .G., (2012):** Inhibition of acetylcholinesterase activity and Fe²⁺-induced lipid peroxidation in rat brain *in vitro* by some citrus fruit juices. J. Med. Food .15, 1-7.
- Baldissera, M.D., Souza, C.F., Grando, T.H., Doleski, P.H., Boligon, A.A., Stefani, L.M and Monteiro, S.G., (2017):**Hypolipidemic effect of beta-caryophyllene to treat hyperlipidemic rats. Nau.Schm.Arch .Pharm. 390,215-223.
- BenGara,A,BenAbdallah,R.K.,Rim,C.,Nadia,H.,Majed,K.,Francesco, P.P.,Abdelfattah,E.,Lotfi,F.,Hafedh,B.,and Karima,B.,(2017):** protection of liver-kidney functions by Cystoseira crinita sulphated polysaccharide in high-fat diet-fed rats.biomedicine and pharmacotherapy.85,517-536.
- Bohmer,H.B.,(1971):**micro-determination of creatinine .clin.chem.Acta.32,81-85.
- Capuano ,E.(2017):** The behavior of dietary fiber in the gastrointestinal tract determines its physiological effect. Crit Rev Food Sci Nutr 57,3543-3564.
- Caraway, W.,(1955):** Determination of serum urate .Am.J.Clin.Path.25,840.
- Choi, H.S., (2005):**Characteristic odor components of kumquat (Fortunella japonica Swingle) peel oil. J Agric Food Chem .53,1642-1647.
- Dayse, L. S., Nicolle, C. D. S., Edenil, C. A., Marcelo, E. S. and Dirce, R. D., (2022):** Kinkan orange protects hypercholesterolemic rats against dyslipidemia and oxidative stress. An. Acad. Bras. Cienc. 94,(Suppl. 3), e20201066 DOI 10.1590/0001-376520220201066.
- Devanooru, K., Shrilakshmi, E., and Rao, S., (2015):** Value Added Products from Agriculture: Extraction of Pectin from Agro Waste

- Product *Musa Acuminata* and Citrus Fruit. Res. J. of Agric and Forest. Sci , 3, (3) , 13-18.
- Dubat, A.,(2010):**A new AACC international approved method to measure rheological properties of a dough sample. Cer.Foods W.55, 150.
- EL-Tantawy, W.H., (2015):** Biochemical effects, hypolipidemic and anti-inflammatory activities of *Artemisia vulgaris* extract in hypercholesterolemic rats. J .Clin. Biochem. Nutr., 57,33-38.
- Fossati, P. and Prencipe, I, (1982):** Serum triglycerides determination colorimetrically with an enzyme that produce hydrogen peroxide. Clin.Chem.,28,2077-2083.
- Ghasemi, K, Ghasemi, Y, Ebrahimzadeh, M.A., (2009):** Antioxidant activity, phenol and flavonoid contents of 13 citrus species peels and tissues. Pak. J .Pharm Sci., 3,277–281
- GIglio, R.V, Patti, A. M., Nikolic, D., Volti, G.L., Al-Rasadi, K., Niki, K., Mikhailidis, D.P., Montalto, G., Ivanova, E. Orekhov, A. N. and Rizzo, M. (2016):**The effect of bergamot on dyslipidemia. Phytomed., 23,1175-1181.
- Humaira, A., Masood, S.B., Muhammad, J., Hafiz, A. R.,(2017):**Citrus peel extract and powder attenuate hypercholesterolemia and hyperglycemia using rodent experimental mod. Asian Paci. J. of Trop. Biomed. 7, 10 , 870-880.
- Izli, G., Izli, N., Taskin, O. and Yildiz, G., (2018):** Convective drying of kumquat slices: comparison of different drying temperatures on drying kinetics, colour, total phenolic content and antioxidant capacity. Latin .Amer.App. Res. 48,37-42 .
- Jesch, E.D., and Carr, T.P., (2017):** Food Ingredients That Inhibit Cholesterol Absorption. Prev. Nutr. Food .Sci 22, 67-80.
- Jing, Li , Zhang, Y.u., Fan, .S. , Ming, G.u,Yu,G., Xiong, L.u, Cheng,H., and Zhiqinm, Z., (2013):**Preventive and ameliorating effects of citrus d-limonene on dyslipidemia and hyperglycemia in mice with high-fat diet-induced obesity. Euro. J. of .Pharma, 715, (1–3),46–55.
- Jung, C. H., Cho, I., Ahn, J., Jeon, T.-I., and Ha, T.-Y (2012):**Quercetin reduces high-fat diet-induced fat accumulation in the liver by regulating lipid metabolism genes. Phytotherapy .Research. Hem. 59, (9),4496–4503
- Kiin-kabari, D. and Eki-Ejiofor, J., (2013):**physico-chemical and sensory properties of cakes and cookies produced from composite flours of wheat and plantain. J.Food.Tech.1, (1), 9-13.
- Lee, J.S., Song-H, B., Seon-M, J.,Hye,J.K., Kyung, M. D., Yong,B. P., and Myung,S. C,(2010):** Antihyperlipidemic effects of

- buckwheat leaf and flower in rats fed a high-fat diet. Food Chem., 235–240.
- Lee, R. and Nieman, D. (1996):** Nutrition Assessment. 2nd Ed. Mosby, Missouri, U.S.A.
- Lien, D. N., Nguyen, T. Q., Nguyen, H. Q., Do, V. P. 1 and Nguyen, T. T. N. (2009):** Anti-Obesity and Body Weight Reducing Effect of Fortunella japonica Peel Extract Fractions in Experimentally Obese Mice. KKU Sci. J. 37, (Supplement) 96-104.
- Lim, T. K., (2012):** Citrus japonica 'Marumi'. In: Edible Medicinal And Non-Medicinal Plants. Springer, Dordrecht .647-650.
- Lin, Y, Vermeer, M. A, Bos, W., van, B. L, Schuurbijs, E., Miret-Catalan, S, Trautwein, E. A (2011):** Molecular structures of citrus flavonoids determine their effects on lipid metabolism in HepG2 cells by primarily suppressing ApoB secretion. J Agric Food Chem 59, (9), 4496–4503
- Majzoubi, M., Hedayati, S. M. , Habibi, G , F. and Farahnaky A. (2014):** Effect of corn resistant starch on the physicochemical properties of cake. J. Agric. Sci. Technol. 16, 569-576.
- Maria, C. S., Giuseppe, A., Maria, A., Giovanni, C. D., Giuliana, P., Attilio, M., and Francesco, G., (2022):** Postharvest Technologies of Fresh Citrus Fruit: Advances and Recent Developments for the Loss Reduction during Handling and Storage. Horticulturae., 8, 612.
- McCormick, K., Jesus S., (2017):** IBM SPSS Modeler Essentials: Effective techniques for building powerful data mining and predictive analytics solutions. 238 pages.
- McGill, A. T, Stewart, J. M, Lithander, F. E, Strik, C. M., and Poppitt, S. D., (2008):** Relationships of low serum vitamin D 3 with anthropometry and markers of the metabolic syndrome and diabetes in overweight and obesity. Nutr .J., 7, (1), 4.
- Milessa, S. A. , Renata, P. A. B., Roberta, M. M., Maria, S. F., Valéria, S. N., Eder, R. Q., Marcia, K., Sergio, C., Chin, J., Edna, R. N., and Ana, M. L., (2013):** Dietary phytosterol does not accumulate in the arterial wall and prevents atherosclerosis of LDLr-KO mice. Atheroscl., 442–447.
- Mithun, R., Shubham, J. k., Johra, K., Abdulaziz, B. D., Mohammad, A. A., Mohammad, N. A., Fahad, M. A., Santwana, P., Prashanta, K. D and Rajlakshmi, D., (2022):** Dietary Polyphenols and Their Role in Oxidative Stress-Induced Human Diseases: Insights Into Protective Effects, Antioxidant Potentials and Mechanism(s) of Action. Front Pharmacol. 13, 806470.

- Mollace, V, Sacco, I, Janda, E, Malara, C., Ventrice, D., Colica, C., (2011):** Hypolipemic and hypoglycaemic activity of bergamot polyphenols from animal models to human studies. *Fitoterapia* 82,(3),309-16
- Moretti, V.M., Madonia, G., Diaferia, C., Mentasti, T., Paleari M.A and Panseri, .S,(2004):**Chemical and microbiological parameters and sensory attributes of a typical Sicilian salami ripened in different conditions. *Meat. Sci.* 66,(4),845–54
- Mrabet, A., Rodriguez,G.G., R. Rodriguez,A., Guillen,.B. R., Ferchichi, .A., Sindiand, M.and. A. Jimenez,A. (2016):** Quality characteristics and antioxidant properties of muffins enriched with date fruit (phoeni x dactylifera l.) Fiber concentrates. *J. Food Quality.* 39, 237–244.
- Mulvihill,E .E, Allister, E.M, Sutherland,B.G, Telford, D.E, Sawyez, C.G, Edwards, J.Y, Markle, J.M, Hegele,R.A and Huff, M.W.,(2009):** Naringenin prevents dyslipidemia, apolipoprotein B overproduction, and hyperinsulinemia in LDL receptornull mice with diet-induced insulin resistance. *Diabetes.* 58, 2198-2210
- Ordon, J.D, Gomez, M.A and Vattuone, M.I., (2006):** Antioxidant activities of *Sechium edule* (Jacq.) Swartz extracts. *Food Chem* 97,452–458.
- Pandya, N.; Santani, D. and Jain, S.,(2006):** Antioxidant activity of ezetimibe in hypercholesterolemic rats. *Ind. J. Pharmacol.,*38,205-206.
- Park, H,Y, Park, Y., Lee, Y., Noh ,S.K., Sung ,E.G, Choi, I.,(2012):** Effect of oral administration of water-soluble extract from citrus peel (*Citrus unshiu*) on suppressing alcohol-induced fatty liver in rats. *Food Chem.,* 130,(3),598-604.
- Peng, L.W, Sheu, M.J, Lin, L.Y., Wu, C.T, Chiang, .H.M., (2013):** Effect of heat treatments on the essential oils of kumquat (*Fortunella margarita* Swingle). *Food Chem.* 136,(2),532-537.
- Pereira, R.M, López, B,G.C, Diniz, S,N, Antunes, A,A, Garcia, D.M, Oliveira, C.R and Marcucci,M.C.(2017):** Quantification of flavonoids in Brazilian orange peels and industrial orange juice processing wastes. *Agric Sci.,* 8,631-644.
- Pultrin, A.M.,Galindo, L.K., andCosta,K.,(2017):** Effects of the essential oil from lemon (*Citrus aurantium* L.) peels in experimental anxiety models in mice.*Life Sci.,* 78,(15),1720-1725.
- Quispe, .R, Manalac, R.J, Faridi, K.F., Blaha, M.J, Toth, P.P, Kulkarni, K.R, Nasir, K, Virani,S., Banach, M.,and**

- Blumenthal, R.S.,(2015):**Relationship of the triglyceride to high-density lipoprotein cholesterol (TG/HDL-C) ratio to the remainder of the lipid profile: The Very Large Database of Lipids-4 (VLDL-4) study. *Atherosclerosis.*, 242,(1),243–50.
- Rajendra, C. V., Estari, M. (2013):** Preliminary phytochemical and in vitro anti-diabetic activity of *Ficus racemosa* (L.) stems bark extract. *Online Intern. Inter .discip. Res. J.,Vol-III*, 134-141.
- Ramful, D., Evelyne, T., Okezie, I. A., Emmanuel ,B. and Theeshan, B. (2011):** Polyphenol composition, vitamin C content and antioxidant capacity of Mauritian citrus fruit pulps. *Food Res. Inter.* 44, 2088–2099.
- Reeves, P.G., Nielsen, F.H. and Fahey, G.C. Jr., (1993):** AIN 93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J. Nutr.* 123, (11), 1939-51.
- Reitman, S. and Frankel, S.(1957) :**"A calorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic trasaminases. *Am . J.Clin.*, 28:56.
- Roy, S.E.(1970):**Colorimetric determination of serum allkalin phosphatase .*clin .of .chem.*,16,432-432.
- Sadek, E.S, Makris, D.P., and Kefalas, P., (2009):** Polyphenolic composition and antioxidant characteristics of kumquat (*Fortunella margarita*) peel fractions. *Plant Foods .Hum .Nutr.* 64, 297-302.
- Saeedeh, A and Asna, U., (2007):** Antioxidant properties of various solvent extracts of mulberry (*Morus indica* L.) leaves. *Food Chem* 102,1233– 1240.
- Sohn, H.Y., Son, K.H., Know, C. S.and Kang, S.S. (2016):** Cytotoxic activity of 18 prenylated flavonoids isolated from medicinal plants: *Morusvalba* L. , *Morus mongolica* Schneider , *Broussnetiavpapyrifera* (L.) Vent , *Sophora flavescens* Ait and *Echinosophora koreensis*”, Nakai. *Phytomed.* 11, 666-672.
- Toth, P.P, Patti, A. M., Nikolic, D., Giglio, R. V., Castellino, G., Biancucci, .T., Geraci, F. , David, S., Montalto, G., Rizvi, A. and Rizzo, M.,(2016):** Bergamot Reduces Plasma Lipids, Atherogenic Small Dense LDL, and Subclinical Atherosclerosis in Subjects with Moderate Hypercholesterolemia: A 6 Months Prospective Study. *Front Pharmacol.*, 6,299.
- Trinder,P.(1969):**Enzymatic Calorimetric Determination of Triglycerides by GOP-PAP Method. *Ann.of Clin. Biochem.*, 6, 24-27.

- Valeria, I, Rosa, R., Antonio, .G., Alessandra, D. B. and Amalia, P.,(2021):Functionalized Biscuits with Bioactive Ingredients Obtained by Citrus Lemon Pomace. Foods 10,(10),2460.
- Wang, Y. ,Zeng, W. , Xu,P;Lan,Y.;Zhu.,R. , Zhong,K. , Huang,Y and Gao,H., (2012): Chemical Composition and Antimicrobial Activity of the Essential Oil of Kumquat (*Fortunella crassifolia* Swingle) Peel. Int. J. Mol. Sci., 3382-3393.
- Wang,M.X, Hasegawam, J., Kitamura,Y, Wang, Z., Matsuda, .A Shinoda, W., (2011): Effects of hesperidin on the progression of hypercholesterolemia and fatty liver induced by high-cholesterol diet in rats. J. Pharmacol .Sci .117,(3),129-38
- Wieland, H.; and Seidel, D. (1981): HDL. Cholesterol determination after separation of high density lipoprotein. Art Ztl. Lab., 27,141-154.

المستخلص العربي:

زيادة كوليسترول الدم هو عامل الخطر الرئيسي لأمراض القلب والأوعية الدموية، وبالتالي، تهدف الدراسة الحالية الى تقييم تاثير اضافة الكيمكوات والليمون (كامل وقشر) على النمو والسمنة و،الاختبارات البيوكيميائية الحيوية في الجرذان المصابة بفرط كوليسترول الدم، بالإضافة إلى تقييم الخصائص الحسية لمفاتيح تعريف الارتباط المخبوزة التي تحتوي على جرعات ٥٪ من الكمكوات والليمون (كامل وقشر) تم تغذية ثلاثين من ذكور الجرذان البيضاء بنظام غذائي أساسي كمجموعة (سيطرة) مكونة من ٥ جرذان لكل منهما ؛ تتغذى المجموعة (٢) على نظام غذائي عالي الكوليسترول والمجموعات المتبقية (٣، ٤، ٥، ٦) على نظام غذائي يحتوي على ٥٪ من الكمكوات والليمون (كامل وقشر) مع نظام غذائي عالي الكوليسترول لمدة أربعة أسابيع، انخفض مستوى الكوليسترول والدهون الثلاثية في الدم ، والدهون منخفضة الكثافة والمنخفضة الكثافة جدا وعلاوة على ذلك انخفضت انزيمات الكبد مع الكرياتينين وحمض البوليك، انخفاضاً كبيراً في المجموعات المكملة بالكيمكوات والليمون (كامل وقشر) بنسبة ٥٪. لذلك ، قد يتكون فكرة عن كون الكيمكوات والليمون من كاستراتيجية جيدة من الفينولات التي يمكن استخدامها في السيطرة على فرط كوليسترول الدم وعواقبه الضارة وتعزيز الحماية الكلوية في الفئران المصابة بفرط كوليسترول الدم. كما أوضحت نتائج التقييم الحسي أن البسكويت المحضر من الكمكوات والليمون (كامل وقشر) بنسبة ٥٪ تم قبوله من قبل أعضاء المحكمين بتقييمات مختلفة.

الكلمات المفتاحية: فواكه حمضية، ليمون كيمكوات، تلخبط في دهون الدم، السمنة.