

The Protective Action of Cephalotyre (Ras) Cheese Supplemented with Jalapenos Red Pepper on Inflammation and Colon Cancer

Eman F. EL-Haggar ¹, Samah M. El-Sayed ², Adel M. M. Kholif ², Osama A. Ibrahim ^{2,*}

¹ Nutrition and Food Science Department, Faculty of Home Economics, Arish University, North Sinai, Egypt; eman_nrc@hotmail.com (E.E.);

² Dairy Science Department, Industries and Nutrition Research Institute, National Research Centre, Cairo, Egypt; samah_mosbah80@yahoo.com (S.E.); adelkholif@yahoo.com (A.K.); osama_nrc@hotmail.com (O.I.);

* Correspondence: osama_nrc@hotmail.com;

Scopus Author ID 56819417700

Received: 30.05.2024; Accepted: 6.10.2024; Published: 14.02.2025

Abstract: Recently, public health strategies have been used for immunonutrition to effectively suppress disease as a tool of preconception and precaution restrictions. The present applied research is concerned with consumer demand for a specific type of cheese, such as Cephalotyre (Ras) cheese, the most popular hard cheese in Egypt, that met its satisfaction in terms of form, taste, nutritional value, and good quality. Thus, the current study aimed to investigate the protective impact of Ras cheese supplemented with Jalapeno red pepper (JRP) with two levels *in vivo* and *in vitro* systematic with multipurpose. The results showed that JRP Ras cheese had a favorable role in weight control, hepatoprotective effect, and dualistic role in suppression of hyperlipidemia and colorectal cancer. It could be concluded that JRP Cephalotyre (Ras) cheese might provide the market with functional cheese that supports consumer immunity with antioxidant and antitumor properties in addition to a protective role for metabolic syndrome.

Keywords: cephalotyre (Ras) cheese; jalapeno red pepper; proinflammatory; hyperlipidemia; oxidative stress; colon cancer.

© 2025 by the authors. This article is an open-access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Since coronavirus is prevalent globally, public health strategies are currently focused on prioritizing immunonutrition as a preventative tool and using good nutrition practices, as achieving infection recovery and viral clearance requires activation of the host's immune response. The target of this study was a response to popular demand to supply the local market with functional food products that support immunity and suppress metabolic syndrome-related diseases.

Colorectal cancers, alongside cardiovascular disease (CVD), are the highest-ranked prevalence and costliest chronic conditions leading to death globally in 2020. Meanwhile, this rank is relevant to a positive relationship between metabolic syndrome and colorectal cancer [1,2]. Metabolic syndrome has multifaceted disadvantages on health impact connected to several diseases such as cardiovascular disease, diabetes mellitus, and cancer prolonged to inflammation and immunity disorders [3]. Inflammation is a protective reaction characterized by accumulating specific subsets of leukocytes to sites of infection or tissue damage, migration of leukocytes directional, and selective process. However, atherosclerosis is a progressive

disease resulting from the negative effects of oxidative stress that provokes complications such as hypercholesterolemia, hyperglycemia, obesity, hypertension, tumor progression, and aging, promoting vascular inflammation and endothelial activation [4]. Atherosclerosis can affect any artery in the coronary or cerebral circulation. The arterial wall is damaged by the oxidation of LDL, which induces the activation of endothelial cells, fostering the nascence and growth of plaques through the atherogenesis process [5-8]. The severity of thrombotic complications of atherosclerosis occurs when proteolytic enzymes and intense immune and inflammatory activity destroy the fibrous cap in the plaque, which is linked to transforming the stable plaque to unstable [5]. Tumor necrosis factor α (TNF- α) and interleukin-6 (IL-6) act as early inflammatory biomarkers predicting CVD events in the short term and are involved in the initial phase of cell interaction and atheroma formation. Interleukin-6 is a multifactor and recently has a fundamental role in lipid metabolism [9,10]. Nonetheless, preconception and precaution restrictions are presumed to be effective suppression. Changes in lifestyle and dietary patterns are pertinent, equitable, effective, negative, or positive.

Natural phytochemicals support protection against cardiovascular and metabolic diseases. Moreover, it exhibits anti-inflammatory activity and is considered a potential drug [11,12]. Jalapenos red pepper (JRP) (*Capsicum annuum*) is derived from the Capsicum group of the family Solanaceae. It was defined as an herbal product that is red and spicy when mature, and its hotness is produced by capsaicin; it is used as a flavoring, coloring, and preserving agent in food and medicine [13,14].

Dairy foods offer a bounty of health benefits due to macro- and micro-nutrients and high-quality protein, including bioactive peptides, milk fat globules, prebiotics, and probiotics. Dietary guidelines for Americans 2025 recommend that dairy products should be approximately two to three servings per day based on dietary intake for calcium, which ranges from 500-1300 mg/day. Calcium consumption from dairy products is linked to body weight regulation, diabetes control, bone and digestive health [15-17].

Processing and manufacturing milk into cheese preserves a perishable food and converts it into a stable and storable product its manifold uses. There are different varieties of cheese. Ras cheese is Egypt's most popular hard cheese, similar to the Greek "Cephalotyre" cheese [18]. Furthermore, health problems are a major anxiety to the world population, though there are certain challenges in the development of functional foods that promote health-promoting bioactive combinations. Thus, these ingredients were advanced and used as agents for improving flavor and appearance, and this change occurred after the progress of food additives in food manufacturing [19-22].

Recent dietary recommendations and guidelines for lifestyle management to reduce cardiovascular risk are surrounded by ambiguous controversy about using fat in dairy products. Using full-fat milk in dairy products, half, low, or even free, puzzles the consumer and impresses them. Proponents of using full fat proclaim it has been associated with several cardiometabolic benefits. At the same time, opponents denounce that it is due to saturated fat and cholesterol content, which lead to CVD and cancer development. As with so many issues of controversy, the goal of this article is not to convince nor dissuade individuals from using fat in dairy products. Rather, the main aim of this study is to clarify the role of antioxidant power and anticancer activity of Cephalotyre (Ras) cheese supplemented with JRP through implementation *in vivo* and *in vitro* scale to evaluate their health impact.

2. Materials and Methods

2.1. *Cephalotyre (Ras) cheese manufacture.*

Ras cheese was prepared as described by El-Sayed *et al.* [23]. Four Ras cheese treatments supplemented with JPR in the level of 2, 4, and 6% to the cheese curd. Hence, the findings of Ras cheese characteristics, including chemical, rheological, and sensorial during its ripening period at $12 \pm 2^\circ\text{C}$ for 4 months [23], led to the conduct of two cheese treatments (JPR in the level of 2 and 4%) in suitable animal model as *in vivo* and *in vitro* experiments in comparison of control Ras cheese.

2.2. *Experimental design and diet preparation.*

The present study was performed on 30 male rats with Sprague Dawley strain with body weight (100 ± 5 g as mean \pm SD) obtained from the National Research Centre, Egypt. The rats were housed in metabolic stainless-steel cages under hygienic conditions at a temperature of $25 \pm 3^\circ\text{C}$. Food and water were allowed, as well as ad libitum.

This study followed the recommendations of the Good Medical and Laboratory Practice guidelines, as well as the Institutional Animal Care and Use Committee (IACUC) guidelines and recommendation, and World Health Organization (WHO) rules regarding the ethics of scientific research according to the Medical Research Ethics Committee, National Research Centre (NRC), Cairo, Egypt (Approval code 20193).

Experimental rats were divided into 5 groups (6 rats each); rat diets were prepared and divided into five groups as follows: Group 1 (negative group) fed on basal diet was semi-synthetic and nutritionally adequate (AIN-93 M), vitamins mixture and minerals mixture were prepared as described by Reeves *et al.* [24]. Group 2 (positive group) fed on a high protein/high-fat diet designed as described by Liu *et al.* [25] with slight modification. Group 3 fed on control Ras cheese (RC) mixed with a positive diet, while groups 4 and 5 fed on Ras cheese supplemented with JRP in two levels, 2% (RJL) and 4% (RJH) mixed with a positive diet, respectively. All diets contained the same level of vitamins, minerals, and fiber through the experiment period of 10 weeks. At the end of the experiment, the rats were anesthetized, and blood samples were collected and then stored at -70°C until biochemical analysis.

2.3. *Nutritional evaluation.*

The nutritional evaluations of the diets were carried out by determination of feed intake, body weight gain (BWG %), feed efficiency ratio (FER), organ weight (%), and body weight (%), according to Chapman *et al.* [26].

2.3.1. Biochemical analysis.

Liver function: AST and ALT activities were determined calorimetrically, according to Reitman and Frankel [27]. Kidney function: urea and creatinine were estimated as described by Fawcett and Scott [28] and Bartles *et al.* [29]. Lipid profiles were evaluated as Triacylglycerol, Chowdhury *et al.* [30]; Total cholesterol, Lopes-Virella *et al.* [31]. LDL-cholesterol and VLDL-cholesterol were determined according to Warnick *et al.* [32]. The atherogenic index was calculated as described by Goh *et al.* [33]; Lipid peroxides were estimated as described by Ohkawa *et al.* [34]; Tumor necrosis factor and interleukin-6 were measured by using an ELISA kit.

2.3.2. Anticancer activity.

The colorectal cancer cell line (HT29) was obtained from Nawah Scientific Inc. (Mokatam, Cairo, Egypt). Cells were maintained in RPMI media supplemented with 100 mg/mL of streptomycin, 100 units/mL of penicillin, and 10% of heat-inactivated fetal bovine serum in a humidified, 5% (v/v) CO₂ atmosphere at 37°C.

According to Allam *et al.*, the anticancer activity of Ras cheese supplemented with JRP was tested using the cell line technique [35]. Cytotoxicity assay of colorectal carcinoma cells (HT29) was assessed using a sulforhodamine B (SRB) assay. Aliquots of 100 µl cell suspension (5x10³ cells) were in 96-well plates and incubated in complete media for 24h. Cells were treated with another aliquot of 100 µl media containing drugs at various concentrations. After 72h of drug exposure, cells were fixed by replacing media with 150 µl of 10% TCA and incubated at 4°C for 1h. The TCA solution was removed, and the cells were washed 5 times with distilled water. Aliquots of 70 µl SRB solution (0.4% w/v) were added and incubated in a dark place at room temperature for 10 min. Plates were washed 3 times with 1% acetic acid and allowed to air-dry overnight. Then, 150 µl of TRIS (10 mM) was added to dissolve the protein-bound SRB stain; the absorbance was measured at 540 nm using a BMGLABTECH®-FLUOstarOmega microplate reader (Ortenberg, Germany).

2.4. Statistical analysis.

Statistical results were analyzed using a one-way ANOVA procedure to analyze variance using SAS software [36]. The results were expressed as mean ± standard deviation, and the differences between means were tested for significance using Duncan's multiple range at ($p \leq 0.05$).

3. Results and Discussion

Nowadays, applied research is no longer based on the pronounced production of high-quality cheese and interesting flavors but is also concerned with the commercialization of cheese as functional food marketing. Instead, consumers are concerned about the safe use of preservatives.

Cephalotyre (Ras) cheese, as a fermented cheese, expands to supply important and essential nutrients such as high-quality protein, mainly bioactive components created during the proteolysis stages of ripening as well as lactose and fat are broken down by fermentation and lipolysis. The ripened cheese reflects a savory quality in the final product represented to consumers [37]. This applied study was conducted from previous work, including chemical analysis, color development, texture profile analysis, and sensory evaluation of Ras cheese samples supplemented with JRP, which were assessed during the ripening of cheese for 4 months. However, the addition of ripened JRP improves the quality of Ras cheese as well as the overall acceptability and nutritive impact [23].

However, the nutritional evaluation of Ras cheese supplemented with Jalapenos red pepper with respect to studies of the hyperlipidemia association with proinflammatory in rat model results showed that all rats were generally healthy throughout the feeding experiment period. Hyperlipidemia occurred in experimental rats due to induction of a high protein/high-fat diet [38], which was confirmed by lipid profile analysis. Mild degrees of hyperlipidemia indicated a predisposing factor to vascular disease. Hazards of inflammation include plaque formation and vulnerability to weakened arterial walls. In that respect, atherosclerosis is a

progressive disease accumulation of lipids and fibrous elements in the arteries concomitant to cardiovascular disease [39].

3.1. Nutritional evaluation.

Nutritional parameters of different experimental groups are illustrated in Table 1. It could be noticed that the addition of JRP to Ras cheese led to a significant ($p \leq 0.05$) difference in final body weight and body weight gain of rats. In contrast, these RJL and RJH groups were significantly ($p \leq 0.05$) decreased compared to a positive control group (RC). Also, no significant ($p \leq 0.05$) differences were observed between the negative control and RJH groups. The feed efficiency ratio showed non-significant ($p \leq 0.05$) changes among RJH, RJL, and negative control groups compared to the RC group. Table 1 shows feed intake revealed a significant ($p \leq 0.05$) increase in RC, RJL, and negative control group. As shown in the nutritional parameters in Table 1, rat weights were reduced gradually in groups fed Ras cheese with JRP compared to the positive control group. Although there was an increase in feed intake, it could be due to the effect of the antioxidant power of JRP, which contains total polyphenols. However, they showed a low percentage in weight gain compared to the positive control group. It could refer to the high metabolic rate of JRP that accelerates weight loss [40,41].

Table 1. Nutritional parameters of different rat groups.

Parameters	NC	PC	RC	RJL	RJH
Initial body weight [g]	101.50±2.26 ^a	100.33±1.86 ^a	100.83±1.47 ^a	100.83±3.06 ^a	101.33±2.16 ^a
Final body weight [g]	263.33±2.74 ^d	295.83±1.47 ^a	284.5±2.34 ^d	273.83±3.25 ^b	262.00±7.66 ^c
Gain body weight [g]	161.83±4.43 ^d	195.50±3.08 ^a	183.67±3.08 ^d	173.00±4.00 ^b	160.67±5.61 ^c
Feed intake [g.day ⁻¹]	14.50±32.86 ^b	16.00±42.43 ^a	15.00±53.67 ^{ab}	15.83±70.14 ^a	15.33±30.98 ^{ab}
Feed efficiency ratio	0.186±0.008 ^{bc}	0.204±0.009 ^a	0.205±0.013 ^c	0.183±0.015 ^{ab}	0.175±0.011 ^{bc}
Relative liver weight	2.453±0.05 ^d	2.996±0.027 ^b	2.342±0.038 ^c	3.176±0.050 ^a	3.092±0.083 ^b

NC: negative group; PC: positive group; RC: control Ras cheese; RJL: Ras cheese supplemented with JRP low dose; RJH: Ras cheese supplemented with JRP high dose. All parameters are represented as means ± standard deviation. Means in the same line with different superscript letters are significantly different at $p \leq 0.05$.

3.2. Lipid profile.

Dietary supplementation of Cephalotyre (Ras) cheese supplemented with JRP groups declined total cholesterol (Figure 1) and triglyceride (TG) values (Figure 2) which significantly ($p \leq 0.05$) decreased. Moreover, there was an increase of HDL values in the plasma compared with those in the positive control group, while LDL values decreased significantly ($p \leq 0.05$) by these treatments (Figure 1).

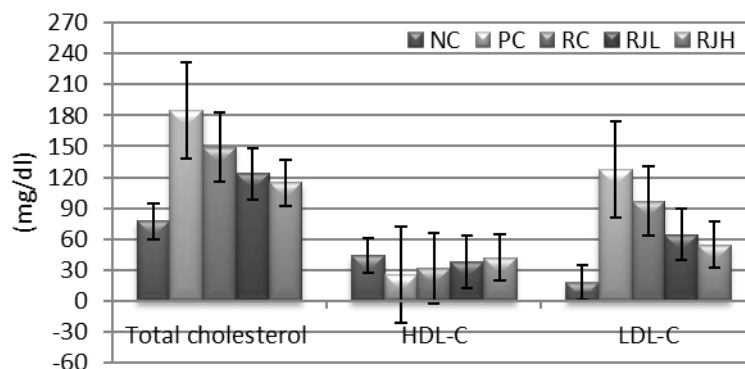


Figure 1. Total cholesterol, HDL-C, and LDL-C of different rat groups. NC: negative group; PC: positive group; RC: control Ras cheese; RJL: Ras cheese supplemented with JRP low dose; RJH: Ras cheese supplemented with JRP high dose.

However, there was a negative correlation among parameters total cholesterol (Figure 1), LDL-C (Figure 1), lipid peroxidation (Figure 3), and atherogenic index (Figure 4) with the RJH group, while RJL group and RC group is comparable with a positive control group. However, TG and VLDL-C had a null effect perceived between RC and RJL groups. Also, there were no significant ($p \leq 0.05$) differences observed in HDL-C for the negative group and RJH group. However, HDL-C showed a significant ($p \leq 0.05$) increase in the RJL group followed by the RC group compared to the positive control group.

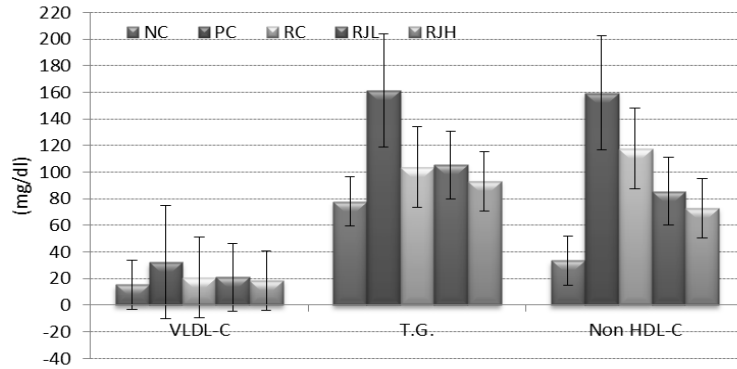


Figure 2. VLDL-C, triglycerides, and non-HDL-C of different rat groups. NC: negative group; PC: positive group; RC: control Ras cheese; RJL: Ras cheese supplemented with JRP low dose; RJH: Ras cheese supplemented with JRP high dose.

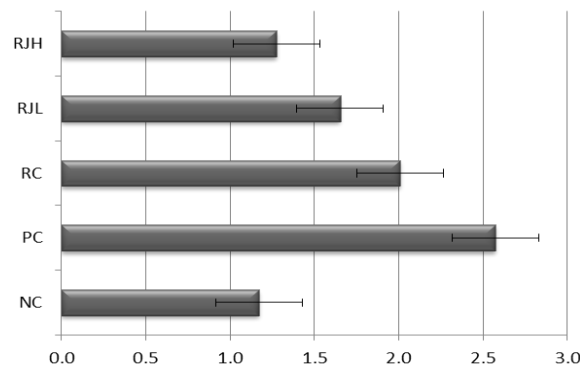


Figure 3. Lipid peroxidation of different rat groups. NC: negative group; PC: positive group; RC: control Ras cheese; RJL: Ras cheese supplemented with JRP low dose; RJH: Ras cheese supplemented with JRP high dose.

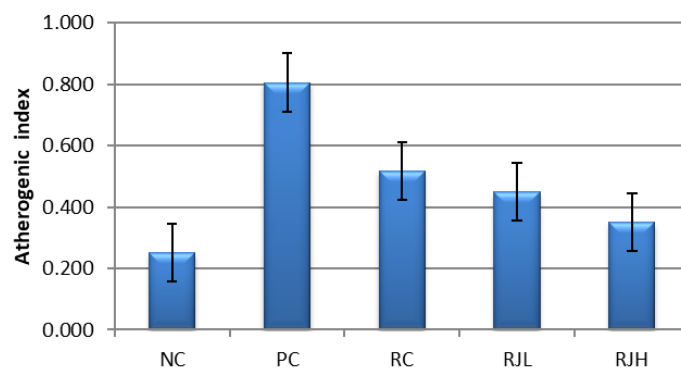


Figure 4. Atherogenic index plasma of different rat groups. NC: negative group; PC: positive group; RC: control Ras cheese; RJL: Ras cheese supplemented with JRP low dose; RJH: Ras cheese supplemented with JRP high dose.

Lipid profile changes are related to excessive cholesterol load to the liver's permissible acceptance level of its normal physiological limit. This causes the liver to be unable to metabolize the lipids, which accelerates atherosclerosis [41]. This aids our result as

hyperlipidemia occurs due to a postprandial high-fat diet reflected in increasing cholesterol levels, triglycerides, and VLDL particles in positive control group rats. Oxidative stress leads to the oxidation of LDL cholesterol, which contributes to atherosclerosis, heart attack, cerebral stroke, and peripheral artery disease. The alteration in lipid profile, increase in total cholesterol, triacylglycerol, and LDL-C, and reduction in HDL-C are key factors in cardiovascular disease progression [42].

As shown in Figure 1, the obtained results showed a significant ($p \leq 0.05$) increase in HDL-C value in the group fed on Ras cheese supplemented with JRP and a reduction value in the positive control group due to the hyperlipidemia effect. Moreover, the addition of JRP in the high-dose group ameliorates normal conditions. Also, the phenolic compounds found in JRP had a hypocholesterolemic effect that could be partly attributed to lower total cholesterol and low-density lipoprotein (LDL)-cholesterol concentrations in tested JRP groups. These findings agreed with Chen *et al.* [43], who reported that the capsaicinoid content of JRP could suppress hyperlipidemia.

VLDL particles ultimately become LDL. So, the higher the LDL and VLDL values, the more risk of heart disease. In this study, there were improvements in lipid particles VLDL and TG in the RC and JRP groups, which could be due to the antioxidant power of JRP through sulfur-containing amino acids of Ras cheese and JRP. However, the animal and cereal proteins containing methionine, cysteine, homocysteine, and taurine had the scavenging ability of free radicals due to sulfhydryl content [44-45].

Producing lipid peroxides due to eliminated free radicals causing cell damage by action of reactive oxygen species (ROS) appeared in the positive group. It significantly decreased in the group fed on Ras cheese group supplied with JRP with the high level it could due to the antioxidant power and polyphenols content of JRP [23]. This finding was in line with Szallasi [46,47].

Furthermore, our results showed the hypolipidemic effect of jalapeno pepper increment in two levels. Ras cheese supplemented with JRP could suppress hyperlipidemia, whereas JRP is rich in polyphenol and vitamins A, C, and K. In addition, carotenoid content, especially lycopene, has an important role in preventing cardiovascular diseases. The antioxidant properties of lycopene play a vital role in the oxidative process as they protect LDL from oxidation. So, it can retract cholesterol synthesis because it contains conjugated diene, which protects native LDL from oxidation [48-50].

There are controversies about dairy products as major food sources of saturated fat, contributing to an increased risk of cardiovascular disease. Proponents proclaim that full-fat dairy products increase coronary heart diseases and obesity because they are significant sources of saturated fats and trans-fatty acids, especially those of industrial origin [51-52]. From another point of view, authors found that cheese had a slight effect on LDL levels compared to butter [43].

The reason for this is the reduction of CVD risk factors or the decrease in cholesterol. From a point of view, not only was it restricted to using milk fat, but the real reason was a decline in total energy intake from saturated fat and a changing lifestyle. Changing dietary patterns may be one way to prevent the onset of these conditions. Lauric acid, myristic acid, and oleic acid are fatty acids in dairy products related to lipid profile metabolism [53,54]. Supplemented hard cheese with vegetable sources of fats, polyunsaturated fat, and fiber involved in preventing CVD is associated with a lower risk of CVD. Jalapeno pepper is rich in fatty acids such as linoleic and linolenic acid, and Ras cheese is rich in these fatty acids. It was

reported that linolenic had positive implications for human health because these fatty acids reduce the frequency of cardiovascular disease and type 2 diabetes [55,56]. Also, linolenic acid had hypocholesterolemic effects in both animals and humans. It was reported that feeding rats a rich linolenic acid diet lowered total cholesterol; dairy products with plenty of conjugated linoleic acid (CLA) daily could also accelerate fat loss [40,41].

Dairy products are a premium source of minerals, protein, and other nutrients effective in osteoporosis and osteopenia [57]. As for children, adults, and the aged who often avoid drinking milk for gastrointestinal symptoms, including abdominal pain, flatulence, and diarrhea, or have lactose intolerance, fermented dairy products could be excellent choices. Cheese contains paramount minerals that may related to central adiposity and blood regulation. Ras cheese supplemented with JRP provides calcium source from cheese and JRP. On the other hand, other researchers reported that dairy products can provide up to 60% of the recommended daily allowance (RDA) of calcium. Also, 13% of total calories from cheese could decrease LDL levels compared to butter. It could contribute to dismantling cholesterol and calcium content or the beneficial role of fermentation and microorganisms in the digestion of fatty acids [58-60]. This aids and supports our criteria and data that dairy fat is not associated with the risk of CVD; moreover, produced functional cheese supplemented with natural antioxidants has beneficial health impacts.

3.3. Liver and kidney functions.

Data represented in Figure 5 shows the effect of feeding rats on Ras cheese supplemented with JRP on liver enzyme activities. AST and ALT activities were significantly ($p \leq 0.05$) increased in rats fed on a positive control diet (RC) compared to the negative control diet. Also, it could be noticed that the RJH group recorded a significant ($p \leq 0.05$) decrease in tested liver enzymes compared to the positive control group. ALT in the RC group, which fed on Ras cheese supplemented with a high dose of JRP, revealed no significant ($p \leq 0.05$) observed compared to the negative control group, while no significant ($p \leq 0.05$) observed in AST between the RC group and RJL which fed on Ras cheese supplemented with a low level of JRP.

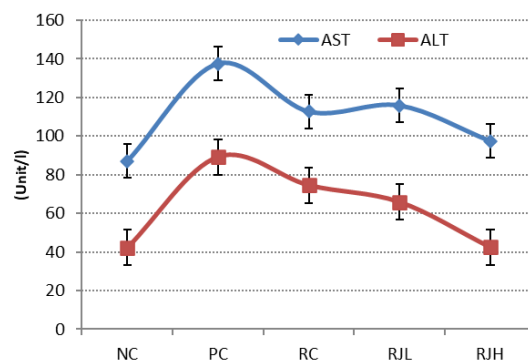


Figure 5. Aminotransferase enzymes of different rat groups. NC: negative group; PC: positive group; RC: control Ras cheese; RJL: Ras cheese supplemented with JRP low dose; RJH: Ras cheese supplemented with JRP high dose.

Any elevation in AST and ALT levels may indicate a liver problem/damage or dysfunctions. Elevated transaminases indicate inflammation or cellular injury, whether minor or severe [61,62]. Figure 5, plasma activities of AST and ALT were elevated significantly in positive control rats, indicating liver dysfunction due to hyperlipidemia. Two groups fed cheese

supplemented by JRP showed an impressive reduction in AST and ALT activity compared to the positive control group. This study shows that adding JRP could have a gradual hepatoprotective effect with the dose. So, the effect of the addition of JRP as an excellent source of vitamins, minerals, and antioxidants could ameliorate liver functions.

3.4. Proinflammatory parameters.

Proinflammatory parameters of different experimental groups are presented in Figure 6. The positive group significantly ($p \leq 0.05$) elevates TN α and IL6 in hyperlipidemic rats compared to negative control rats. In addition, TN α of the RJH group and RJL group showed a more significant ($p \leq 0.05$) pronounced effect than the positive control group. In contrast, no significant ($p \leq 0.05$) changes appeared for IL6 between the RJH group and negative control diet.

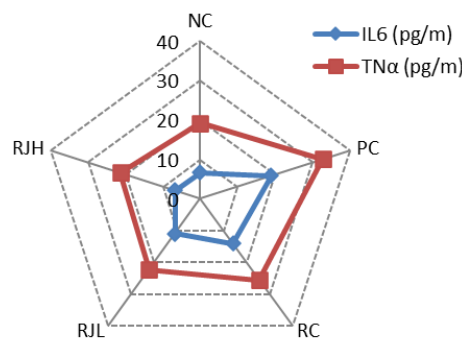


Figure 6. Tumor necrosis factor-alpha and Interleukin-6 of different rat groups. NC: negative group; PC: positive group; RC: control Ras cheese; RJL: Ras cheese supplemented with JRP low dose; RJH: Ras cheese supplemented with JRP high dose.

Interleukin-6 plays essential roles in the immune response, the central nervous system, and lipid metabolism. IL-6 role in liver and lipid metabolism appears to induce apolipoproteins; moreover, it stimulates catabolism of TG and phospholipid metabolism in the liver [63]. Elevated proinflammatory adipocytokines such as IL-6 and TN α in the adipose tissue macrophages were observed in the renal inflammation in rats. As observed in our results, there was a reduction in body weight in each group of Ras cheese supplemented with jalapeno pepper. Still, the high dose reflects more effects it could reclaim to increase the expression of enzymes involved in fatty acid oxidation. Inflammation has a central role in the pathogenesis of the atherosclerotic process, as anti-inflammatory medication could reduce atherosclerotic changes despite a disturbed lipid profile [64-65]. Rats fed on positive diets showed significant elevation by IL6 and TN α compared to the negative control due to oxidative stress reflected by inflammation processes; the significant reduction appears in cheese groups supplemented by any addition of JRP gradually.

3.5. Anti-cancer activity.

The result of the cytotoxicity effect of Ras cheese supplemented with JRP explored the IC₅₀ dose was >300 μ g/ml. The survival curve of the colorectal carcinoma cell line (HT29) is illustrated in Figure 7. In accordance with our results, Aune *et al.* [66] reported that dairy products have a protective effect in counteracting colorectal cancer risk due to their high calcium content, which may bind proinflammatory. Moreover, bile and ionized fatty acids may reduce cell proliferation and promote cell differentiation. In agreement with Leischner *et al.*

[67], Wolf *et al.* [68], Zampaglione *et al.* [69], and Sun *et al.* [70], antitumor or cytotoxic effects of cow's milk on cells from different tumor types, such as intestinal epithelial tumor cell line HT-29 and hepatocellular cell line HepG2 refer to protein components such as α -lactalbumin, β -lactoglobulin or bovine serum albumin, milk fat components, such as conjugated linoleic acid, milk fat globule membrane, or butyrate, as well as calcium and other protein components such as lactoferrin, lactoferricin, and casomorphines. Furthermore, β -LG was used as a nanocarrier for hydrophobic acid labile drugs for oral administration like irinotecan, a potent agent in colorectal cancer treatment, which showed more cytotoxic effectively against human gastric carcinoma AGS cells and colon carcinoma HT-29 cells than the free drug. In addition, the power of inhibitory effects of capsaicin on cancer onset, development, progression, and metastasis [71].

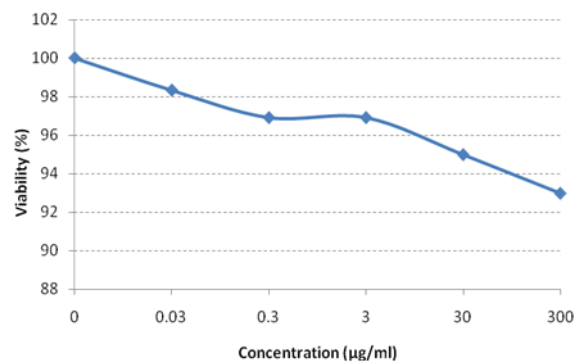


Figure 7. The survival curve of colorectal carcinoma cell line (HT29).

4. Conclusions

Adding Jalapenos red pepper improves the quality of Kefalotyri (Ras) Cheese and its overall acceptability. Moreover, Ras cheese supplemented with JRP had hypolipidemia associated with proinflammatory effects due to its polyphenolic compounds, vitamins, and minerals content; it can also be attributed to its sulfur-containing amino acids and fiber contents. Therefore, it is considered a high-quality functional dairy product that may offer vital nutrients to consumers who desire tasty foods and health benefits such as antioxidants, antitumor, hepatoprotective effect, and suppression of hyperlipidemia.

Funding

This research received no external funding.

Acknowledgments

Declared none.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Flore, G.; Deledda, A.; Lombardo, M.; Armani, A.; Velluzzi, F. Effects of Functional and Nutraceutical Foods in the Context of the Mediterranean Diet in Patients Diagnosed with Breast Cancer. *Antioxidants* **2023**, *12*, 1845, <https://doi.org/10.3390/antiox12101845>.

2. American Heart Association. Heart Disease and Stroke Statistics At-a-Glance. Feb 19, **2019**. <https://www.acc.org/latest-in-cardiology/ten-points-to-remember/2019/02/15/14/39/aha-2019-heart-disease-and-stroke-statistics>
3. Yasin, M.; Li, L.; Donovan-Mak, M.; Chen, Z.-H.; Panchal, S.K. *Capsicum* Waste as a Sustainable Source of Capsaicinoids for Metabolic Diseases. *Foods* **2023**, *12*, 907, <https://doi.org/10.3390/foods12040907>.
4. Oladapo, O.O.; Ojora, K.A.; Quadri, O.M.; Ajani, R.S. Lipidemic effects of common edible oils and risk of atherosclerosis in diabetic Wistar rats. *ARYA Atheroscler.* **2017**, *13*, 14-19.
5. Aziz, M.; Yadav, K.S. Pathogenesis of Atherosclerosis A Review. *Med. Clin. Rev.* **2016**, *2*, 3-22, <https://doi.org/10.21767/2471-299X.1000031>.
6. Marchio, P.; Guerra-Ojeda, S.; Vila, J.M.; Aldasoro, M.; Victor, V.M.; Mauricio, M.D. Targeting Early Atherosclerosis: A Focus on Oxidative Stress and Inflammation. *Oxidative Med. Cell. Longev.* **2019**, *2019*, 8563845, <https://doi.org/10.1155/2019/8563845>.
7. Ao, Z.; Huang, Z.; Liu, H. Spicy Food and Chili Peppers and Multiple Health Outcomes: Umbrella Review. *Mol. Nutr. Food Res.* **2022**, *66*, e2200167, <https://doi.org/10.1002/mnfr.202200167>.
8. Wolf, D.; Stachon, P.; Bode, C.; Zirlik, A. Inflammatory mechanisms in atherosclerosis. *Hämostaseologie* **2014**, *34*, 63-71, <https://doi.org/10.5482/HAMO-13-09-0050>.
9. Zuliani, G.; Volpato, S.; Blè, A.; Bandinelli, S.; Corsi, A.M.; Lauretani, F.; Paolisso, G.; Fellin, R.; Ferrucci, L. High interleukin-6 plasma levels are associated with low HDL-C levels in community-dwelling older adults: The InChianti study. *Atherosclerosis* **2007**, *192*, 384-390, <https://doi.org/10.1016/j.atherosclerosis.2006.05.024>.
10. Hashizume, M.; Mihara, M. IL-6 and lipid metabolism. *Inflamm. Regen.* **2011**, *31*, 325-333, <https://doi.org/10.2492/inflammregen.31.325>.
11. Kumar, S.; Andy, A. Health promoting bioactive phytochemicals from Brassica. *Int. Food Res. J.* **2012**, *19*, 141-152.
12. Zhang, Y.-J.; Gan, R.-Y.; Li, S.; Zhou, Y.; Li, A.-N.; Xu, D.-P.; Li, H.-B. Antioxidant Phytochemicals for the Prevention and Treatment of Chronic Diseases. *Molecules* **2015**, *20*, 21138–21156, <https://doi.org/10.3390/molecules201219753>.
13. Shahverdi, A.; Kheiri, F.; Faghani, M.; Rahimian, Y.; Rafiee, A. The effect of use red pepper (*Capsicum annum* L) and black pepper (*Piper nigrum* L) on performance and hematological parameters of broiler chicks. *Euro. J. Zool. Res.* **2013**, *2*, 44-48.
14. Akgül, A. Spices Science and Technology. Turkish Association of Food Technology, Ankara, **1993**.
15. Abou-Donia, S.A. Recent developments in Ras cheese research: a review. *Egypt. J. Dairy Sci.* **2002**, *30*, 155–166.
16. Dror, D.K.; Allen, L.H. Dairy product intake in children and adolescents in developed countries: trends, nutritional contribution, and a review of association with health outcomes. *Nutr. Rev.* **2013**, *72*, 68-81, <https://doi.org/10.1111/nure.12078>.
17. U.S. Department of Health and Human Services and U.S. Department of Agriculture. 2015 – 2020 Dietary Guidelines for Americans. 8th Edition. December 2015. Available at <https://odphp.health.gov/our-work/food-nutrition/previous-dietary-guidelines/2015>. Dietary Guidelines Advisory, C.; Hhs; Office of Disease, P.; Health, P.; Usda; Center for Nutrition Policy, P. *Dietary guidelines for Americans 2015-2020*; Government Printing Office: **2015**.
18. Roberfroid, M.B. Global view on functional foods: European perspectives. *Br. J. Nutr.* **2002**, *88*, S133–S138. <https://doi.org/10.1079/BJN2002677>.
19. Gibson, G.R.; Williams, C.M. Functional Foods: Concept to Product (Woodhead Publishing in Food Science and Technology), 1st Edition; CRC Press: **2000**.
20. Ibrahim, O.A.E.-H.; Mohamed, A.G.; Bahgaat, W.K. Natural peppermint-flavored cheese. *Acta Sci. Pol. Technol. Aliment.* **2019**, *18*, 75-85, <https://doi.org/10.17306/J.AFS.0607>.
21. Abdella, M.A.A.; Ahmed, S.A.; Ibrahim, O.A. Statistical improvement of protease production from a new isolate *Bacillus thuringiensis* strain-MA8 and its application in the production of enzyme-modified cheese. *Int. J. Biol. Macromol.* **2023**, *225*, 361-375, <https://doi.org/10.1016/j.ijbiomac.2022.11.073>.
22. Ibrahim, O.A.; Refaat, O.G.A.; Rabeh, N.M.; Abu zeid, A.S. Characterization of Cephalotyre (Ras) Cheese Supplemented with Turmeric Powder. *Egyptian J. Ntr. Health* **2023**, *18*, 23-38, <https://doi.org/10.21608/EJNH.2023.317757>.
23. El-Sayed, S.M.; Ibrahim, O.A.; Kholif, A.M.M. Characterization of novel Ras cheese supplemented with Jalapeno red pepper. *J. Food Process. Preserv.* **2020**, *44*, e14535, <https://doi.org/10.1111/jfpp.14535>.

24. Reeves, P.G.; Nielsen, F.H.; Fahey, G.C. 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.* **1993**, *123*, 1939-1951, <https://doi.org/10.1093/jn/123.11.1939>.
25. Liu, X.; Blouin, J.-M.; Santacruz, A.; Lan, A.; Andriamihaja, M.; Wilkanowicz, S.; Benetti, P.-H.; Tomé, D.; Sanz, Y.; Blachier, F.; Davila, A.-M. High-protein diet modifies colonic microbiota and luminal environment but not colonocyte metabolism in the rat model: the increased luminal bulk connection. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2014**, *307*, G459-G470, <https://doi.org/10.1152/ajpgi.00400.2013>.
26. Chapman, D.G.; Castillo, R.; Campbell, J.A. Evaluation of protein in foods. I. A method for the determination of protein efficiency ratios. *Can. J. Biochem. Physiol.* **1959**, *37*, 679-686.
27. Reitman, S.; Frankel, S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am. J. Clin. Pathol.* **1957**, *28*, 56-63, <https://doi.org/10.1093/ajcp/28.1.56>.
28. Fawcett, J.K.; Scott, J.E. A RAPID AND PRECISE METHOD FOR THE DETERMINATION OF UREA. *J Clin Pathol.* **1960**, *13*, 156-159, <https://doi.org/10.1136/jcp.13.2.156>.
29. Bartels, H.; Böhmer, M.; Heierli, C. Serum kreatininbestimmung ohne enteissein. *Clin. Chim. Acta* **1972**, *37*, 193-197, [https://doi.org/10.1016/0009-8981\(72\)90432-9](https://doi.org/10.1016/0009-8981(72)90432-9).
30. Chowdhury, F.R.; Rodman, H.; Bleicher, S.J. Glycerol-like contamination of commercial blood sampling tubes. *J. Lipid Res.* **1971**, *12*, 116.
31. Lopes-Virella, M.F.; Stone, P.; Ellis, S.; Colwell, J.A. Cholesterol determination in high-density lipoproteins separated by three different methods. *Clin. Chem.* **1977**, *23*, 882-884, <https://doi.org/10.1093/clinchem/23.5.882>.
32. Warnick, G.R.; Knopp, R.H.; Fitzpatrick, V.; Branson, L. Estimating low-density lipoprotein cholesterol by the Friedewald equation is adequate for classifying patients on the basis of nationally recommended cutpoints. *Clin. Chem.* **1990**, *36*, 15-19, <https://doi.org/10.1093/clinchem/36.1.15>.
33. Goh, V.H.H.; Tain, C.F.; Tong, T.Y.Y.; Mok, H.P.P.; Wong, M.T. Are BMI and other anthropometric measures appropriate as indices for obesity? A study in an Asian population. *J. Lipid Res.* **2004**, *45*, 1892-1898, <https://doi.org/10.1194/jlr.M400159-JLR200>.
34. Ohkawa, H.; Ohishi, N.; Yagi, K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* **1979**, *95*, 351-358, [https://doi.org/10.1016/0003-2697\(79\)90738-3](https://doi.org/10.1016/0003-2697(79)90738-3).
35. Allam, R.M.; Al-Abd, A.M.; Khedr, A.; Sharaf, O.A.; Nofal, S.M.; Khalifa, A.E.; Mosli, H.A.; Abdel-Naim, A.B. Fingolimod interrupts the cross talk between estrogen metabolism and sphingolipid metabolism within prostate cancer cells. *Toxicol. Lett.* **2018**, *291*, 77-85, <https://doi.org/10.1016/j.toxlet.2018.04.008>.
36. SAS. Statistical Analysis System. User's Guide: Statistics. SAS Institute. Inc, Cary NC 275513 USA, **1999**.
37. El-Hofi, M.A.; Ismail, A.A.; Abd Rabo, F.H.R.; El-Dieb, S.M.; Ibrahim, O.A. Studies on acceleration of Ras cheese ripening by aminopeptidase enzyme from buffaloes' pancreas. II—Utilization of Buffaloes' pancreas aminopeptidase in acceleration of Ras cheese ripening. *New York Sci. J.* **2010**, *6*, 575-581.
38. Libby, P. Inflammation in Atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* **2012**, *32*, 2045-2051, <https://doi.org/10.1161/ATVBAHA.108.179705>.
39. Karam, I.; Ma, N.; Yang, Y.J.; Li, J.Y. Induce Hyperlipidemia in Rats Using High Fat Diet Investigating Blood Lipid and Histopathology. *J. Hematol. Blood Disord.* **2018**, *4*, 104, <https://doi.org/10.15744/2455-7641.4.104>.
40. Mah, E.; Chen, O.; Liska, D.J.; Blumberg, J.B. Dietary Supplements for Weight Management: A Narrative Review of Safety and Metabolic Health Benefits. *Nutrients* **2022**, *14*, 1787, <https://doi.org/10.3390/nu14091787>.
41. Raziani, F.; Tholstrup, T.; Kristensen, M.D.; Svanegaard, M.L.; Ritz, C.; Astrup, A.; Raben, A. High intake of regular-fat cheese compared with reduced-fat cheese does not affect LDL cholesterol or risk markers of the metabolic syndrome: a randomized controlled trial. *Am. J. Clin. Nutr.* **2016**, *104*, 973-981, <https://doi.org/10.3945/ajcn.116.134932>.
42. Packard, C.J.; Satio, Y. Non-HDL Cholesterol as a Measure of Atherosclerotic Risk. *J. Arterioscler. Thromb.* **2004**, *11*, 6-14, <https://doi.org/10.5551/jat.11.6>.
43. Chen, L.; Tang, W.; Wu, X.; Zhang, R.; Ding, R.; Liu, X.; Tang, X.; Wu, J.; Ding, X. Eating Spicy Food, Dietary Approaches to Stop Hypertension (DASH) Score, and Their Interaction on Incident Stroke in Southwestern Chinese Aged 30–79: A Prospective Cohort Study. *Nutrients* **2023**, *15*, 1222, <https://doi.org/10.3390/nu15051222>.
44. Bin, P.; Huang, R.; Zhou, X. Oxidation Resistance of the Sulfur Amino Acids: Methionine and Cysteine. *BioMed Res. Int.* **2017**, *2017*, 9584932, <https://doi.org/10.1155/2017/9584932>.

45. Gorissen, S.H.M.; Crombag, J.J.R.; Senden, J.M.G.; Waterval, W.A.H.; Bierau, J.; Verdijk, L.B.; van Loon, L.J.C. Protein content and amino acid composition of commercially available plant-based protein isolates. *Amino Acids* **2018**, *50*, 1685-1695, <https://doi.org/10.1007/s00726-018-2640-5>.
46. Szallasi, A. Capsaicin and cancer: Guilty as charged or innocent until proven guilty?. *Temperature* **2023**, *10*, 35-49, <https://doi.org/10.1080/23328940.2021.2017735>.
47. Szallasi, A. Dietary Capsaicin: A Spicy Way to Improve Cardio-Metabolic Health?. *Biomolecules* **2022**, *12*, 1783, <https://doi.org/10.3390/biom12121783>.
48. Arab, L.; Steck, S. Lycopene and cardiovascular disease. *Am. J. Clin. Nutr.* **2000**, *71*, 1691S-1695S, <https://doi.org/10.1093/ajcn/71.6.1691S>.
49. Agarwal, S.; Rao, A.V. Tomato lycopene and its role in human health and chronic diseases. *CMAJ* **2000**, *163*, 739-744.
50. Riccioni, G.; Mancini, B.; Di Ilio, E.; Bucciarelli, T.; D'orazio, N. Protective effect of lycopene in cardiovascular disease. *Eur. Rev. Med. Pharmacol. Sci.* **2008**, *12*, 183-190.
51. Huth, P.J.; Park, K.M. Influence of dairy product and milk fat consumption on cardiovascular disease risk: a review of the evidence. *Adv. Nutr.* **2012**, *3*, 266-285, <https://doi.org/10.3945/an.112.002030>.
52. Forouhi, N.G.; Krauss, R.M.; Taubes, G.; Willett, W. Dietary fat and cardiometabolic health: evidence, controversies, and consensus for guidance. *BMJ* **2018**, *361*, k2139, <https://doi.org/10.1136/bmj.k2139>.
53. Çetinkaya, A.; Öz, F. Changes in cholesterol and free fatty acid content of Kars Gravyer Cheese (A Turkish dairy product produced by the traditional method). *Ukr. Food J.* **2018**, *7*, 409-420, <https://doi.org/10.24263/2304-974X-2018-7-3-6>.
54. Haug, A.; Høstmark, A.T.; Harstad, O.M. Bovine milk in human nutrition--a review. *Lipids Health Dis.* **2007**, *6*, 25, <https://doi.org/10.1186/1476-511x-6-25>.
55. Pérez-Gálvez, A.; Garrido-Fernández, J.; Mínguez-Mosquera, M.I.; Lozano-Ruiz, M.; Montero-de-Espinosa, V. Fatty acid composition of two new pepper varieties (*Capsicum annuum* L. cv. Jaranda and Jariza). Effect of drying process and nutritional aspects. *J. Am. Oil Chem. Soc.* **1999**, *76*, 205-208.
56. Bhandari, S.R.; Bashyal, U.; Lee, Y.-S. Variations in proximate nutrients, phytochemicals, and antioxidant activity of field-cultivated red pepper fruits at different harvest times. *Hortic. Environ. Biotechnol.* **2016**, *57*, 493-503, <https://doi.org/10.1007/s13580-016-1008-6>.
57. Thorning, T.K.; Raben, A.; Tholstrup, T.; Soedamah-Muthu, S.S.; Givens, I.; Astrup, A. Milk and dairy products: good or bad for human health? An assessment of the totality of scientific evidence. *Food Nutr. Res.* **2016**, *60*, 32527, <https://doi.org/10.3402/fnr.v60.32527>.
58. Jacobsen, R.; Lorenzen, J.K.; Toubro, S.; Krog-Mikkelsen, I.; Astrup, A. Effect of short-term high dietary calcium intake on 24-h energy expenditure, fat oxidation, and fecal fat excretion. *Int. J. Obes.* **2005**, *29*, 292-301, <https://doi.org/10.1038/sj.ijo.0802785>.
59. Lordan, R.; Tsoupras, A.; Mitra, B.; Zabetakis, I. Dairy Fats and Cardiovascular Disease: Do We Really Need to Be Concerned? *Foods* **2018**, *7*, 29, <https://doi.org/10.3390/foods7030029>.
60. Hess, J.M.; Jonnalagadda, S.S.; Slavin, J.L. Dairy Foods: Current Evidence of their Effects on Bone, Cardiometabolic, Cognitive, and Digestive Health. *Compr. Rev. Food Sci. Food Saf.* **2016**, *15*, 251-268, <https://doi.org/10.1111/1541-4337.12183>.
61. Giannini, E.G.; Testa, R.; Savarino, V. Liver enzyme alteration: a guide for clinicians. *CMAJ* **2005**, *172*, 367-379, <https://doi.org/10.1503/cmaj.1040752>.
62. Kim, S.H.; Park, D.H.; Lim, Y.J. Impact of Diet on Colorectal Cancer Progression and Prevention: From Nutrients to Neoplasms. *Korean J. Gastroenterol.* **2023**, *82*, 73-83, <https://doi.org/10.4166/kjg.2023.079>.
63. Miguel, N.A.; Andrade, S.F.; Nai, G.; Laposy, C.B.; Nascimento, F.F.; Dinallo, H.R.; Melchert, A. Effects of resveratrol on liver function of obese female wistar rats. *Cienc. Anim. Bras.* **2016**, *17*, 402-410, <https://doi.org/10.1590/1089-6891v17i332990>.
64. Silva, J.L.; Santos, E.A.; Alvarez-Leite, J.I. Are We Ready to Recommend Capsaicin for Disorders Other Than Neuropathic Pain? *Nutrients* **2023**, *15*, 4469, <https://doi.org/10.3390/nu15204469>.
65. Ibrahim, S.M.; Gomaa, R.S.; Ismail, S.I.; Ibrahim, H.S.G. Role of inflammation versus hypercholesterolemia in the development of atherosclerosis in male albino rats. *Al-Azhar Intern. Med.* **2018**, *16*, 58-65, https://doi.org/10.4103/AZMJ.AZMJ_54_18.
66. Aune, D.; Lau, R.; Chan, D.S.M.; Vieira, R.; Greenwood, D.C.; Kampman, E.; Norat, T. Dairy products and colorectal cancer risk: a systematic review and meta-analysis of cohort studies. *Ann. Oncol.* **2012**, *23*, 37-45, <https://doi.org/10.1093/annonc/mdr269>.

67. Leischner, C.; Egert, S.; Burkard, M.; Venturelli, S. Potential Protective Protein Components of Cow's Milk against Certain Tumor Entities. *Nutrients* **2021**, *13*, 1974, <https://doi.org/10.3390/nu13061974>.
68. Wolf, T.G.; Cagetti, M.G.; Fisher, J.-M.; Seeberger, G.K.; Campus, G. Non-communicable Diseases and Oral Health: An Overview. *Front. Oral. Health* **2021**, *2*, 725460, <https://doi.org/10.3389/froh.2021.725460>.
69. Zampaglione, L.; Ferrari, J.; Pedica, F.; Goossens, N. HCC in metabolic syndrome: current concepts and future directions. *Hepatoma Res.* **2021**, *7*, 55, <https://doi.org/10.20517/2394-5079.2021.22>.
70. Sun, J.; Song, J.; Yang, J.; Chen, L.; Wang, Z.; Duan, M.; Yang, S.; Hu, C.; Bi, Q. Higher Yogurt Consumption Is Associated With Lower Risk of Colorectal Cancer: A Systematic Review and Meta-Analysis of Observational Studies. *Front. Nutr.* **2022**, *8*, 789006, <https://doi.org/10.3389/fnut.2021.789006>.
71. Hudáková, T.; Šemeláková, M.; Očenáš, P.; Kožurková, M.; Krochtová, K.; Sovová, S.; Tóthová, Z.; Gulášová, Z.; Popelka, P.; Solár, P. Chili pepper extracts, capsaicin, and dihydrocapsaicin as potential anticancer agents targeting topoisomerases. *BMC Complement. Med. Ther.* **2024**, *24*, 96, <https://doi.org/10.1186/s12906-024-04394-5>.