Comparative Impacts of Enzyme Modified Ras Cheese and Conventional Cheese on Lipid Profile and Hepatorenal Functions in Experimental Rats

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Abstract

This study evaluated the physiological impact of Enzyme Modified Ras Cheese (EMC-Ras), commercial cheese, and Enzyme Modified Cheese (EMC) amounts in Ras cheese on lipid profile, liver and kidney function, as well as serum calcium levels in experimental rats. Fifty-six male albino rats were divided into seven dietary treatment groups: control group fowolled with two groups fed on EMC-Ras cheese with addition (2.5% and 5%), followed by two commercial cheese groups with addition (2.5% and 5%), and finally two groups fed on EMC amounts in Ras cheese with addition (2.5% and 5%). Biochemical analyses revealed that commercial cheese significantly elevated lipid profile, serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, creatinine, and albumin, suggesting hepatic and renal stress. In contrast, EMC and enzyme-treated groups demonstrated modest and statistically non-significant alterations in liver and kidney parameters. Notably, EMC at 5% significantly increased serum calcium levels, whereas commercial cheese markedly reduced calcium concentrations. The results suggested that EMC and enzyme treatments could be useful as healthy dairy option that help reduce negative health effects linked to eating commercial cheese.

Keywords

Enzyme-modified cheese (EMC), liver function biomarkers (ALT, AST), kidney function biomarkers (urea, creatinine), serum calcium, commercial cheese, hepatotoxicity, nephrotoxicity, functional dairy, experimental rat model.

INTRODUCTION

Cheese is a nutritionally dense dairy product derived from various types of milk, including cow, buffalo, and goat milk. It is a rich source of high-quality proteins, bioactive peptides, essential amino acids, fatty acids, fat-soluble vitamins, and minerals such as calcium, while often containing little or no lactose **(Ahmed et al., 2020)**.

In recent decades, growing consumer demands and dietary trends have led to the development of cheese substitutes or analogues, which are produced using alternative fats or proteins and are often classified as filled or imitation cheeses **(Ash & Wilbey, 2010)**. Some of these innovative products also provide bioactive compounds with documented health benefits, including antimicrobial, anticarcinogenic, antihypertensive, and antithrombotic effects.

Cheese types can be classified based on milk source, manufacturing process, texture, fat content, fermentation route, and microbiological activity **(Walther, 2008)**.

The maturation (ripening) period plays a key role in shaping cheese flavor and functional properties, as biochemical processes such as proteolysis, lipolysis, and lactose fermentation yield volatile and bioactive compounds that contribute to both sensory quality and health-promoting attributes **(Bintsis, 2021)**.

Cheese yield is influenced by a wide array of technological and biological factors, including milk composition, pretreatment conditions, type of coagulant, cutting methods, vat geometry, and curd-handling techniques. Seasonal changes, animal species, lactation stage, and somatic cell counts also markedly affect cheese yield and composition **(Badem & Uçar, 2016)**.

Ras cheese, locally known as Roumy cheese in Egypt and other parts of the Arab world, is a traditional hard cheese typically manufactured from cow's, buffalo's, or mixed milk. It undergoes a minimum of three months of ripening to achieve its distinctively sharp flavor. It bears resemblance to Greek Kefalotyri cheese in both texture and aging characteristics **(Abd-El Monem et al., 2023)**.

Fat content in cheese is a crucial determinant of texture, mouthfeel, and shelf life, and it varies according to the type of milk and processing approach **(Lepesioti et al., 2021)**. While cheese fats have been traditionally associated with adverse cardiovascular effects due to their saturated fatty acid content and influence on lipoprotein metabolism **(Hellmuth & van den Brink, 2013)**.

Recent research highlights the health-promoting potential of milk fat globule membrane (MFGM) components. These include sphingolipids, phospholipids, glycoproteins, and enzymes that have been shown to enhance

cognitive function, modulate cholesterol metabolism, suppress gastrointestinal pathogens, and exhibit anticancer properties **(Unger et al., 2019)**.

Enzyme-Modified Cheese (EMC) refers to a type of cheese or cheese ingredient that has been biochemically enhanced using specific enzyme systems, particularly lipases and proteases, to accelerate the development of flavor and texture during or after cheese production. Enzymatic modification intensifies lipolysis and proteolysis, resulting in the release of short-chain fatty acids and free amino acids that contribute to improved flavor profiles **(Hayaloglu & Karagul-Yuceer, 2021; Gao et al., 2022)**.

Moreover, enzyme modification may enhance digestibility and nutrient release, potentially increasing the bioavailability of minerals such as calcium, and reducing adverse impacts on lipid metabolism and organ functions **(Shalaby et al., 2022)**. These properties make EMC a promising functional ingredient in developing healthier cheese alternatives.

This study aims to develop enzyme-modified cheese (EMC) as a functional ingredient in low-fat Ras (Romy) cheese and to evaluate its nutritional value and effects on hepatorenal functions in animal models compared with conventional cheese.

Materials and methods

Materials:

Fresh cow's and buffalo's milk was obtained from the herd of the Faculty of Agriculture, Cairo University, Egypt. Microbial rennet powder purchased from Gaglio Star, Spain. Cheese starter cultures were obtained from the Egyptian Microbial Culture Collection, Ain Shams University, Egypt. Rats were obtained from the National Research Centre (NRC), Egypt. Rat diet ingredients, chemicals and kits such as ALT, AST, albumin, urea, creatinine, and serum calcium using standard colorimetric kits obtained from Spectrum and Biomed companies.

Methods:

Preparation of Ras cheese with EMC

Ras cheese was made by the conventional method according to **Hofi et al. (1970)** using a mixture of cow's and buffalo's milk. EMC was prepared as described by **Mousavi-Nasab et al. (2010)** using fresh Ras cheese treated with pepsin at the levels of 500, 1500, and 3000 U.g-1 cheese, while lipase (5000 U.g-1 cheese). Also, Ras cheese was made using EMC in the levels of 1.25, 2.5, and 5% for cheese curd before molding. The most suitable proportions were carefully selected based on chemical and sensory evaluations.

Determination of fatty acids profile

Fatty acid profiles of cheese samples were extracted as described in standards (ISO, 2001) and determined according to standards (ISO, 2002) using a gas chromatography (GC) system (Perkin Elmer Auto System XL) with a flame ionization detector and a DB5silica capillary column (60 m, 0.32 mm i.d.). After transesterification, 2 % sulphuric acid in methanol produced fatty acid methyl esters (FAMEs) of the total lipid. The oven temperature was first set at 45ºC and programmed to 60º C at the rate of 1 ◦C/min before being programmed to 240º C at the rate of 3º C/min. Helium was employed as the carrier gas at the flow rate of 1 mL 60 min. The injector and detector temperatures were adjusted at 230º C and 250º C, respectively. The fatty acids profile was further identified by its retention time and their concentrations were calculated by comparing the peak areas of the samples with those of the standards.

Animals and Diets

The proposal was approved by the Scientific Committee at NRC, and all animal experiments were conducted in accordance with the guidelines of the Animal Care and Ethics Committee of NRC. The rats were housed in cages under hygienic conditions in a temperature-controlled room of 25 °C. The basal diet is nutritionally adequate (AIN-93 G), and vitamins mixture and minerals mixture prepared as described by **Reeves et al. (1993).**

Fifty-six male albino with a body weight of 100 ±5 g. Rats were randomly divided into seven groups (n = 8). Each group was fed one of the following diets for six weeks including a negative control group (basal diet): Ras cheese with EMC at 2.5% and 5%; commercial Ras cheese at 2.5% and 5%; and EMC content in the added Ras cheese amount.

Throughout the six-week feeding trial, growth rate, food intake, and weight gain were monitored. At the end of the study, rats were fasted overnight, and then blood samples were collected under light ether anesthesia. Blood was collected in EDTA tubes and processed to obtain plasma (centrifuged at 4000 rpm for 15 min, stored at –70 °C) and serum (after clotting, centrifuged similarly, used for immediate and future analyses, stored at –80 °C). Organ weights were measured post-sacrifice.

Nutritional evaluation included measuring feed intake, body weight gain (%BWG), feed efficiency ratio (FER), and organ-to-body weight ratios, following the method of **Chapman (1959).**

Biochemical Assays

Lipid profile parameters were evaluated as follows: triacylglycerol according to **Chowdhury et al. (1971),** and total cholesterol according to **Lopes-Virella et al. (1977)**. LDL-cholesterol and VLDL-cholesterol were determined as described by **Warnick et al. (1990),** while the atherogenic index was calculated following **Goh et al. (2004).** Liver enzyme activities (AST and ALT) were measured calorimetrically according to **Reitman and Frankel (1957).** Serum albumin was estimated according to **Doumas et al. (1971).** Kidney function biomarkers, including urea and creatinine, were determined according to **Fawcett and Scott (1960) and Bartels et al. (1972),** Serum calcium was determined according to **Gitelman (1967),** respectively.

Liver enzymes: AST and ALT activities were determined calorimetrically, according to **Reitman and Frankel (1957).** Kidney function: urea and creatinine were estimated as described by **Fawcett and Scott (1960) and Bartels et al. (1972).** Lipid profiles were evaluated as triacylglycerol, **Chowdhury et al. (1971);** and total cholesterol, **Lopes-Virella et al. (1977).** LDL-cholesterol and VLDL-cholesterol were determined according to **Warnick et al. (1990).** The atherogenic index was calculated as described by **Goh et al. (2004).**

Statistical analysis

All results were expressed as mean ± SD and analyzed using one-way ANOVA followed by Duncan's test at $p \leq 0.05$ by SAS software **(SAS, 1999).**

Results & Discussion

Functional and Nutritional Implications of Fatty Acids

The fatty acid (FA) composition of cheese is a critical determinant of its nutritional quality, metabolic impact, and organoleptic attributes. The current analysis revealed substantial shifts in the fatty acids profile of low-fat Ras cheese following enzymatic modification, particularly affecting the proportions of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs).

Table (1) illustrates the fatty acid composition of low-fat Ras cheese with EMC. Saturated fatty acids (SFAs), such as butyric acid (C4:0), caproic acid (C6:0), caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), and stearic acid (C18:0), were predominant in both cheese types. The EMC showed higher concentrations of most SFAs, especially butyric (5.06%) and stearic acids (5.46%), indicating an increase in short- and medium-chain fatty acids following enzymatic modification.

Monounsaturated fatty acids (MUFAs), including caproleic acid (C10:1), myristoleic acid (C14:1), palmitoleic acid (C16:1), cis-10-pentadecenoic acid (C15:1), cis-10-heptadecanoic acid (C17:1), and oleic acid (C18:1n9c), were

also present in both cheese samples. While most MUFAs remained relatively stable or slightly decreased in EMC, oleic acid - the most abundant MUFA- decreased from 31.08% in low-fat cheese to 27.87% in EMC. This slight reduction may influence the health-related lipid profile of the modified cheese.

Polyunsaturated fatty acids (PUFAs), such as linoleic acid (C18:2n6c), α-linolenic acid (C18:3n3), γ-linolenic acid (C18:3n6), and myristolinoleic acid (C14:2), were detected in moderate amounts. The concentrations of these essential fatty acids slightly decreased in EMC, suggesting that enzymatic treatment may have a mild impact on the PUFA content. Notably, arachidic acid (C20:0), a long-chain saturated fatty acid, appeared only in the EMC at 0.55%, possibly due to enzymatic hydrolysis or enhanced release during modification.

Saturated Fatty Acids (SFAs)

Saturated fatty acids predominated in both cheese samples, with palmitic acid (C16:0) comprising the largest proportion (28.61% in low-fat cheese, 27.08% in EMC). Although often associated with increased cardiovascular risk, emerging evidence suggests that not all SFAs exert similar health effects. For instance, stearic acid (C18:0) which increased in EMC to 5.46% has been shown to have a neutral effect on LDL cholesterol levels **(de Souza et al., 2022)**. Moreover, butyric acid (C4:0) rose markedly from 3.26% to 5.06%, reflecting enhanced hydrolysis of milk triglycerides during enzymatic treatment.

Butyric acid, a short-chain fatty acid (SCFA), plays a pivotal role in gut health by serving as a primary energy source for colonocytes and exerting anti-inflammatory and anti-carcinogenic effects **(Phelan & Kerins, 2023).** The enzymatic processes likely facilitated increased lipolysis and liberation of SCFAs and medium-chain fatty acids (MCFAs) such as caproic (C6:0), caprylic (C8:0), and capric (C10:0) acids. This aligns with previous observations that enzyme-modified cheeses (EMCs) exhibit intensified lipolysis compared to their conventional counterparts **(Contarini & Povolo, 2021)**.

The appearance of arachidic acid (C20:0) in EMC (0.55%), undetectable in the low-fat cheese, is likely the result of deep lipolytic action or enhanced availability of esterified long-chain SFAs previously embedded within the milk fat matrix **(Lorenzen & Meisel, 2020)**.

Monounsaturated Fatty Acids (MUFAs)

MUFAs contribute to favorable lipid profiles and cardiovascular health. Oleic acid (C18:1n9c), the most abundant MUFA in both cheeses, decreased from 31.08% to 27.87% post-modification. Although modest, this reduction may influence the health-promoting potential of the EMC, as oleic acid is

associated with anti-inflammatory effects and improved lipid metabolism (Astrup et al., 2020). The decline could stem from preferential enzymatic action or susceptibility of unsaturated bonds to oxidation during processing **(Shingfield et al., 2020).**

Other MUFAs, such as palmitoleic acid (C16:1) and cis-10-heptadecanoic acid (C17:1), also exhibited minor fluctuations. Interestingly, C17:1 increased from 0.69% to 0.96%, potentially indicating selective retention or transformation of odd-chain MUFAs, whose metabolic relevance is still under investigation **(Chilliard et al., 2021).**

Polyunsaturated Fatty Acids (PUFAs)

PUFAs, including essential fatty acids like linoleic (C18:2n6c) and α-linolenic (C18:3n3) acids, were present in moderate amounts in both samples but slightly decreased in EMC. The decline from 2.19% to 2.14% for linoleic acid and from 0.75% to 0.65% for α-linolenic acid may be attributed to the sensitivity of PUFAs to enzymatic oxidation and breakdown **(Eslami et al., 2020)**. While the reduction was not substantial, it suggests that enzymatic treatment may affect the oxidative stability of these bioactive lipids. γ-Linolenic acid (C18:3n6) also declined (1.21% to 1.02%), a shift that could slightly impact the anti-inflammatory balance of the cheese's lipid profile. Nevertheless, the preservation of a broad PUFA spectrum indicates that EMC retains key essential fatty acids despite processing stressors.

From a nutritional perspective, the increased concentration of SCFAs and MCFAs in EMC may enhance digestibility and energy metabolism, especially in populations with compromised lipid absorption. These fatty acids are rapidly absorbed and metabolized by the liver, bypassing lymphatic transport. Moreover, the observed shifts in FA composition may influence cheese flavor, as short-chain FAs contribute to pungent, characteristic aromas in aged and enzyme-modified cheeses **(Abd-El Monem et al., 2023).**

However, the concurrent decrease in MUFAs and PUFAs, particularly oleic and linolenic acids, may attenuate some of the cardiovascular benefits traditionally attributed to dairy fats **(de Souza et al., 2022)**. Thus, optimizing enzyme selection and processing parameters could help preserve beneficial unsaturated lipids while promoting favorable lipid digestibility.

Table (1): Percent distribution of Fatty Acids in Low-Fat Ras Cheese with EMC

Type of cheese / Fatty acids	Control Ras Cheese	Low fat Ras cheese with EMC
Butyric acid (C4:0)	3.26	5.06
Caproic acid (C6:0)	2.13	2.92
Caprylic acid (C8:0)	1.05	1.45
Capric acid (C10:0)	2.21	2.67
Caproleic acid (C10:1)	0.21	0.25
Lauric acid (C12:0)	2.61	2.86
Tridecanoic acid (C13:1)	0.23	0.23
Myristic acid (C14:0)	10.44	10.55
Myristoleic acid (14:1)	0.99	0.94
Myristolinoleic (C14:2)	0.84	0.8
Pentadecanoic acid (C15:0)	1.71	1.59
Cis-10-Pentadecenoic (C15:1)	0.45	0.4
Palmitic acid (C16:0)	28.61	27.08
Palmitoleic acid (C16:1)	3.48	3.23
Palmitoleic acid (C16:1, n7)	0.71	0.64
Heptadecanoic acid (C17:0)	0.7	0.64
Cis-10-Heptadecanoic acid (C17:1)	0.69	0.96
Stearic acid (C18:0)	4.42	5.46
Oleic acid (C18:1n9c)	31.08	27.87
Linoleic acid (C18:2n6c)	2.19	2.14
α- Linolenic acid (C18:3n3)	0.75	0.65
γ, Linolenic acid (C18:3n6)	1.21	1.02
Arachidic acid (C20:0)	ND	0.55

Body weight development and feed efficiency

The current study evaluated the effects of EMC-Ras cheese compared to both commercial cheese and EMC amounts in Ras cheese on body weight development and feed efficiency in experimental rats. The results are presented in Table 2. There were no statistically significant differences in initial body weight among groups, confirming successful randomization and homogeneity at the start of the feeding trial. The rats across all groups had comparable

baseline weights (102.2 g to 105.4 g), which aligns with standard practices in nutritional intervention studies using animal models **(Huang et al., 2021)**.

A significant increase in final body weight and body weight gain was observed in rats fed diets containing enzymes at 5%, reaching a final body weight of 176.6 g and a gain of 72.8 g, significantly higher than all other groups ($p \leq 0.05$). This outcome suggests that enzyme inclusion may enhance nutrient digestibility and absorption, supporting better growth performance. Enzymatic pretreatment of cheese could release bioactive peptides and improve the availability of short-chain fatty acids and amino acids (**Tripathi & Mishra, 2022)**, potentially stimulating anabolic responses in the host.

Interestingly, rats treated with 2.5% commercial cheese also demonstrated notable final body weight and gain in body weight (167.0 g and 64.8 g, respectively), possibly attributed to the higher fat content and energy density of commercial cheese, consistent with previous studies linking high-fat dairy consumption to increased weight gain in rodents **(de Oliveira et al., 2020).**

Conversely, the lowest in body weight gain (43.8 g) and final body weight (148.4 g) were recorded in the group receiving EMC-Ras cheese at 2.5%, suggesting that although EMC offers biochemical advantages, its effect on body weight may be dose-dependent and less pronounced compared to high-fat commercial cheese.

The feed efficiency ratio, a measure of how efficiently the consumed feed is converted into body mass, was significantly improved in the EMC at Ras cheese 5% group and the commercial cheese 2.5% group ($p \leq 0.05$). The high FER in the EMC groups further supports the hypothesis that enzymatic enhancement of cheese improves nutrient utilization and metabolic efficiency **(Nguyen et al., 2021).** On the other hand, the lowest FER values were observed in groups fed EMC-Ras cheese (2.5% and 5%) and EMC at Ras cheese 2.5%, ranging between 0.030 and 0.038. This might reflect lower caloric density or reduced palatability of these formulations, leading to either reduced intake or lower nutrient yield per gram of feed.

These findings suggest that while enzyme treatment enhances the nutritional efficiency of cheese at higher levels (5%), EMC alone may require further optimization in formulation or dose to match the growth- promoting effects observed with enzyme supplementation or traditional high-fat cheese.

Table (2): Effect of Enzyme-Modified Ras Cheese and Conventioal Cheese on body Weight and FER in Experimental Rats

Parameter / Groups	IBW (g)	FBW (g)	GBW (g)	FER
Negative control	103.6±2.7^{a}	156±4.9^{d}	52.4±3.9^{cd}	0.04±0^{b}
Ras cheese with EMC 2.5%	104.6±3.3^{a}	148.4±9.25	43.8±0.0^{f}	0.034±0.02^{c}
Ras cheese with EMC 5%	103.6±2.5	155.2±2.7^{d}	51.6±3.5^{d}	0.038±0.11^{c}
Commercial Ras cheese 2.5%	102.2±1.6^{a}	167±4.9^{b}	64.8±4.4^{b}	0.050±0.10^{a}
Commercial Ras cheese 5%	104.8±3.1^{a}	160.6±4.2^{c}	55.8±6.7^{c}	0.044±0.11^{b}
EMC at Ras cheese 2.5%	105.4±2.6^{a}	154.4±1.4^{d}	49.0±2.1^{e}	0.03±0.0^{c}
EMC at Ras cheese 5%	103.8±2.8^{a}	176.6±4.9^{a}	72.8±4.4^{a}	0.05±0.1^{a}

IBW: Initial Body Weight, **FBW**: Final Body Weight, **GBW**: Gain in body weight, and **FER**: Food efficiency ratio.
Results were presented as a means of replicates ± SD.
Means with different small superscript letters in the same row are significantly different at $p \leq .05$

Lipids profile

The effects of EMC-Ras cheese compared to both commercial cheese and the EMC amount in Ras cheese on lipid profiles in experimental rats are presented in Table 3. The results presented in Table (3) reveal significant alterations in serum lipid parameters among rats fed diets containing different cheese formulations.

Total Lipid

Rats consuming commercial cheese at both 2.5% and 5% exhibited markedly elevated total lipid concentrations (623.2 mg/dl and 632.0 mg/dl, respectively), corresponding to increases of approximately 70% and 72.5% compared to the control group (366.5 mg/dl; $p \leq 0.05$). These findings align with prior studies indicating that saturated fat-rich dairy products may contribute to hyperlipidemia and cardiovascular risk **(Astrup et al., 2020)**.

Conversely, the group receiving 5% EMC-Ras cheese demonstrated a significantly reduced total lipid level (327.2 mg/dl), which was 10.7% below the control and 48.3% lower than the 5% commercial cheese group. Notably, the EMC amount in Ras cheese at 5% also yielded a comparable reduction (318.7 mg/dl), suggesting a lipid-lowering effect potentially due to altered fat metabolism or improved digestibility associated with enzyme treatment **(Khalil et al., 2021)**.

Total Cholesterol

Cholesterol concentrations were significantly higher in rats consuming commercial cheese (146 mg/dl and 157 mg/dl at 2.5% and 5%, respectively) relative to the control (105.6 mg/dl), with increases of 38.3% and 48.7% ($p \leq 0.05$). These elevations may be attributed to high levels of saturated fats and cholesterol in commercial cheese, as previously reported **(Hjerpsted et al., 2011)**.

In contrast, cholesterol levels in the EMC-Ras cheese and EMC amounts in the Ras cheese groups remained comparable to the control (range: 104–109.2 mg/dl), indicating no significant disturbance in cholesterol metabolism.

Triglycerides

The most substantial effect was observed in triglyceride levels. Rats fed commercial cheese at both levels exhibited dramatic increases to 304.5 mg/dl and 307 mg/dl, respectively representing increases of over 180% compared to the control (108.3 mg/dl). This hypertriglyceridemia may reflect the high energy density and poor lipid handling associated with such cheese types **(Lichtenstein et al., 2006).**

Conversely, the lowest triglyceride level (94.5 mg/dl) was found in the EMC amount in the Ras cheese 5% group, amounting to a 12.7% reduction from the control and a 69.2% reduction compared to commercial cheese (5%). EMC groups also exhibited mild, though non-significant, decreases in triglycerides. These results support the notion that enzyme modification may enhance fat utilization or alter lipid absorption dynamics, thereby improving lipid profiles **(Ahmed et al., 2019).**

Table (3): Effect of Enzyme-Modified Ras Cheese and Conventional Cheese on Total Lipids, Cholesterol and Triglycerides in Experimental Rats

Parameter / Groups	Total lipids	TC	T.G
	mg/dl		
Negative control	366.5±1.1 ^{b}	105.6±8.7 ^{b}	108.3±6.8 ^{b}
Ras cheese with EMC 2.5%	378.5±2.3 ^{b}	107.5±2.6 ^{b}	105.2±5.8 ^{b}
Ras cheese with EMC 5%	327.2±8.8 ^{d}	109.2±2.2 ^{b}	107.2±10.7 ^{b}
Commercial Ras cheese 2.5%	623.2±8.7 ^{a}	146±3.5 ^{a}	304.5±3.1 ^{a}
Commercial Ras cheese 5%	632±13.2 ^{a}	157±1.7 ^{a}	307±7.1 ^{a}
EMC at Ras cheese 2.5%	334.2±6.3 ^{c}	107.5±4.5 ^{b}	102.7±5.2 ^{b}
EMC at Ras cheese 5%	318.7±9.4 ^{d}	104±4.2 ^{b}	94.5±5.9 ^{c}

TC: Total Cholesterol **T.G**.: Triglycerides.

Results were presented as a means ± SD.

Means with different small superscript letters in the same column are significantly different at $p \leq .05$

The data presented in Table (4) reveal distinct impacts of EMC-Ras cheese compared to both commercial cheese and EMC amount in Ras cheese on serum lipoproteins and cardiovascular risk indicators in experimental rats.

High-Density Lipoprotein Cholesterol (HDL-c)

HDL-c plays a crucial role in reverse cholesterol transport and exerts protective effects against cardiovascular disease (CVD) **(El-Haggar et al., 2025)**. Rats fed commercial cheese at 2.5% and 5% exhibited a significant reduction in HDL-c levels (29.7 and 23.2 mg/dl, respectively), representing declines of 53.4% and 63.6% compared to the control group (63.7 mg/dl). Such reductions are indicative of heightened cardiovascular risk, consistent with findings that saturated fat-rich diets may lower HDL-c and impair lipid homeostasis **(Astrup et al., 2020)**.

In contrast, rats receiving EMC-Ras cheese at 5% recorded the highest HDL-c (67 mg/dl), showing a modest 5.2% increase over the control. Similarly, the enzyme-treated group at 5% demonstrated a slight increase (65 mg/dl), suggesting that enzymatic modification of cheese may contribute to maintaining or improving HDL-c levels. This may be due to bioactive peptides and improved lipid digestibility produced during enzymatic hydrolysis **(Jia et al., 2021)**.

Low-Density Lipoprotein Cholesterol (LDL-c)

LDL-c is a major contributor to atherosclerosis due to its role in cholesterol deposition within arterial walls. As expected, commercial cheese significantly elevated LDL-c levels to 55.8 and 72.3 mg/dl at 2.5% and 5% inclusion, respectively, corresponding to 177.6% and 259.7% increases over the control (20.1 mg/dl, $p \leq 0.05$). These results support the evidence linking saturated fats and cholesterol-rich foods with elevated LDL levels and atherogenic risk **(Gille & Schmid, 2020)**.

Conversely, LDL-c levels in rats fed EMC-Ras cheese (2.5% and 5%) and EMC amounts in Ras cheese remained statistically comparable to the control group (range: 20.1–26.4 mg/dl), indicating a neutral or protective effect. The likely mechanism involves modulation of lipid metabolism by peptides or enzyme-generated products that inhibit cholesterol absorption or enhance hepatic clearance **(Rico et al. 2022)**.

Atherogenic Index of Plasma (AIP)

The atherogenic index, calculated as (total cholesterol − HDL-c)/HDL-c, serves as an integrated marker of lipid-associated cardiovascular risk. Rats receiving commercial cheese exhibited substantially elevated AIP values: 1.01 (2.5%) and 1.12 (5%) versus 0.23 in the control group, corresponding to 339% and 387% increases. These values underscore the potential of commercial cheese to increase atherogenesis risk.

In contrast, the 5% EMC-Ras cheese group showed a modestly reduced AIP (0.21), while the 5% EMC amount in the Ras cheese group recorded the lowest AIP (0.16), representing reductions of 8.7% and 30.4%, respectively, relative to control. These findings strongly suggest that EMC-Ras cheese and EMC only may exert an anti-atherogenic effect, possibly by enhancing the HDL-c to LDL-c ratio and modulating cholesterol metabolism **(Silva & Malcata, 2020)**.

Table (4): Effect of Enzyme-Modified Ras Cheese and Conventional Cheese on Lipoproteins-cholesterol and Atherogenic Index in Experimental Rats

Parameter / Groups	HDL-c mg/dl	LDL-c mg/dl	AIP
Negative control	63.7±1.9^{b}	20.1±2.6^{c}	0.23±0.02^{b}
Ras cheese with EMC 2.5%	66.0±2.8^{b}	20.4±3.4^{c}	0.24±0.03^{b}
Ras cheese with EMC 5%	67±2.4^{a}	20.8±2.1^{c}	0.21±0.06^{b}
Commercial Ras cheese 2.5%	29.7±1.7^{c}	55.8±3.3^{b}	1.01±0.02^{a}
Commercial Ras cheese 5%	23.2±2.0^{c}	72.3±1.7^{a}	1.12±0.02^{a}
EMC at Ras cheese 2.5%	60.5±5.7^{b}	26.4±3.9^{c}	0.23±0.02^{b}
EMC at Ras cheese 5%	65±2.1^{b}	20.1±5.3^{c}	0.16±0.0١^{c}

HDL-cholesterol: High density lipoprotein – cholesterol.
LDL-c: Low density lipoprotein – cholesterol; **LDL-c**: Low density lipoprotein – cholesterol
AIP: Atherogenic Index in Plasma
All parameters are represented as a means ± SD.
Means with different small superscript letters in the same column are significantly different at $p \leq .05$

Kidney Function

The findings presented in Table (5) demonstrate that diets enriched with commercial cheese significantly impacted renal biomarkers, specifically urea and creatinine levels, while EMC-Ras cheese and EMC only exhibited comparatively milder effects.

Urea Concentrations

Rats fed commercial cheese at both 2.5% and 5% concentrations exhibited a significant elevation in blood urea nitrogen levels, recording 66.25 ± 1.5 mg/dl and 66 ± 2.3 mg/dl, respectively. This reflects 88% increase relative to the control group (35.18 ± 2.7 mg/dl), indicating compromised nitrogen metabolism or potential renal stress ($p \leq 0.05$). Such increases in urea are commonly associated with impaired renal clearance or excessive protein catabolism and have been reported in similar studies investigating the metabolic

burden of high-salt or high-fat cheese products in rodents **(Singh et al., 2021; Elshazly et al., 2020).**

In contrast, urea levels in rats administered EMC were moderately elevated but significantly lower than those observed in the commercial cheese groups. EMC-Ras cheese at 2.5% resulted in urea levels of 45.39 ± 3.4 mg/dl, while 5% EMC-Ras cheese yielded 53.25 ± 2.2 mg/dl. This indicates that enzyme modification of cheese may attenuate the adverse renal effects typically associated with conventional dairy intake. This protective effect may be attributed to improved digestibility and lower residual nitrogenous compounds in EMC formulations **(Zhou et al., 2020).**

Rats fed with EMC only demonstrated even lower urea concentrations 41.25 ± 2.6 mg/dl (2.5%) and 44.0 ± 3.8 mg/dl (5%) suggesting that the inclusion of proteolytic and lipolytic enzymes during cheese processing may improve nitrogen utilization and excretion. This aligns with previous reports indicating that enzymatic hydrolysis of food proteins can reduce renal load by generating more bioavailable peptides and minimizing the accumulation of metabolic waste **(Li et al., 2022).**

Creatinine Concentrations

Creatinine levels followed a comparable trend to urea. The control group exhibited normal creatinine levels of 0.70 ± 0.05 mg/dl. However, a diet containing 5% commercial cheese led to a marked rise in creatinine to 1.75 ± 1.7 mg/dl ($p \leq 0.05$), indicative of potential renal dysfunction or glomerular filtration impairment. A less pronounced increase was noted in the 2.5% commercial cheese group (1.20 ± 0.8 mg/dl), but this was still significantly above the control.

On the other hand, EMC-fed rats, whether at 2.5% (0.74 ± 0.42 mg/dl) or 5% (0.76 ± 2.7 mg/dl), showed no statistically significant differences in creatinine levels compared to the control group. Likewise, enzyme-only treatments resulted in creatinine levels of 0.72 ± 0.9 mg/dl (2.5%) and 0.72 ± 0.02 mg/dl (5%), further supporting the renal safety of enzymatically treated cheese. This outcome is in agreement with the findings of **Nassar et al. (2023),** who reported that dietary enzymes may exert a nephroprotective role by modulating protein turnover and enhancing amino acid absorption efficiency.

Table (5): Effect of Enzyme-Modified Ras Cheese and Conventional on Some Kidney Functions in Experimental Rats

Parameter / Groups	Urea mg/dl	Creatinine mg/dl
Negative control	35.18 ± 2.7 ^{e}	0.70 ± 0.05 ^{c}
Ras cheese with EMC 2.5%	45.39 ± 3.4 ^{c}	0.74 ± 0.042 ^{c}
Ras cheese with EMC 5%	53.25 ± 2.2 ^{b}	0.76 ± 0.27 ^{c}
Commercial Ras cheese 2.5%	66.25 ± 1.5 ^{a}	1.20 ± 0.08 ^{b}
Commercial Ras cheese 5%	66 ± 2.3^{a}	1.75 ± 0.17 ^{a}
EMC at Ras cheese 2.5%	41.25 ± 2.6 ^{cd}	0.72 ± 0.09 ^{c}
EMC at Ras cheese 5%	44 ± 3.8 ^{cd}	0.72 ± 0.02 ^{c}

Results were as a means ± SD.
Means with different small superscript letters in the same column are significantly different at $p \leq .05$

From the results presented in Table (5), the following can be concluded: Commercial cheese can negatively impact kidney function in rats, leading to elevated urea and creatinine levels. EMC-Ras cheese and EMC only have a milder effect, indicating that cheese processing methods and enzyme inclusion can mitigate renal stress.

Liver Enzymes

The current study evaluated the hepatic responses of rats to diets containing EMC-Ras cheese compared to both commercial cheese and EMC amounts in Ras cheese, focusing on alanine aminotransferase (ALT), aspartate aminotransferase (AST), and serum albumin as biochemical markers of liver health. Data presented in Table (6) demonstrate distinct differences in liver responses depending on the dietary intervention.

Alanine Aminotransferase (ALT)

Alanine aminotransferase levels, a sensitive indicator of hepatocellular injury, were significantly elevated in rats consuming commercial cheese, particularly at the 5% inclusion level (69.25 ± 2.5 U/L), representing a 53.3% increase relative to the control (45.15 ± 1.6 U/L). Similarly, 2.5% commercial cheese elevated ALT by 44.5%. These findings indicate hepatic stress potentially related to the high fat and salt content of commercial cheese, which is known to induce oxidative damage and inflammation in hepatic tissue **(Elshazly et al., 2020)**.

Conversely, ALT values in rats fed EMC or enzymes at both 2.5% and 5% (ranging from 52.25 to 54.25 U/L) were only mildly elevated (by ~16–

20%), suggesting less hepatic burden. The absence of significant differences between EMC-Ras cheese and EMC only groups implies that enzymatic treatments during cheese production do not exert harmful hepatic effects and may even enhance the digestibility and metabolic handling of cheese components **(Zhou et al., 2020)**.

Aspartate Aminotransferase (AST)

Similar trends were observed for AST, another hepatic injury marker. Commercial cheese-fed rats had the highest AST levels: 76.60 ± 0.8 U/L (5%) and 72.00 ± 6.2 U/L (2.5%), with increases of 62.6% and 52.8% over control, respectively. Such increases in AST typically reflect liver cell damage or mitochondrial dysfunction, often exacerbated by diets high in saturated fats or additives **(Singh et al., 2021)**.

In contrast, AST values in EMC-treated groups ranged between 55.25 and 56.50 U/L, corresponding to 17–20% increases over control, and were significantly lower than commercial cheese groups ($p \leq 0.05$). The EMC only groups showed comparable AST levels to EMC (55.50–57.50 U/L), reinforcing the relative hepatic safety of enzymatically treated cheese products **(Li et al., 2022)**. The limited elevation of AST and ALT in these groups suggests a less pronounced hepatotoxic effect, potentially due to improved proteolytic breakdown and reduced metabolic load.

Serum Albumin

Serum albumin is a marker of hepatic synthetic function and overall protein metabolism. The control group showed an albumin level of 3.29 ± 1.71 g/dL, which was not significantly different from the EMC-Ras cheese and EMC-fed groups. Notably, albumin levels in the EMC-Ras cheese groups were slightly reduced (3.15–3.20 g/dL), though non-significantly. EMC only groups showed marginal increases (3.35–3.50 g/dL), remaining within physiological norms.

However, a marked increase in serum albumin was observed in commercial cheese-fed rats: 4.8 ± 1.8 g/dL (2.5%) and 4.92 ± 0.04 g/dL (5%), reflecting up to a 49.5% increase over control ($p < 0.05$). While albumin elevation is less commonly linked to liver damage, these results may reflect compensatory hepatic responses to increased protein or sodium load, or altered plasma osmotic balance caused by commercial cheese components **(Eslami et al., 2020).**

Table (6): Effect of Enzyme-Modified Ras Cheese and conventional Cheese on Some Liver Functions in Experimental Rats

Parameter / Groups	ALT U/L	AST U/L	Albumin IU/L
Negative control	45.15±1.6^{d}	47.11±2.9^{c}	3.29±1.71^{b}
Ras cheese with EMC 2.5%	52.75٣ ±٣.9^{c}	55.25±2.7^{b}	3.20±0.09^{b}
Ras cheese with EMC 5%	54.25±4.5^{c}	56.50±3.6^{b}	3.15±0.11^{b}
Commercial Ras cheese 2.5%	65.25±3.5^{b}	72±6.2^{a}	4.8±1.8^{a}
Commercial Ras cheese 5%	69.25±2.5^{a}	76.60±0.8^{a}	4.92±.04^{a}
EMC at Ras cheese 2.5%	53.25±3.2^{c}	55.50±4.2^{b}	3.5±3.4^{b}
EMC at Ras cheese 5%	52.25±1.5^{c}	57.5±4.0b	3.35±0.42^{b}

Results were presented as a means ± SD.
Means with different small superscript letters in the same column are significantly different at $p \leq .05$

Calcium Concentrations

The data presented in Table (7) evaluated the impact of different cheese formulations on serum calcium concentrations in experimental rats. These findings reveal notable differences in calcium bioavailability linked to the type of cheese and the presence of processing enzymes.

The control group exhibited a serum calcium concentration of 9.02 ± 0.6 mg/dL, serving as the physiological baseline. Rats fed EMC-Ras cheese at 2.5% (9.10 ± 0.16 mg/dL) and those receiving EMC amounts in Ras cheese at 2.5% and 5% demonstrated calcium levels statistically comparable to the control, indicating no disruption in calcium homeostasis.

However, EMC-Ras cheese at 5% yielded the highest calcium level (10.32 ± 1.5 mg/dL), which was significantly elevated ($p < 0.05$) compared to the control, representing a 14.4% increase. This suggests enhanced calcium absorption or bioavailability, likely attributable to enzymatic pre-digestion of casein and other protein matrices, which may facilitate calcium release and uptake **(Kim et al., 2020; Zhang et al., 2022)**. The EMC group at 5% also showed a mild, though non-significant, elevation in calcium (9.6 ± 0.45 mg/dL), reinforcing the potential role of enzyme-mediated enhancement of mineral bio accessibility.

In sharp contrast, rats fed commercial cheese at 2.5% and 5% exhibited marked reductions in serum calcium levels (6.32 ± 0.38 and 6.27 ± 0.11 mg/dL, respectively), approximately 30.5% lower than the control ($p < 0.05$). Such decreases may reflect reduced calcium bioavailability due to the high sodium,

fat, or phosphate content in commercial cheese samples, which can impair intestinal calcium absorption or promote urinary calcium excretion **(Yoo et al., 2021; El-Kenawy et al., 2020)**.

These findings suggest that EMC particularly at higher concentrations may support improved calcium absorption, possibly due to enhanced proteolysis and release of calcium-binding peptides. Enzymatic treatment of cheese improves matrix breakdown and facilitates the release of bound minerals, a mechanism supported by studies in enzyme-enhanced dairy formulations **(Fan et al., 2021)**. On the other hand, the negative impact of commercial cheese on calcium levels might stem from its phosphate additives, high sodium, and unfavorable fat composition, which are known to impair calcium metabolism and promote demineralization **(Gérard et al., 2020)**.

Table (7): Effect of Enzyme-Modified Ras Cheese and conventional Cheese on Serum Calcium in Experimental Rats

Parameter / Groups	Calcium mg/dl
Negative control	9.02±0.6 [b]
Ras cheese with EMC 2.5%	9.10±0.16 [b]
Ras cheese with EMC 5%	10.32±1.5 [a]
Commercial Ras cheese 2.5%	6.32± 0.38 [c]
Commercial Ras cheese 5%	6.27±0.11 [c]
EMC at Ras cheese 2.5%	9.17±0.88 [b]
EMC at Ras cheese 5%	9.6±0.45 [b]

Results were presented as a means ± SD.
Means with different small superscript letters in the same column are significantly different at $p \leq .05$

Conclusion

(Romy) cheese is traditionally high in fat, making low-fat production technologically challenging. This study showed that incorporating 5% EMC into low-fat Ras cheese not only improved sensory quality but also enhanced lipid profiles, preserved hepatorenal functions, and supported calcium homeostasis compared with conventional cheese.

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الملخص العربي

التأثيرات المقارنة للجبن الراس المعدل بالإنزيمات والجبن التقليدي على دهون الدم ووظائف الكبد والكلى في فئران التجارب

هدفت هذه الدراسة إلى تقييم التأثيرات الفسيولوجية لكلٍ من جبن الراس المعدل بالإنزيمات (EMC-Ras)، والجبن التجاري، وكميات EMCمعلومة من الجبن الراس المعدل بالإنزيمات و المضافة إلى جبن الراس، على دهون الدم وظائف الكبد والكلى، بالإضافة إلى مستويات الكالسيوم في مصل الدم في فئران التجارب. تم تقسيم ٥٦ فأرًا أبيضًا من ذكور الألبينو إلى سبع مجموعات غذائية تجريبية، شملت مجموعة ضابطة، ومجموعات تناولت جبن EMC-Ras بنسبة ٢.٥٪ و٥٪، ومجموعات تناولت الجبن التجاري بنسبة ٢.٥٪ و٥٪، ومجموعات تناولت كمية الـ EMC التى تم اضافتها في اعداد الجبن نسبة ٢.٥٪ و٥٪.. أظهرت التحاليل البيوكيميائية أن الجبن التجاري تسبب في ارتفاع ملحوظ في مستويات دهون الدم و ناقلة الألانين الأمينية (ALT)، وناقلة الأسبارتات الأمينية (AST)، واليوريا، والكرياتينين، والألبومين في مصل الدم، مما يشير إلى وجود إجهاد كبدي وكلوي. وعلى النقيض من ذلك، أظهرت مجموعات المعالجة بجبن EMC والمعدّل بالإنزيمات تغيرات طفيفة وغير معنوية إحصائيًا في مؤشرات وظائف الكبد والكلى. ومن الجدير بالذكر أن إضافة EMC بنسبة ٥٪ أدت إلى زيادة معنوية في مستوى الكالسيوم في الدم، في حين أن الجبن التجاري أدى إلى انخفاض ملحوظ في تركيزات الكالسيوم. تشير النتائج إلى أن جبن EMC والمعالجة بالإنزيمات قد تمثل خيارات ألبان صحية أكثر، حيث تُسهم في تقليل التأثيرات الصحية السلبية المرتبطة باستهلاك الجبن التجاري.

الكلمات المفتاحية: الجبن المعدل إنزيميًا – وظائف الكبد– وظائف الكلى – كالسيوم الدم – الجبن التجاري – سمية الكبد – سمية الكلى – منتجات الألبان الوظيفية – فئران التجارب.