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Extending Shelf Life of Cookies Using Cloves and Study It's Anti-Hypercholesterolemic Effect of Male Rats

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Abstract

This study was carried out to investigate the effect of different levels 1, 1.5, and 2%, of roasted clove powder on the quality attributes, nutritional and stability during storage of cookies at room temperature 28°C in addition to compared with cookies containing synthetic antioxidant 0.4% BHT furthermore study the protective effect of roasted clove powder against hypercholesterolemia of male rats. Chemical, physical, sensory and microbiological analysis were investigated. Treatments A (1, 1.5 and 2% roasted cloves powder) Treatments B (0.4 %BHT) and control (without anti-oxidant) were studied. Objective and sensory evaluations were conducted to determine the acceptability of the final products. Sensory results showed the overall acceptability of the different cookies samples. Samples using roasted clove powder were found to have a more intense color. Male rats (n=24) weighted 150±5g were used to the experimental design. Group 1 was fed basal diet, group2 fed hypercholesterolemic diet for 4 weeks. Groups3 and 4 as the same of group 2 and were fed roasted cloves powder 2% which was the highest general acceptability among all cookie's formulas and 0.4% BHT as a synthetic antioxidant, respectively. Body weight gain, feed efficiency ratio, lipid profile, atherogenic index, coronary risk index, liver enzymes, kidney enzymes, oxidative and anti-oxidative biomarkers were determined. Heart and aorta were histologically examined. The results showed that the roasted clove powder was able to delay the oxidative products in cookies when compared to control (without anti-oxidant) and synthetic antioxidant. The examined microbes' growth indicated that roasted clove powder was a more effective antibacterial agent. When compared to cookies with synthetic antioxidant and control, the cookies with roasted clove powder displayed more antioxidant qualities. Obtained results refers to observed improvement in groups 3 and 4 compared to +ive control specially rats fed 2% roasted cloves powder(G3). Furthermore, the histological examination showed an improvement in the groups treated with cloves and BHT compared with +ive group which was compatible with the above observations. Adding clove powder to cookies improved their physicochemical, nutritional, and bioactive characteristics, as well as their storability, without significantly impacting their sensory acceptance and can contribute to extending the food products' shelf life in addition to its safety for health as well as roasted cloves may play a role against hypercholesterolemia so, it's may beneficial for human being.

Keywords: Hypercholesterolemia, Eugenol, Clove, Cookies, Lipid oxidation, Antioxidant activity.

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1. Introduction

A medical condition known as hypercholesterolemia is characterized by increased plasma cholesterol levels resulting from an increase in cholesterol levels, while plasma triglycerides remain normal and Apo lipoprotein B (Apo B)-rich lipoproteins, identified lipoprotein with low density (LDL) [1]. A significant increased LDL-cholesterol (LDL-C) from birth is a characteristic of homozygous familial hypercholesterolemia (HoFH). Individuals with aortic and supra-aortic stenosis usually experience the onset of cardiovascular disease (CVD) in the second half of life [2]. Hypercholesterolemia is a metabolic disorder characterized by high levels of serum, low-density lipoprotein, and blood cholesterol. The World Health Organization (WHO) estimated that 17.3 million people died from cardiovascular diseases (CVD) in 2008 and warned that 23.6 million people will die annually from CVD by 2030.Hypercholesterolemia indicates a strong risk factor for the development of ischemic heart diseases. These include angina, myocardial infarction, atherosclerosis and all chronic inflammatory conditions [3]. Clove is known for its therapeutic properties such as antioxidant, anti-inflammatory, anti-aging, anti-fungal, anti-microbial, anti-diabetic, pain relieving and activity against pathogenic organisms. These activities are attributed to the presence of unsaturated phenolic compounds mainly eugenol, eugenol acetate and β-caryophyllene. Eugenol is the main constituent responsible for the medicinal properties of the clove bud. In addition, eugenol possesses strong antioxidant activity, which is comparable to the activities of synthetic antioxidants like butylated hydroxytoluene (BHT) [4].

Cookies are the most common bakery product manufactured and consumed in great amounts over the world due to their palatability, low prices, storability, variety of flavors, and ready for consumption status. A recent study focused on supplementing cookies with various dietary supplements or plant materials renowned for their high nutritional and phytochemical content. As a result, the fortification of foods or formulations of new food products with health-promoting effects such as antidiabetic, anti-inflammatory, anticancer, and antioxidant properties is on a rise [5]**.** The production of cookies is complicated by microbial growth and lipid oxidation, which reduces the product's shelf life. The development of rancidity in bread products has a significant impact on texture, color, and organoleptic characteristics as well as on nutritional value decreases. Preservation agents and antioxidants may even be able to avoid these problems. The use of synthetic antioxidants like butylated hydroxytoluene (BHT) has limited use as an antioxidant, as evidenced by certain findings showing potential negative impacts (carcinogenic and tumor-promoting) in animal models [6]. As a result, efforts are being designed to lower the level of synthetic antioxidants in food by using natural antioxidants instead. Determining the antibacterial activity of aromatic oils is largely dependent on their molecular structure and content. Eugenol, a naturally present phenol molecule found in clove oil, is an antioxidant that also protects tissue from oxidative damage and scavenges and fights free radicals. Clove has the strongest capacity to release hydrogen which avoids lipid peroxidation, and it has greater antioxidant action than conventional BHT [7]. Dried clove buds have 20% essential oil, with eugenol accounting for 70-90%. Other phytochemicals extracted from clove essential oil include eugenol acetate, β-caryophyllene, anti-inflammatory and antithrombotic effects [8]. The American Food and Drug Administration (FDA) confirmed the safety of clove buds, clove oil, and some clove components as a dietary supplement, whereas the WHO has set the recommended daily intake of cloves in humans as 2.5 mg/kg body weight [9]. The objective of this investigation was to assess the effect of roasted clove powder on cookies preservation throughout a 21-day duration of storage at room temperature (28˚C) and study the anti hypercholesterolemic effects of clove compared with the synthetic antioxidant (BHT) of adult male albino rats.

2. Materials and Methods

2.1. Materials

- The source of Clove buds was purchased from Crops Research Institute, Agricultural Research Center, Ministry of Agriculture, Giza, Egypt.
- Wheat flour (refined), sugar, milk powder, egg, butter, and vanilla were obtained from the local market, Cairo, Egypt.
- All other chemicals, reagents, and solvents of proximal determination were obtained from El-Gomhoria Pharmaceutical Company, Cairo, Egypt.
- Casein, cellulose, vitamins mixture, minerals mixture and formalin were purchased from El-Gomhoria Company, Cairo, Egypt.
- Twenty-four male albino rats weighed 150±5g were obtained from Helwan Station, Cairo, Egypt.
- Kits for blood analysis were purchased from Gama Trade Company for chemical, Giza, Egypt.

2.2. Methods

2.2.1. Chemical composition of the raw materials

2.2.1.1. Gas chromatography – mass spectrometry (GC – MS) analysis

The sample was analyzed using a gas chromatography (Agilent 8890 GC System) coupled to a mass spectrometer according to method used in (National Institute of Standard and Technology, NIST).

2.2.2. HPLC conditions

HPLC analysis was performed on an Agilent 1260 series. The separation was performed using a Zorbax Eclipse Plus C8 column [10].

2.2.3. Preparing of roasted Powdered Cloves

Using a coffee grinder, the roasted clove buds were ground into a powder. In a coffee grinder, clean clove buds were ground at a low speed for about three minutes. A 60-mesh sieve was used to filter the powder.

2.2.4. Preparing the Cookies

The cookies were made using a modified variation of a standard recipe by [11] with a slight amount of modification with the following formula: egg (25.5 g), sugar (200 g), softened butter (100 g), wheat flour (200 g), and baking powder (5 g), tablespoon pure vanilla extract (5 g), and roasted clove powder was added in different samples of cookies as shown in Table 1. Preheat oven to 175°C, Cream butter and sugar, add egg, vanilla and beat well, mix in flour (The ingredients were mixed for five minutes to achieve a consistent consistency), Drop by rounded teaspoonful onto parchment-lined cookie sheet and bake 10-12 minutes. The cookies were weighed once they had cooled. After being collected, the samples were packed in plastic bags to be kept at room temperature (28°C). To present five samples, cookies were prepared. As a control, the first sample did not contain an antioxidant ingredient, as a natural antioxidant, roasted clove powder was added to the second, third, and fourth samples at three different amounts (1, 1.5, and 2%). The fifth sample was produced by adding a synthetic antioxidant (BHT) (Figure 10)**.** The steps for overall treatments can be summarized as the following:

1. Control: without treatment.
2. Treatment (A): roasted clove powder cookies samples prepared with addition of 1, 1.5, and 2 % roasted clove powder/100g wheat flour.
3. Treatment (B): synthetic antioxidant (BHT) cookies sample prepared with an addition of 0.4% BHT /100g wheat flour.

2.2.5. Percent change in weight (%) and area (cm^2)

The percentage change in the treated samples weight after backing was calculated (%). Areas were measured (cm^2) using Planimeter to determine uniformity under the adding clove powder to treatments against control [12].

$$\% = \frac{weigt\ befor\ baking\ (g) - weight\ after\ bakig(g)}{weight\ before\ baking\ (g)}\ X\ 100$$

$$\% = \frac{area\ befor\ baking\ cm^2 - area\ afterbakingcm^2}{area\ befor\ baking\ cm^2}\ X\ 100$$

Product's volume $(cm)^3$ was determined by rapeseed displacement method according to Penfield and Campbell, (1990) [13].

2.2.6. Panel test of different cookies sample

Sensory evaluation was determined to detect color, odor, Degree of chawing, taste, texture, and overall acceptability. Organoleptic characteristics were evaluated using a scale of 1 to 5, with 1 representing very poor and 5 representing very good general acceptance as stated by [13]. Evaluation was carried out on 20 well trained panelists from Nutrition and Food Science Department, Faculty of Home Economics, Helwan University, Cairo, Egypt by using a scoring sheet.

2.2.7. Oxidative stability test

The measurement of acid value was used to regularly determine the amount of oxidation of lipid from cookies. (AV) during 0, 7, 14, 21, and 21 days of storage at room temperatures. Acid value (AV) was carried out in accordance with the official method [14].

2.2.8. Peroxide value (PV)

Peroxide value was determined according to the method of [15].

2.2.9. Determination of total aerobic bacterial count

The total aerobic bacterial count CFU/g was performed using the plate count agar medium as recommended by [16].

2.2.10. Determination of total yeast and molds count

The yeast and mold count CFU/g were calculated according to [17].

2.2.11. Experiment Design

Rats were fed AIN-93 basal diet which was formulated according to [18] for one week as acclimatization period after that rats were divided into four groups as follow:

- Group one was fed a basal diet all over the experiment period and served as -ve control.
- Group two (+ve control) fed high cholesterol diet contains 2.43% cholesterol with 0.49% cholic acid [19].
- Groups three and four as the same of group 2 and fed 2% cloves powder according to the more acceptable sample of the panel test and 0.4% BHT, respectively.

The experiment period was 4 weeks and carried out at the Post Graduated Lab of Faculty of Home Economics, Helwan University, Cairo, Egypt. Rats were weighted weekly and feed intake (FI) was recorded daily all over the experimental period. At the end of the experiment, body weight gain (BWG) and feed efficiency ratio (FER) were calculated in accordance with the method of [20]. Rats were fasted over night for 12hs and then sacrificed. Blood samples were obtained from each rat and centrifuged at 3000rpm for 15 minutes to extract serum for biochemical analysis. Heart and aorta were weighted and kept for histopathological examination by using the method described by [21].

2.2.12. Biochemical assays

Serum total cholesterol was determined according to [22]. Triglyceride was calculated according to [23]. High density lipoprotein was assayed according to [24]. Low density lipoprotein was calculated, and very low-density lipoprotein was determined according to [25]. Atherogenic index (AI) and coronary risk index (CRI) were calculated according to [26]. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined according to [27] and [28], respectively. Creatinine and uric acid were determined according to the methods described by Fossati et al., (1980) and Bartels et al., (1972) respectively [29-30]. Reduced Glutathione (GSH) and malondialdehyde (MDA) were determined according to the methods described by Habig et., (1974) and Ohkawa et al., (1979) respectively [31-32].

2.3. Statistical analysis

Statistical analysis was conducted using the analysis of variance (ANOVA) test and statistical analysis program [33]. The results were expressed as mean ± SE at $P < 0.05$ significance.

3. Results and discussion

3.1. Chemical composition of the essential oil of clove (%)

Table 2 shows the results of roasted clove oil content % which includes eugenol 44.56%, β-caryophyllene 35.51%, α-humulene 5.73%, copaene 5.62 %, eugenol acetate 2.57 %, and β-cadinene 1.18 %. Eugenol and β-caryophyllene recorded the highest concentrations of clove oil content with values 44.56 and 35.51%, respectively. The constituents of the oil also include 2-heptanone, 2-heptyl acetate, β-Ocimene, methyl salicylate, chavicol, α-cubebene and δ-cadinene are also found in clove oil in lower concentrations. In this study, eugenol represented the main component 44.56 %, and it is considered as the responsible for the numerous of activities a number of studies. In the same line Kaur et al., (2019) reported that eugenol is the active substance in clove, the amount of eugenol in a study can differ from study to study [34]. There have been maintains that the process used to extract essential oils is linked to a decrease in eugenol. Generally, these oils have the same main components. A variety of factors, including genetics, environment, and production methods, can influence the amounts of certain compounds present [35].

3.2. Polyphenolic compounds content of clove oil (µg/ml) and (µg/g)

Table 3 shows the results of polyphenolic compounds content of clove oil(µg/ml) and (µg/g). Clove is one among the primary vegetal sources of phenolic chemicals. The highest concentration of polyphenolic compounds was gallic acid, caffeic acid, ellagic acid and chlorogenic acid 373.75 µg/ml 7475.01 µg/g ,140.52 µg/ml 2810.41 µg/g, 134.62 µg/ml 2692.41 µg/g and 105.77 µg/ml 2115.36 µg/g, respectively.

Other phenolic acids found in clove are the catechin, syringic acid, pyro catechol, vanillin, ferulic acid and rosmarinus acid are also found in clove in lower concentrations. Results of polyphenolic compounds content of clove were converged with the results reported by Shan et al., (2005), Gallic acid is the phenolic acid compound that has the highest concentration [36]. In collaboration with academic institutions and commercial organizations, the US Department of Agriculture recently developed a database containing information about the polyphenol content and antioxidant activity of numerous foods. Using this database, we categorized the top 100 polyphenol-rich food sources. The foods with the highest polyphenol content, according to the results, clove was the spice with the highest concentration of antioxidant compounds and polyphenols [37]. In this part of study, selected parameters have been studied to evaluate all treatments with different additives: Treatments A and treatments B compared with control (Table 4). Objective evaluation results of cookies samples are illustrated in (Table 3). It was found that weight after baking, volume and area after baking were higher than that of the control. Roasted clove powder was added to the mixture, which significantly increased its capacity to hold both oil and water. These results are consistent with Jeddou et al., (2017), they mentioned that the increased hydroxyl group in the fiber and polyphenols of clove powder, allowing the binding of more water and oil, is probably the cause of the increase in water and oil holding capacities, this indicated that fiber increased water adsorption in bakery products. The results of our investigation generally demonstrate that the functional qualities of the cookies mixture were enhanced by the addition of clove powder [38].

3.3. Sensory evaluation

An important factor for determining the quality of food is its sensory evaluation. To determine if trained panelists would find the various cookie samples acceptable. Sensory quality attributes of cookies (color, taste, odor, texture, degree of chewing also, overall acceptability) of cookies fortified with different amounts of roasted clove powder and 0.4% BHT were shown in Figures 1-6 The tabulated data demonstrated that there was no significant difference between all treatments ($p<0$ .05) in the color, taste, odor, texture, degree of chewing and overall acceptability of cookies fortified with different levels of roasted powdered cloves compared with 0.4 % BHT and control during of storage period. Meanwhile, cookies fortified with roasted clove powder treatments A (1, 1.5, 2 %) had higher significantly ($p<0$ .05) scores for color, odor, texture and degree of Chewning during of storage period. When clove was added to wheat flour, the textural qualities chewiness and cohesiveness of the flour gel significantly increased as compared to the control (wheat flour alone) [39]. The interaction between the polyphenols and essential oil components in the clove and the starch in wheat flour may be responsible for the improved pasting qualities with the addition of clove powder [40]. These results suggest that adding clove powder to the cookies mixture enhanced its pasting abilities. With regard to the cookies sample containing 2 % of roasted clove powder had significant increase in odor attribute. Nikousaleh and Prakash (2016) indicated that spices that have been roasted release flavors with various compositions [41]. They produce flavor and color in food products and effect on the acceptability of consumers. These outcomes are consistent with those stated by Przygodzka et al., (2015) who confirmed that the sensory, antioxidant, and Maillard reaction profiles of cakes enhanced with included clove showed that the antioxidant capacity, phenolic content, browning properties, and overall acceptability of cakes enriched with clove, allspice, and spice was the best [42]. Overall, the physical and textural qualities of the cookies were improved by the addition of roasted clove powder.

3.4. Oxidative Stability Test

Oxidative test of different cookies samples stored for 21 days at 28°C. The effects of roasted clove powder and synthetic antioxidants (0.4% BHT) on acid value (AV), and peroxide value (PV) of cookies samples kept at 28°C for storage were shown in Figures 7-8. When different roasted clove powders were added, there was a decrease in PV and AV value when compared with sample containing 0.4% BHT and control (without additives). AV changes for cookies samples are contained in Figure 7. After 21 days in storage, an increase in AV was observed in every sample. The increase in the cookies sample containing 0.4% BHT was 2.98, but the control sample (without additives) had a higher increase of 5.90. Cookies with roasted clove powder demonstrated lower AV value than 0.4% BHT, control, and also showed when higher in the roasted clove powder level AV value was decreased in samples. So, 2 % cloves sample was 1.43 less than from 1.5% was1.58 and 1.5% followed by 1% 1.73 in AV values, during 21 days of storage. After 21 days of storage, the acidity of the control and cookie samples containing 0.4% BHT was higher than cookies containing varying quantities of roasted clove powder. An antioxidant called aromadendrene, which is present in roasted clove powder, prevented the cake from being rancid. It has previously been observed that after 28 days of storage, cake samples containing clove essential oil (CEO) showed reduced oxidative stability when compared to synthetic antioxidant-rich cake [10]. Figure 8 proved that the addition of roasted clove powder resulted in the decrease in PV of cookies with comparison to the PV of the control and sample containing 0.4% BHT. peroxide value in all treatments. A ranged from 6.33: 2.98 mg /kg compared to control was 8.62 mg /kg at 21 days of storage. PV increased consistently in all cookie samples when being stored. The value of peroxide reached significantly to its peak at the end of storage. The effect of the addition of 0.4% BHT in the cookies reduce of peroxide value 5.91 mg/kg compared with the peroxide value of control was 8.62 mg /kg at 21 days of storage. The increment rate in the value reduced as the concentration of roasted clove powder increased from 1:2%, indicating that roasted clove powder improves the storability of cookies and helps in the release of antioxidant compounds (all aromadendrene), which retard the PV value of cookies samples, with control cookies having the generally highest values. Moreover, clove has the highest capacity to give off hydrogen and reduce lipid peroxidation.

With respect to the lipid peroxidation, the inhibitory activity of clove determined using a linoleic acid emulsion system indicated a higher antioxidant activity than the standard BHT (Butylated hydroxyl toluene), the use of natural antioxidants in foodstuffs is developed to attenuate the use of synthetic antioxidants in foods [43]. The clove powder addition to the cookies also had an effect on the total bacteria, yeast, and mold counts. Cookies that contain more clove powder have the lowest amounts of these microorganisms. Throughout storage, data in Figure (9) shows that there were significant differences ($p<0.05$) noticed for aerobic bacterial count in the tested samples at the time of storage for 14 days between control (6X10Aa), BHT (2X10), and clove 1 gm 1X10. Bacterial count reached its peak at the end of storage, although the least grow was observed in cookies with higher quantities of roasted clove powder. Furthermore, it is obvious that samples containing roasted clove powders at concentrations of 1.5 and 2% exhibited considerable antibacterial action. The antibacterial activity increased with the addition of roasted clove powder from 1 to 2%. The findings reveal the possible antibacterial properties of roasted clove powder, which are consistent with prior observations. Idowu et al., (2021) who reported that, clove play role as an antimicrobial agent killed and inhibited the growth and reproduction of bacterial [44]. Conversely, there was no detected growth for molds and yeasts during the 21 days of storage period for all treatments with roasted clove powder and BHT compared with control showed of 1x10 cfu/g at the day 14 of storage, and 3x10 cfu/g at the day21 of storage (Table 9). Data in Table 5 indicates that in all cases there wase no visible yeast and mold in all treated samples still day 21. The antimicrobial activities of clove have been proved against fungal strains. These results are in agreement with Devi et al., (2010) who demonstrated that chromatographic evaluations revealed that eugenol was the primary component responsible for anti-fungal activity attributed to spore and micelle lysis [45]. Eugenol has a similar mechanism of action that damages membranes and causes macromolecule deformation. In regard to microbiological standards, microorganisms are thought to have an impact on organoleptic qualities and food safety. Data presented in Table 6 showed the effect of cloves on feed intake (g/day), body weight gain% and feed efficiency ratio in rats inducted with hypercholesterolemia. Data showed that, the mean values of feed intake (g/day) of the positive control group fed on cholesterol diet decreased as compared to the negative control group which fed on basal diet with mean values 19.50 vs19.78g/day, respectively. The third group which fed 2% clove decreased when contrasted with mean values in a positive control group 19.08 vs19.50 g/day, respectively. Whereas feed intake of rates fed 0.4 % BHT (group4) increased when compared with positive control group with mean values 19.64 vs 19.50 g/day, respectively. Regarding body weight gain (BWG), data showed that the mean value of BWG in the positive control group exhibited a significant decrease when compared to the negative control group with mean values 28.67±3.05 vs.30.00±2.97%, respectively. Mean value of rats in group 3 which fed on basal diet with 2% clove significant decreased as compared to the positive control group 16.50±1.27 vs. 28.67±3.05%, respectively. Mean value of rats in group 4 which fed on diet fortified with 0.4% BHT significant increased as compared to the positive control group 30.83±3.41 vs. 28.67±3.05 %, respectively. Regarding feed efficiency ratio, the mean value of FER in the positive control group showed significant decrease as compared to the negative control group 0.0527±0.0034 vs. 0.0544±0.0017, respectively. Mean value of group 3 fed on diet with fortified with 2% clove significant decreased in the mean value of FER as compared to the positive control group 0.0308±0.0033 vs. 0.0527±0.0034, respectively. Mean value of group 4 fed on diet with fortified with 0.4 % BHT significant increase in the mean value of FER as compared to positive control group 0.0585±0.0021vs. 0.0527± 0.0034, respectively. Above results were converged with the results reported by Pérez Gutiérrez and Arrioja (2021), who found that giving clove and curcumin extract to mice fed a high-fat diet at the same time for five weeks resulted in decreased feed intake, weight gain, adipose tissue, liver weight, and lipid profile regulation [46]. Data presented in Table 7 showed the effect of clove on lipid profile of hypercholesterolemic rats. Data showed that the mean value of TC of the positive control group fed on cholesterol diet increased of TC as compared to the negative control group fed on basal diet 219.48±14.09 143.69±06.96mg/dl, respectively for TC. The third group fortified with 2% clove decreased in TC when compared to positive control group 167.95±08.77 vs 219.48±14.09mg/dl, respectively for TC. Whereas rats fed 0.4 % BHT (group 4) decreased when compared with positive control group with mean values 174.82±08.93 vs219.48±14.09mg/dl, respectively. Data showed that the mean value of TG of the positive control group fed with a cholesterol diet raised of TG compared to the negative control group eating a basal diet. 185.76±10.84 vs77.08±04.25mg/dl, respectively for TG. The third group fortified with 2% clove decreased in TG when compared to positive control group 94.70±9.98 vs185.76±10.84mg/dl, respectively for TG. Whereas rats fed 0.4 % BHT (group4) decreased when compared with positive control group with mean values 106.10±4. 32vs 185.76±10.84, respectively for TG. Data showed that, the mean value of HDL of the positive control group fed on cholesterol diet decreased of HDL as compared to the negative control group fed on basal diet 42.07±02.00 vs 65.44 ±01.24mg/dl, respectively for HDL. The third group fortified with 2% clove increased in HDL when compared to positive control group 55.36±02.36 vs 42.07±02.00mg/dl, respectively. Whereas rats fed 0.4% BHT group 4 increased when compared with positive control group with mean values 51.21±01.73 vs 42.07±02.00mg/dl, respectively. The greatest improvement in HDL the group treated with 2% cloves because this group showed significant increase in HDL, as compared to other groups. The data showed that the mean value of LDL of the positive control group fed on cholesterol diet increased in comparison to the negative control group fed on basal diet 140.26±03.56 vs 62.84±02.08 mg/dl, respectively for LDL. The third group fortified with 2% clove decreased in LDL when compared to positive control group 93.65±01.99 vs 140.26±03.56 mg/dl, respectively. Whereas rats fed 0.4 % BHTgroup4 decreased when compared with positive control group with mean values 102.39±00.97 vs140.26±03.56mg/dl, respectively. Data showed that the mean value of VLDL of the positive control group fed on cholesterol diet increased of VLDL as compared with the negative control group fed on basal diet 37.15±01.40vs15.14±00.95mg/dl, respectively for VLDL.

The third group fortified with 2% clove decreased in VLDL when compared to positive control group 18.94±01.84vs37.15±01.40 mg/dl, respectively for VLDL. Whereas rats fed 0.4 % BHTgroup4 decreased when compared with positive control group with mean values 22.22±01.23vs37.15±01.40 mg/dl, respectively for VLDL. The greatest improvement in VLDL the group treated with 2% cloves because this group showed a significant increase in VLDL, as compared to other groups. According to the above findings, rats fed cloves recorded highly improvement in all lipid profile parameters. These results are in compliance with Shyamala et al., (2003) which reported that treatment by clove powder significantly improved the lipid profile [47]. Adefegha et al., (2014) revealed that feeding on clove buds' powder improved hypolipidemic effect except from HDL in high- fat diet induced hyperlipidemic rats [48]. Abd El-Rahman. (2015) found that cloves powder lower the total cholesterol, triglycerides, LDL-c and VLDL-c levels however raised from HDL-c level significantly compared to the positive group [49]. Al-Okbi (2014) found that different mechanisms of action have been observed in the potent effects of clove oil and eugenol on lipid disorders [50]. Pourlak et al., (2020) and Rabeh et al., (2021) showed that cloves extract lower plasma cholesterol, triglycerides, and LDL levels while increasing HDL levels [51-52]. Ramadan et al., (2013) reported that clove included high levels of natural antioxidants phenolic was significance in nutrition as natural antioxidants and might interact with free radicals directly to inhibit lipid peroxidation [53]. Cloves considered as nutritionally unusual for pharmaceutical industries, edible purposes and supply health benefits. Obtained results were in the line with the results reported by Ding et al., (2007) which reported that the clove extract decreased the amount of fat that accumulated in the liver and epididymal adipose tissues, as well as the development of obesity caused by a high-fat diet, body weight, and abdominal adipose tissue weight. It also regulated total triglycerides and low-density lipoprotein cholesterol [54]. Data given in Table 8 showed the effect of cloves on the AI and CRI in rats induced hypercholesterolemia. Data showed that, the mean value of AI of the positive control group fed on cholesterol diet significantly increased as compared to the negative control group fed on basal diet with mean values 3.33±0.001vs0.96± 0.003, respectively. The third group fed basal diet with 2% clove significantly decreased compared to positive control group with mean values 1.69±0.002vs3.33±0.001, respectively. Whereas rats fed basal diet with 0.4 % BHT (group 4) significantly decreased when compared to positive control group with mean values 1.99±0.002 vs 3.33±0.001, respectively. The greatest improvement in AI was in group 3 which fed with 2% cloves. Pertaining to CRI, the positive control group fed on cholesterol diet significantly increased of CRI as compared with the negative control group fed on basal diet with mean values 5.21±0.05 vs 2.19±0.04, respectively. Rats treated with 2% clove in group 3 were significantly lowered in CRI compared to the positive control group with mean values 3.03±0.05 vs 5.21±0.05, respectively. Whereas rats fed 0.4 % BHT in group 4 significantly decreased when compared with positive control group with mean values 3.41±0.02vs 5.21±0.05, respectively. The greatest improvement in CRI was in group 3 which was treated with 2% cloves. Evaluating the risk for atherosclerosis using the atherogenic index was very important to assess the severity of atherosclerosis. Lowering the risk of atherosclerosis is to lower the atherogenic index. Two percent lower in serum cholesterol led to a one percent decrease in the risk of coronary heart disease (CHD) by [55]. As shown by low levels of HDL-C and high levels of TC, TG, and LDL-C, which led to atherosclerosis and other cardiovascular diseases [56]. However, obtained results revealed that clove showed significant improvement in serum's lipid profile by raising the level of HDL-C and lowering the levels of TC, TG, LDL-C, and VLDL-C. Therefore, AI was improved in rats were fed on 2% cloves. Shukri et al., (2011) showed that the consumption of cloves in live animals decreased the tissue damage of cardiac muscles in rats [57]. Atawodi et al., (2010) showed that the polyphenol substances in clove buds have an association with their antioxidant qualities, as well as their capacity to prevent conditions like cardiovascular disease that are linked to oxidative stress [58]. Clove buds contained derivatives of gallic acid, and ellagic acid. Data given in Table 9 showed the effect of cloves on aspartate transaminase (AST) and alanine transaminase (ALT) in rats with induced Hypercholesterolemia. Data showed that, the mean value of ALT and AST of the positive control group was fed cholesterol diet significantly increased as compared to the negative control group which fed on basal diet with mean values 54.32±0.76 vs,31.33±0.71 µ/L, respectively for ALT and 38.41±1.00 vs, 24.50±0.76 µ/L, respectively for AST. The third group with 2% clove significantly decreased in serum ALT and AST when compared to positive control group with mean values 37.09± 0.76 vs 54.32±0.76 µ/L, respectively for ALT and 29.73±0.76 vs 38.41 ±1.00 µ/L, respectively for AST. Whereas rats fed 0.4 % BHT (group4) significantly decreased when compared with positive control group with mean values 42.05±0.054 vs 54.32±0.76 µ/L, respectively for ALT and 32.88±0.76 vs 38.41 ±1.00 µ/L, respectively for AST. Hepatic transaminases, like AST and ALT considered to be the standard for evaluating the liver [59]. This study is in the same line with El-Segaey (2007) who found that clove significantly decreased level of liver enzymes (AST and ALT) in rats and this reduction due to a high level of antioxidants in clove [60]. Abozid and El-Sayed (2013) found that rats treated with clove or clove essential oil prevent from rising plasma levels of AST and ALT compared with hydrogen peroxide treated rats alone [61]. Liver enzymes activity was significantly decreased by clove bud powder (CBP) and showed higher levels from antioxidant (glutathione, ascorbic acid, superoxide dismutase and catalase), compared to the positive control group [48]. Data presented in Table 10 showed the effect of cloves on uric acid and creatinine in rats with induced hypercholesterolemia. Data showed that, the mean value of creatinine and uric acid of the positive control group fed on cholesterol diet significantly increased as compared to the negative control group which fed on basal diet with mean values 2.24 ±0.005 vs. 1.15±0.007 mg/dl, respectively for creatinine and 39.50±0.76 vs. 30.17± 0.70 mg/dl, respectively for. Creatinine and uric acid of rats in group 3 which fed 2% clove significantly decreased compared to positive control group 1.74±0.007 vs. 2.24±0.005 mg/dl, respectively for creatinine and 32.10±0.76 vs.39.50±0.76 mg/dl, respectively for uric acid.

Whereas rates fed 0.4 % BHT (group 4) significantly decreased when compared with positive control group with

mean values 2.09±.008 vs. 2.24 ±0.005 mg/dl, respectively for creatinine and 35.14 ±0.58 vs. 39.50±0.76 mg/dl, respectively for uric acid. Obtained results were in the same line with Rabeh et al., (2021) who reported that S. aromaticum can prevent oxidative damage to renal tissue and lower levels of creatinine and serum uric acid [52]. Moreover, Bakour et al., (2018) found that S. aromaticum can lower liver and kidney harm caused by hydrogen peroxide and protecting the kidney from oxidative damage [62]. Data presented in Table 11 showed the effect of cloves on the activity of GSH and MDA in rats induced Hypercholesterolemia. Data showed that, the mean value of GSH of the positive control group fed on cholesterol diet significantly decreased compared to the negative control group fed on basal diet 50.06±1.99 vs. 59.00±4.39 mmol/dl, respectively. Rats of group 3 which fed basal diet with 2% clove significantly increased compared to positive control group 57.27±4.05vs.50.06±1.99 mmol/dl, respectively. Whereas rates fed 0.4 % BHT (group4) significantly increased when compared with positive control group with mean values 54.00±2.01 vs. 50.06±1.99 mmol/dl, respectively. Regarding MDA, data showed that, the mean value of MDA in the positive control group showed significant increase (P≤0.05) as compared to the negative control group with mean values 49.05±1.98 vs. 38.02±2.36 nmol/dl, respectively. The third group was fed basal diet with 2% clove significantly decreased when compared to positive control group with mean values 40.35±3.62 vs. 49.05±1.98 nmol/dl, respectively. Whereas rates fed 0.4 % BHT (group4) significantly decreased when compared to positive control group with mean values 44.08±2.67 vs. 49.05±1.98 mmol/dl, respectively. Glutathione (GSH) is an important cellular antioxidant whose intracellular damage has been linked to the aging process as well as the development of a variety of diseases such as cardiovascular disease, liver disease, neurodegenerative disease, lung disease, and immunological disorders. GSH also plays a significant function in metabolic syndrome [63]. Glutathione is an antioxidant that prevents cells from oxidative stress by Eugenol and eugenol acetate prevents lipid peroxidation by their phenolic providing hydrogen atoms to catch peroxyl radicals that cause lipid peroxidation [64]. Obtained results were in the same line with Ali et al., (2022) who found that clove flower extract decreases MDA, the level of MDA remarkably decreased concentrations and increased level of GSH when given raw or irradiated clove extract [65]. Its flavonoid and phenolic components, including eugenol, eugenol acetate, and thymol are in control of this antioxidant effect [67]. All these above investigations were in the line with the histopathological examinations of heart and aorta of rats in all four tested groups which was showed in Figures (11-14) for heart and (15-18) for aorta.

Table 1: Ingredients used for cookies preparation and overall treatments under the experimental conditions.

Ingredients(g)	**Control**	**Treatment A**			**Treatment B**
		1%	**1.5%**	**2%**	**0.4%**
Wheat flour	250	247.5	246.25	245	250
Clove powder	0	2.5	3.75	5	0
BHT	0	0	0	0	1
Sugar	100	100	100	100	100
Milk powder	10	10	10	10	10
Egg	60	60	60	60	60
Butter	100	100	100	100	100
Vanilla	5	5	5	5	5
Baking powder	3	3	3	3	3
Salt	3	3	3	3	3

Table 2: Chemical composition of the essential oil of clove (%).

Component Name	(%)
Eugenol	44.56
β-Caryophyllene	35.51
α-Humulene	5.73
Copaene	5.62
Eugenol acetate	2.57
α-Cubebene	1.33
β-Cadinene	1.18
Methyl salicylate	0.63
β-Ocimene	0.59
δ-Cadinene	0.49
2-Heptyl acetate	0.39
Chavicol	0.39
2-Heptanone	0.21

Table 3: Phenolic Compounds Content of clove oil.

Component Name	Conc. (µg/ml)	Conc. (µg/g)
Gallic acid	373.75	7475.01
Chlorogenic acid	105.77	2115.36
Catechin	52.04	1040.75
Caffeic acid	140.52	2810.41
Syringic acid	35.64	712.83
Pyro catechol	34.62	692.32
Ellagic acid	134.62	2692.41
Coumaric acid	1.31	26.19
Vanillin	7.09	141.79
Ferulic acid	0.62	12.49
Naringenin	9.79	195.75
Rosmarinic acid	6.42	128.30
Daidzein	13.26	265.21
Quercetin	2.20	43.92
Cinnamic acid	0.40	7.97
Kaempferol	7.84	156.85
Hesperetin	0.30	5.98

Table 4: Physical properties of Cookies as affected by addition different additives.

Physical Properties	Samples				
	Control	Treatments A			Treatments B
		1 %	1.5 %	2 %	0.04 %
Baking time (min)	7-8	7-8	7-8	7-8	7-8
Wt. before baking (g)	15	15	15	15	15
Wt. after baking (g)	13	14	14	14	13
Volume (cm^3)	35	45	50	53	30
Area before baking (cm^2)	14	14	14	14	14
Area After baking (cm^2)	15	16	17	18	15

Table 5: Clove's effect on yeast and mold count (CFU/g) in cookies samples during storage at 28 °C for 21 days.

Treatments		CFU/g (Storage time (Days))			
		Zero	7	14	21
Control		ND	ND	$1x10^b$	$3x10^a$
Treatments A	1%	ND	ND	ND	ND
	1.5%	ND	ND	ND	ND
	2%	ND	ND	ND	ND
Treatments B	BHT (0.4%)	ND	ND	ND	ND

Reported values are the mean ± SD of three replicates. Means in the same row followed by different subscript letters are significantly different ($p<0.05$). Upper case letters for columns and lower-case letters for rows. The maximum recommended bacterial count is 1×10^4 cfu/g according to [65].

Table 6: Effect of roasted cloves on feed intake (FI), body weight gain (BWG) and feed efficiency ratio (FER) in rats with Hypercholesterolemia.

Parameters / Groups	FI (g/d)	BWG (%)	FER
G1: Control (-ve)	**19.78**	**30.00 ±2.97^a**	**0.0544±0.0017^a**
G2: Control (+ve)	**19.50**	**28. 67±3.05^a**	**0.0527±0.0034^a**
G3: clove 2%	**19.08**	**16.50±1. 27^b**	**0.0308±0.0033^b**
G4: BHT 0.4%	**19.64**	**30. 83±3.41^a**	**0.0585±0.0021^a**

Mean values are expressed as mean ± SE. Means with different superscript letters in the same column are significantly different at $P \leq 0.05$.

Table 7: Effect of cloves on Total Cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL-C), low density lipoprotein (LDL-C) and very low-density lipoprotein (VLDL) in rats with induced Hypercholesterolemia**.**

Parameters / Groups	TC	TG	HDL-C	LDL-C	VLDL-C
	mg/dl				
G1: -ve control	143.69±06.96 ^{d}	77.08±04.25 ^{d}	65.44 ±01.24 ^{a}	62.84 ±02.08 ^{d}	15.41 ±00.95 ^{d}
G2: +ve control	219.48±14.09 ^{a}	185.76±10.84 ^{a}	42.07±02. 00 ^{d}	140.26±03.56^{a}	37.15 ±01.40^{a}
G3: 2% clove	167.95±08.77^{c}	94.70±9. 98^{c}	55.36±02.36^{b}	93.65±01.99 ^{c}	18.94±01.84 ^{c}
G4: 0.4% BHT	174.82 ±08.93 ^{b}	106.10±4. 32 ^{b}	51.21±01.73 ^{a}	102.39 ±00.97 ^{c}	22.22±01.23 ^{b}

Mean values are expressed as means ± SE. Means with different superscript letters in the same column are significantly different at P≤ 0.05.

Table 8: Atherogenic index (AI) and coronary risk index (CRI).

Parameters / Groups	AI	CRI
G1: Control (-ve)	0.96±0.003^{d}	2.19±0.04^{d}
G2: Control (+ve)	3.33±0.001^{a}	5.21±0.05^{a}
G3: clove2%	1.69±0.002^{c}	3.03±0.05^{c}
G4: BHT0.4%	1.99±0.002^{b}	3.41±0.02^{b}

Mean values are expressed as means ± SE. Means with different superscript letters in the same column are significantly different at P≤ 0.05.

Table 9: Effect of cloves on aspartate aminotransferase (AST) alanine aminotransferase (ALT) in rats with induced Hypercholesterolemia.

Parameters / Groups	**ALT**	**AST**
	µ/L	
G1: Control (-ve)	**31.33±0.71^{d}**	**24.50±0.76^{d}**
G2: Control (+ve)	**54.32 ±0.76^{a}**	**38.41 ±1.00^{a}**
G3: clove 2%	**37.09 ± 0.76^{c}**	**29.73±0.76^{c}**
G4: BHT 0.4%	**42.05±0.054^{b}**	**32.88±0.76^{b}**

Mean values are expressed as means ± SE. Means with different superscript letters in the same column are significantly different at P ≤ 0.05.

Table 10: Effect of cloves on creatinine and uric acid in rats induced hypercholesterolemia.

Parameters / Groups	Creatinine	Uric Acid
	mg/dl	
G1: Control (-ve)	1.15 ± 0.007[d]	30.17± 0.70[d]
G2: Control (+ve)	2.24 ±0.005[a]	39.50±0.76[a]
G3: clove2%	1.74±0.007[c]	32.10±0.76[c]
G4: 0.4%BHT	2.09± 0.008[b]	35.14 ±0.58[b]

Mean values are expressed as means ± SE. Means with different superscript letters in the same column are significantly different at $P \leq 0.05$.

Table 11: Effect of cloves on serum concentration of MDA and the activity of GSH in rats induced Hypercholesterolemia.

Parameters / Groups	GSH	MDA
	mmol/dl	
G1: Control (-ve)	59.00±4.39[a]	38.02 ±2.36[d]
G2: Control (+ve)	50.06 ±1.99[d]	49.05±1.98[a]
G3: clove2%	57.27 ±4.05[b]	40.35 ±3.62[c]
G4: 0.4%BHT	54.00±2.01[c]	44.08±2.67[b]

Mean values are expressed as means ± SE. Means with different superscript letters in the same column are significantly different at $P \leq 0.05$.

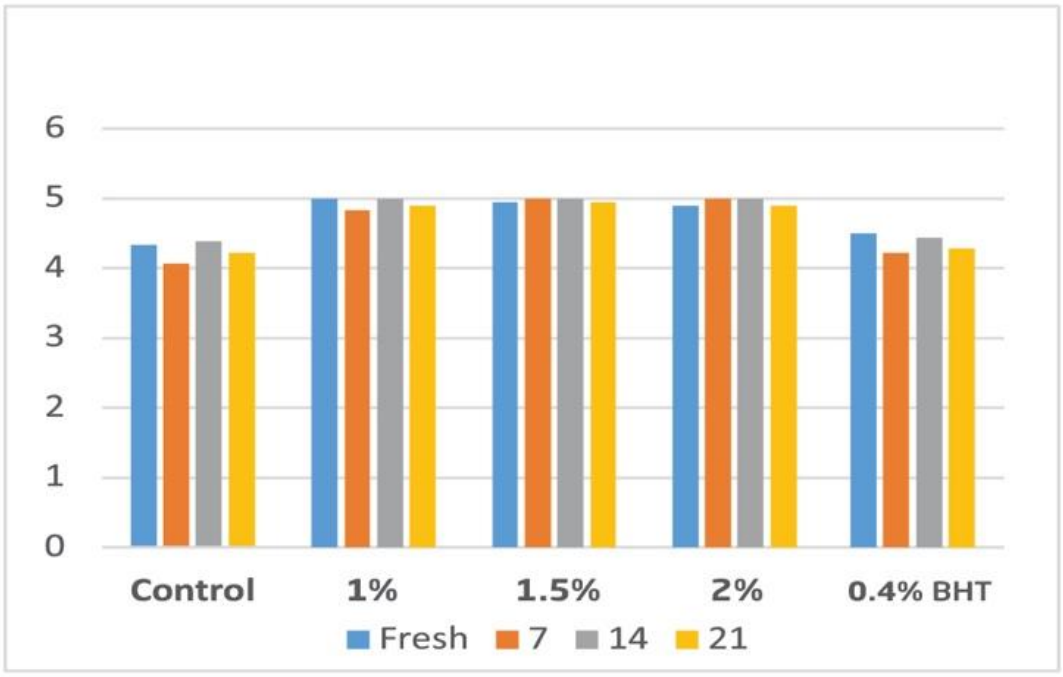

Figure 1: *Mean values of sensory characteristic (color).

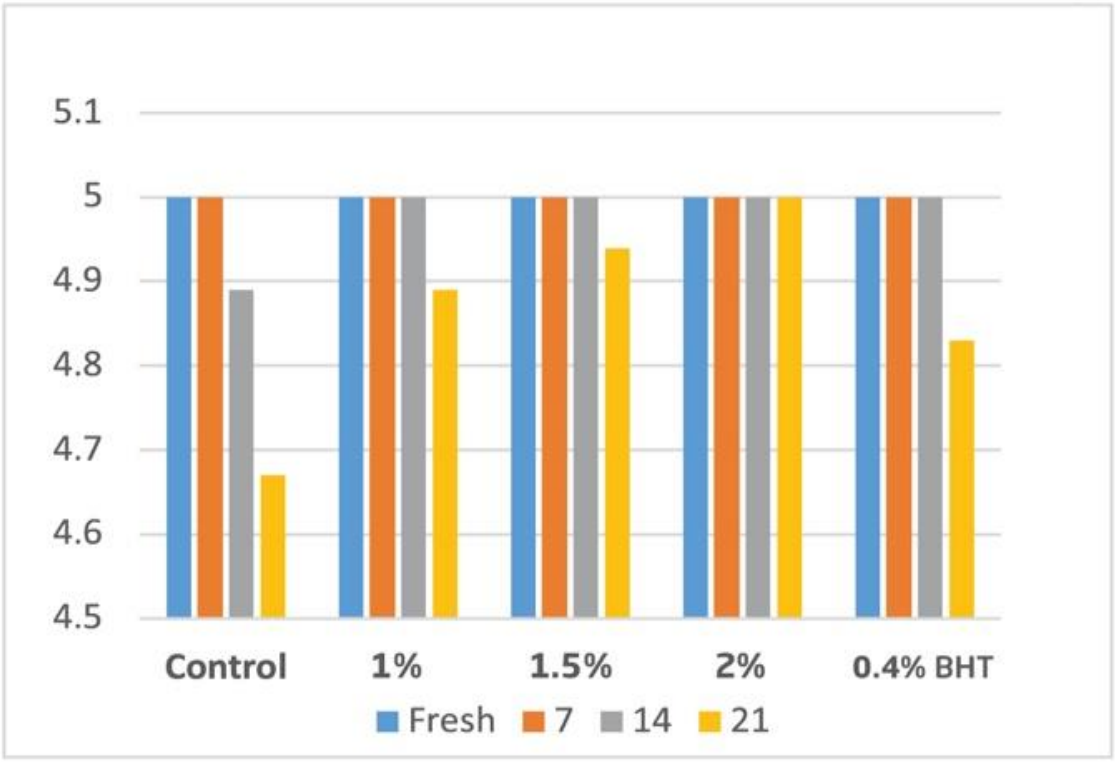

Figure 2: *Mean values of sensory characteristic (odor).

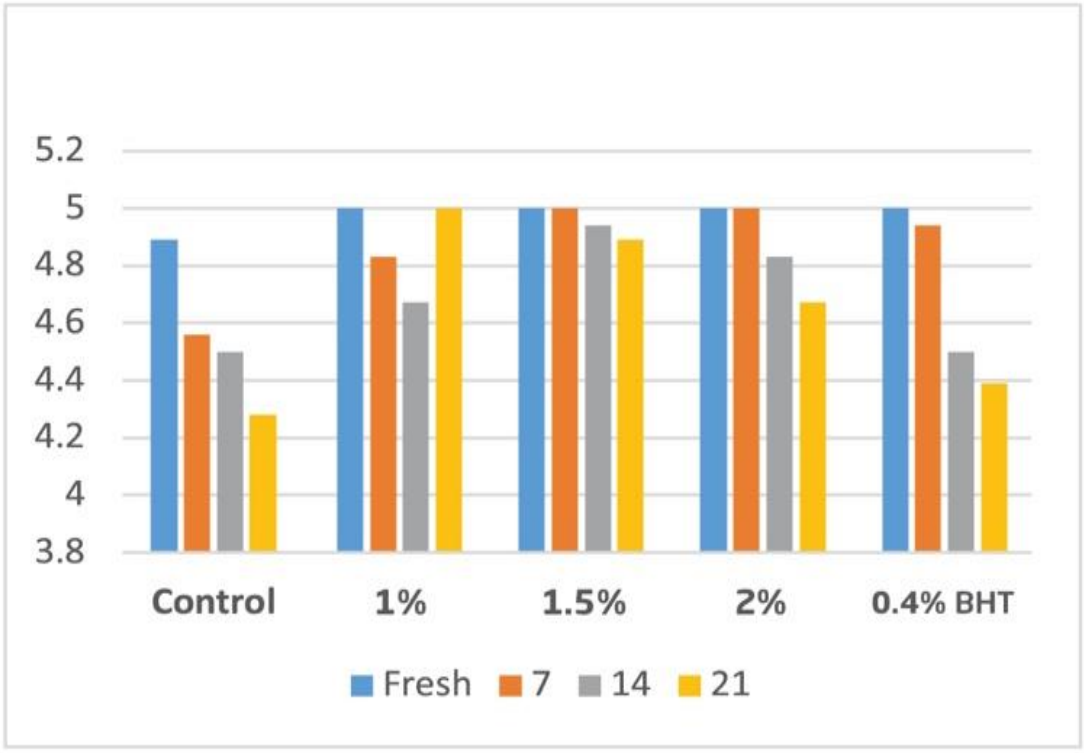

Figure 3: *Mean values of sensory characteristic (degree of chewing).

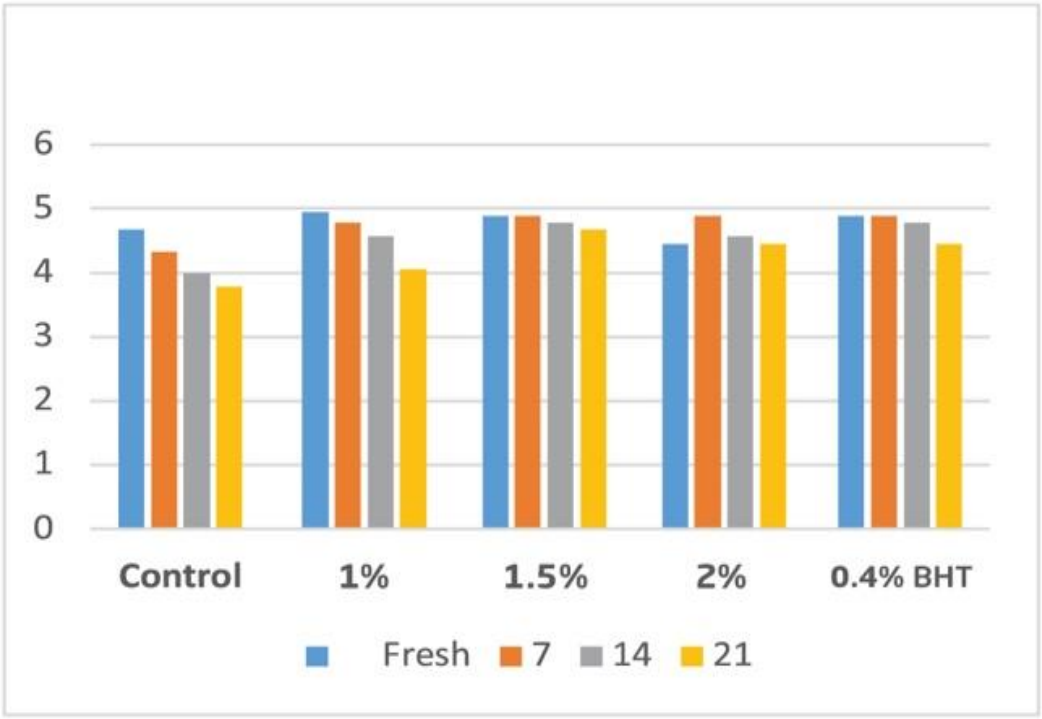

Figure 4: *Mean values of sensory characteristic (taste).

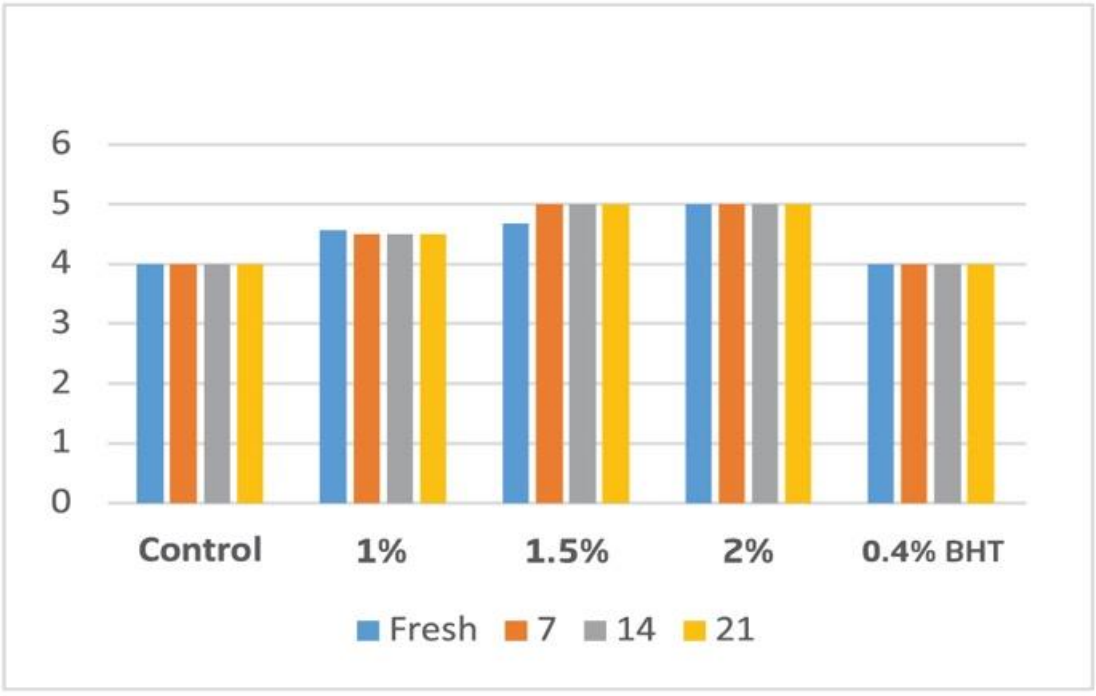

Figure 5: *Mean values of sensory characteristic (texture).

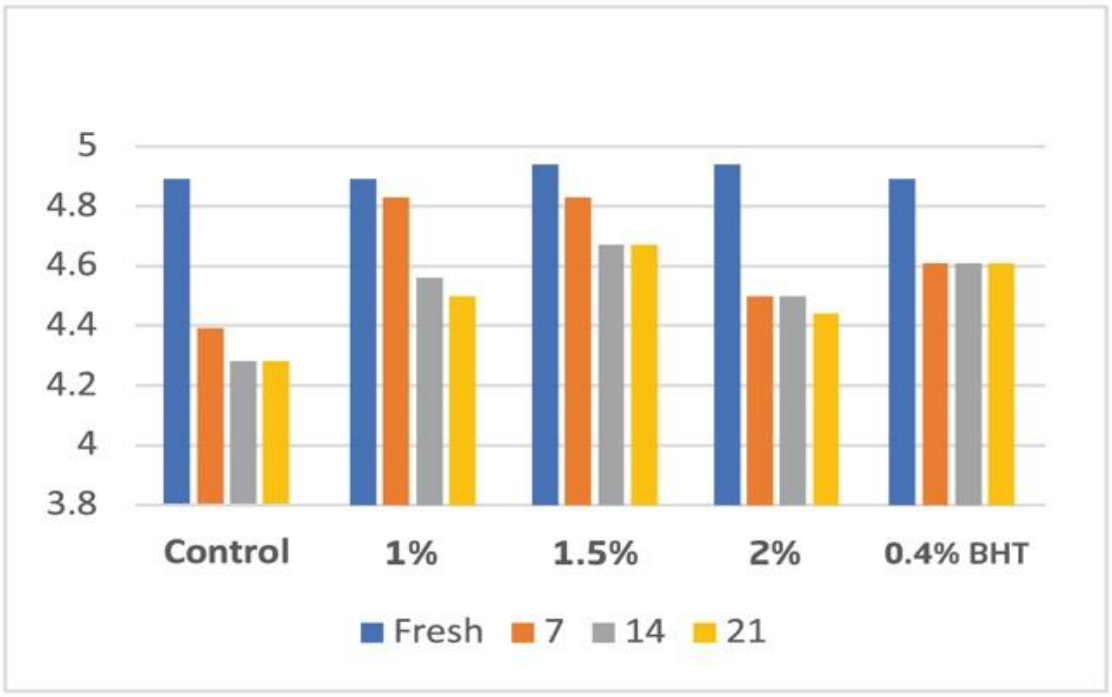

Figure 6: *Mean values of sensory characteristic (overall acceptable).

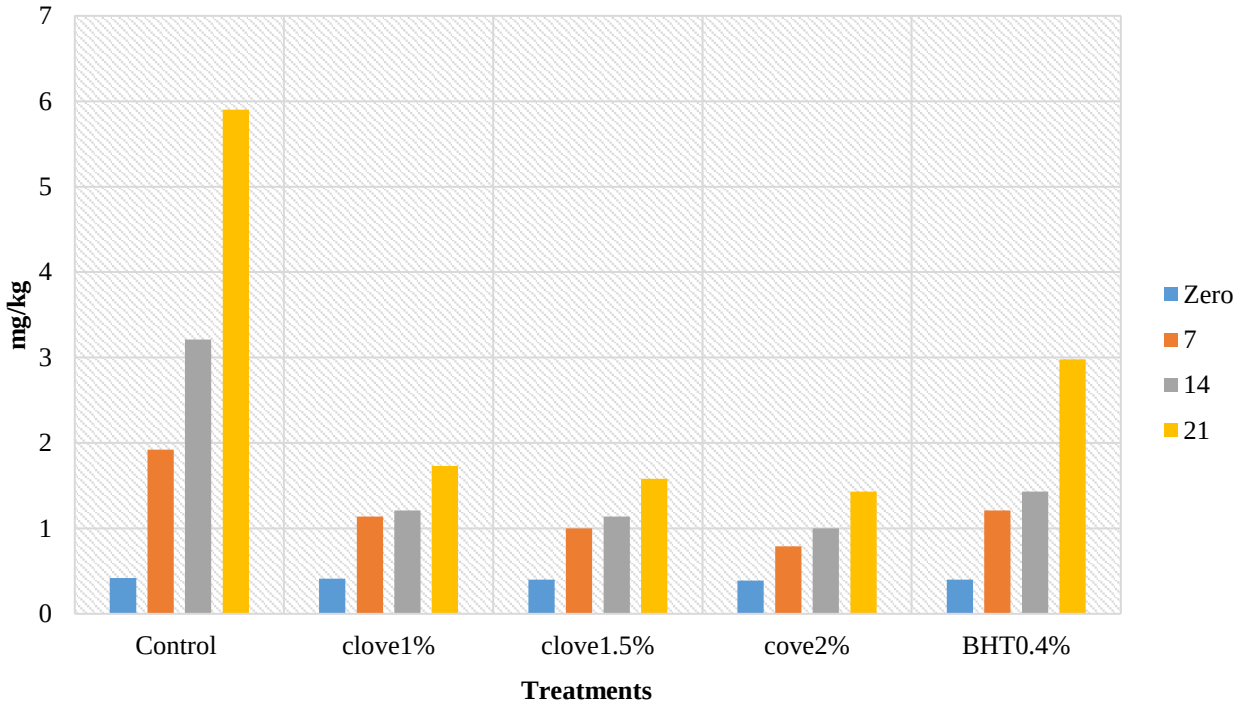

Figure 7: The impact of roasted clove powder and 0.4 % BHT on the acid value (AV) of cookies samples stored at 28°C for 21 days.

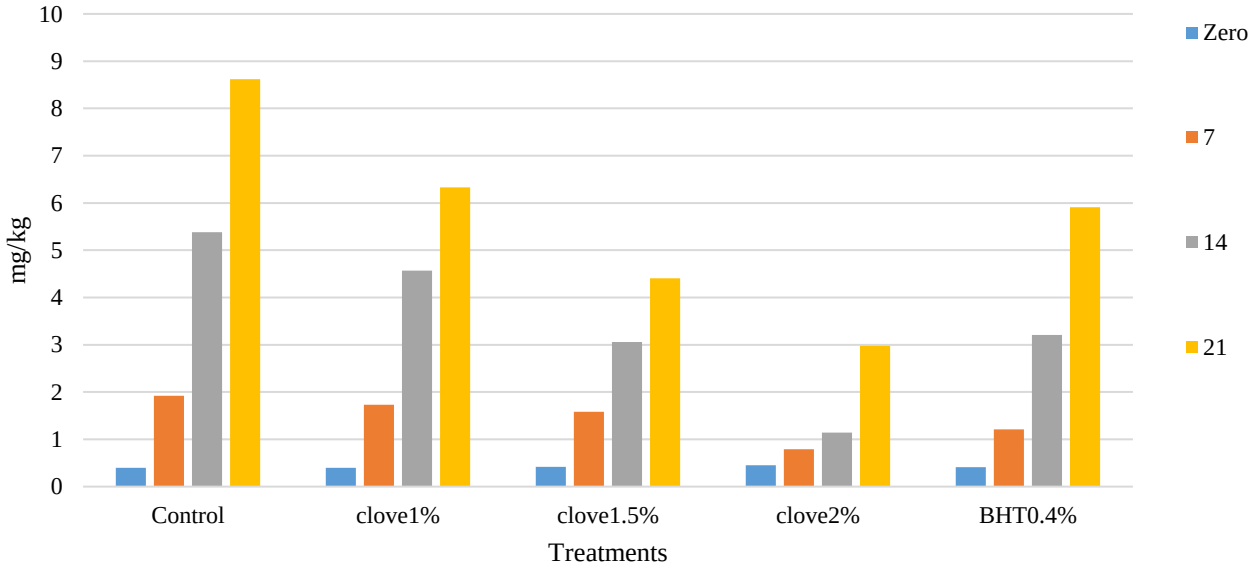

Figure 8: Effect of roasted clove powder and BHT on Peroxide value (PV) of cookies samples during storage at 28°C for 21 days.

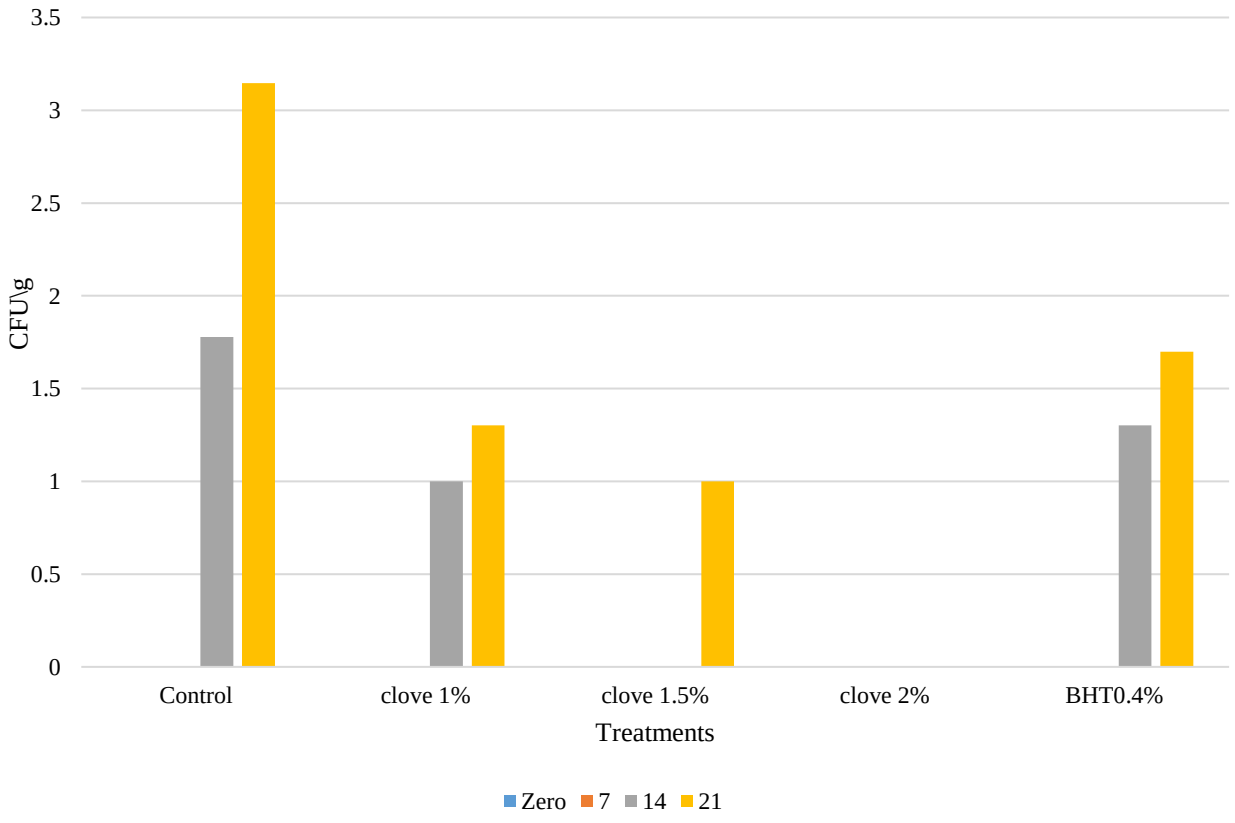

Figure 9: Microbiological examination (Aerobic bacterial count CFU/g) of cookies samples during Storage for 21 days at 28°C.

Control

1% clove

1.5%clove

2%clove

0.4%BHT

Figure 10: Photograph picture of cookies product fortified with different amounts of roasted clove powder and 0.4% BHT as compared to the control**.**

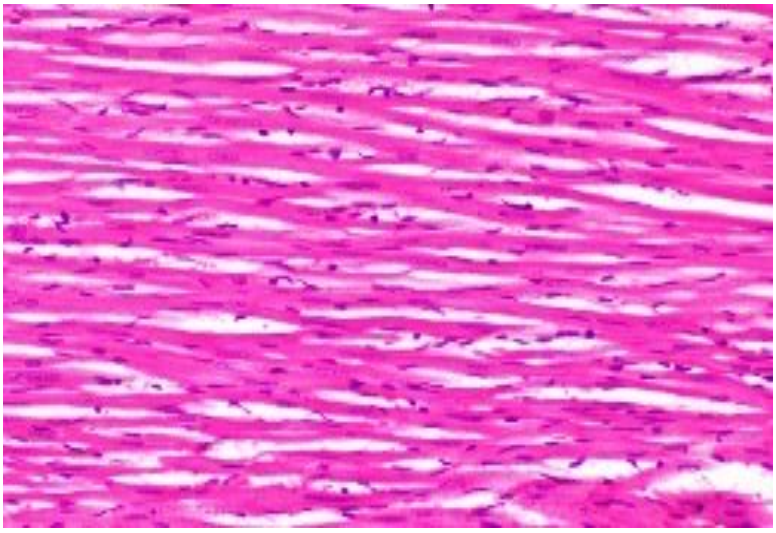

Figure 11: Photomicrograph of heart of normal control rat from (group 1) showing the normal histological structure of cardiac myocytes.

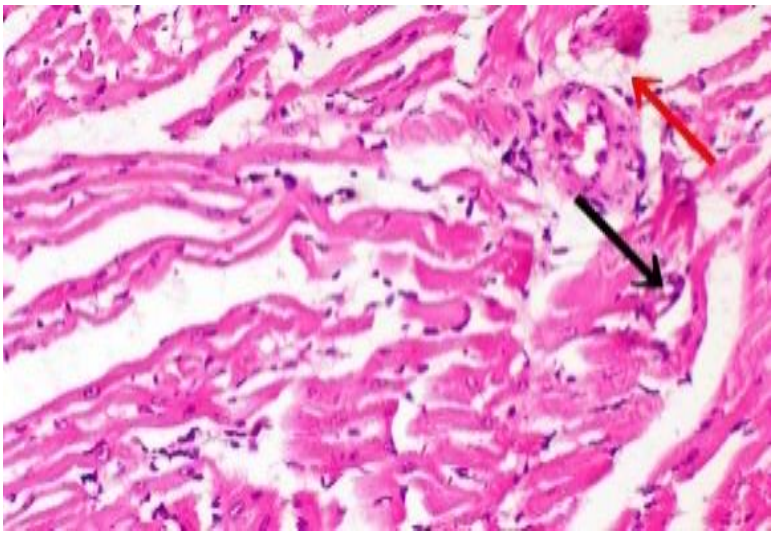

Figure 12: Photomicrograph of heart of rat from control positive group (group2) showing vacuolation of the sarcoplasm of cardiac myocytes (black arrow) and perivascular edema (red arrow).

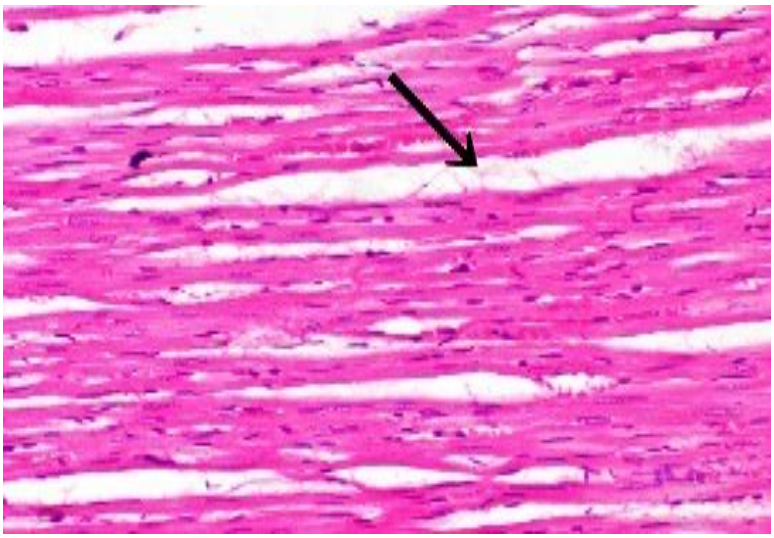

Figure 13: Photomicrograph of heart of rat from treated group with 2% clove (group3) showing slight intramyocardial edema.

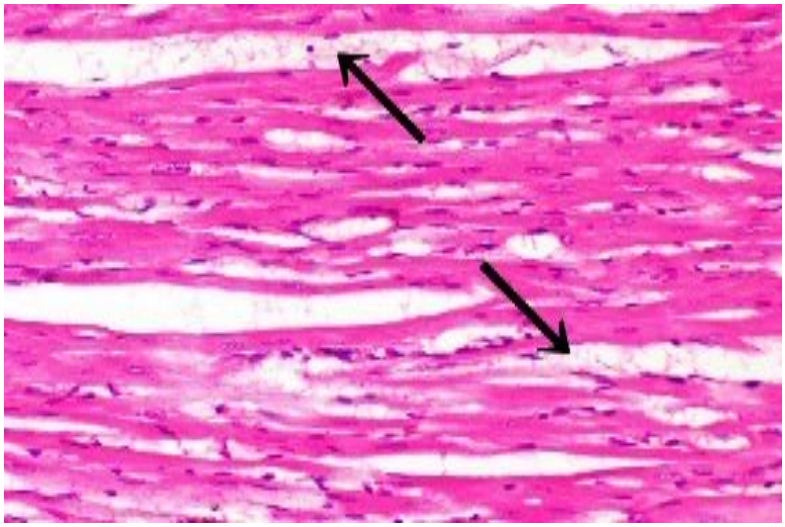

Figure 14: Photomicrograph of heart of rat from group 4 treated with (0.4% BHT) showing slight intramyocardial edema.

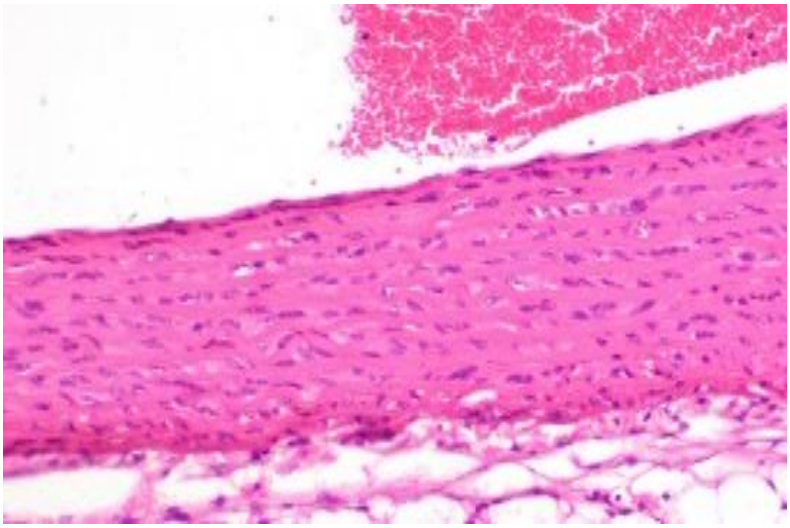

Figure 15: Photomicrograph of aorta of normal control rat from (group1) showing normal histoarchitecture

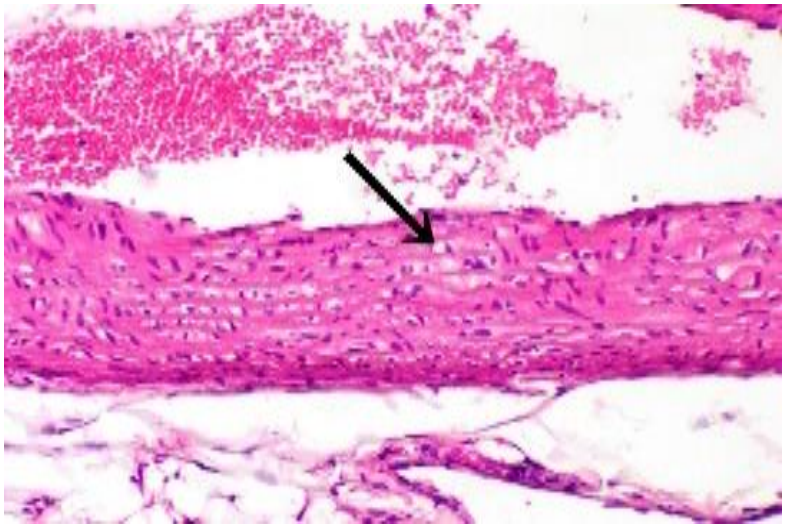

Figure 16: Photomicrograph of aorta of rat from positive control group (group2) showing marked vacuolization in the tunica media of the wall.

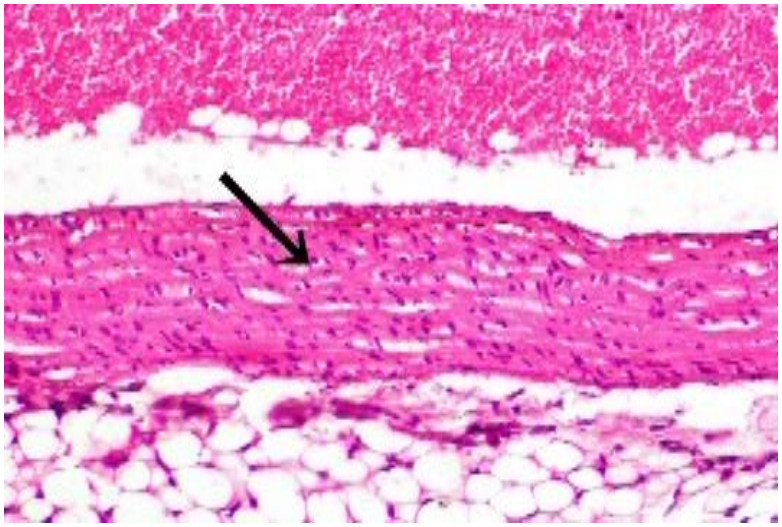

Figure 17: Photomicrograph of aorta of rat from group 3 (treated group with 2% clove) showing slight vacuolization in the tunica media.

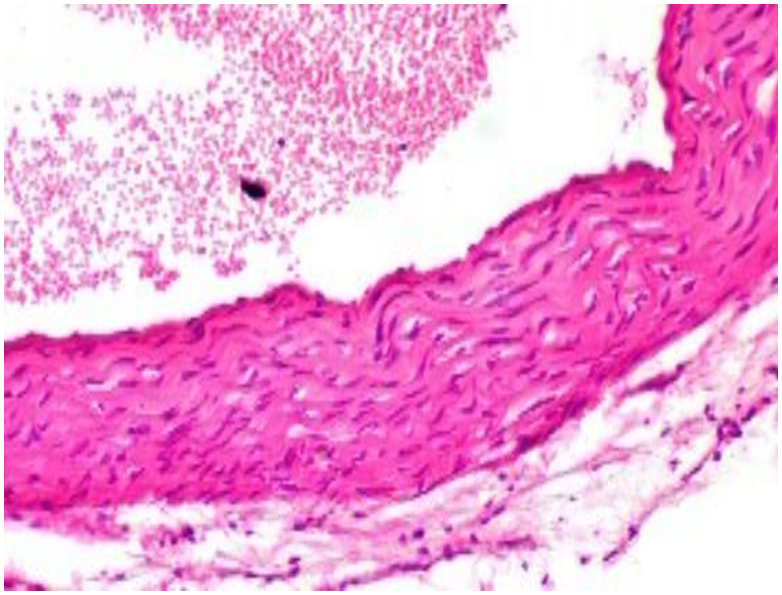

Figure 18: Photomicrograph of aorta of rat from group 4 treated with (0.4% BHT) showing no histopathological alterations.

4. Conclusions

Adding clove powder to cookies improved their physicochemical, nutritional, and bioactive characteristics, as well as their storability, without significantly impacting their sensory acceptance and can contribute to extending the food products' shelf life in addition to its safety for health as well as roasted cloves may play a role against hypercholesterolemia so, it's may beneficial for human being.

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