

Ras Cheese with Different Omega Fatty Acids as Diet Plans: Cytotoxicity & Characteristics

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Abstract: Implantation Ras cheese with different vegetable oils as a ketogenic model in the form to use it as a diet plan to prevent obesity and investigated cytotoxicity mode of these vegetable oils on liver cancer as consequences of obesity complications *in vitro* system by using Huh7 cell line. The chemical and organoleptic characteristics of Ras cheese with different vegetable oils were investigated, as well as phenolic compounds and fatty acid profiles of these oils were identified and determined. Thus, findings show that the Ras cheese with different oil possesses different omega fatty acid ratios, including omega 3, 6, and 9. The total phenolic compound results indicate that sesame oil had a pronounced rank of kaempferol (2478.44 µg/g) content, followed by canola oil (628.15 µg/g). Also, peanuts oil contains the highest value of gallic acid (164.52 µg/g), while canola oil is alone contains hesperetin value (793.44 µg/g). Ras cheese with canola oil had the highest protein and fat contents compared to other Ras cheese treatments. The results showed that Ras cheese with canola and sesame oil had a pivotal role in cancer cells. It is also considered a functional dairy product that may offer vital nutrients and health benefits.

Keywords: Ras cheese; omega fatty acids; ketogenic; obesity; cell line.

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1. Introduction

Overweight and obesity are forms of malnutrition arising due to excess fat deposition. However, obesity promotes disability, disease, and premature death. Overweight and obesity are essential adjustable threat factors to prevent the development of non-communicable diseases (NCDs) and metabolic syndrome. NCDs consisting of diabetes, cardiovascular disease, various cancers, and chronic lung disease account for 63% of annual global deaths [1,2]. Also, NCDs and metabolic syndrome have been identified as independent risk factors for hepatocellular carcinoma (HCC) development is a very commonly diagnosed form of cancer and considered the 5th mutual relevant to deaths. Egypt attained the third and 15th in Africa worldwide [3,4]. Obesity has been recognized as a risk factor for several malignancies, comprises liver cancer inducers with different cellular targets, accretive differences of cytotoxic effects, and participate in the formation of ROS, according to oxidative stress, which has a crucial role in hepatocarcinogenesis. Liver cancer pertinent to excess body weight is probably mediated via the evolution of non-alcoholic fatty liver disease (NAFLD), which is

identified as a condition caused by fat deposition in the liver. It usually occurs among obese patients up to 90%, and almost 25–30% have NASH [5,6]. Reactive oxygen species (ROS) had a dual role as destructive and constructive species. ROS are shared redox-governing activities of the cells to preserve cellular homeostasis. Oxidative stress resulting from multiplying of ROS can cause deleterious processes and is implicated in the damage of cell structures that are the main purpose of various diseases. ROS can facilitate cancer cell proliferation, survival, and adaptation to hypoxia. Furthermore, ROS can evolve antitumorigenic signaling and trigger oxidative stress-induced cancer cell death [7,8].

In recent years, the ketogenic or keto diet (KD) has been a therapeutic intervention for diabetes and obesity. It has also suggested treating some types of cancer in early evidence. KD designed for weight loss plan depends on high fat, low carbohydrate, and adequate protein content. Hence, KD focuses on healthful fats [9,10]. Omega fatty acids act as inflammatory-modulating agents by provoking or suppressing the synthesis of pro-or/anti-inflammatory cell signaling molecules. Omega 3, 6, and 9 fatty acids, namely linoleic and γ -linolenic and oleic acids, respectively, were capable of promoting ROS synthesis. The beneficial health effects of omega fatty acids have presumed effects inhibiting numerous types of tumors.

Nevertheless, their roles as anti-cancer have not been completely established [11,12]. On the other hand, KD starves the tumors. Of point view that, A keto diet constraint cancer cells by using ketones as a source of energy [13,14].

Dairy products, especially cheeses, are an attractive option as a food vehicle to deliver nutrients. The dairy products' effect on human health is quantitatively relevant, which have been chiefly studied for its impact on body weight and adipose tissue [15,16]. However, it seems difficult for some people to understand and follow a keto diet with suitable products in markets [17].

Using *in-vitro* systems in liver research has grown exponentially; Huh-7 cell established cell lines can act as modeling of HCC suitable for studies. The prospective role of using *in vitro* cell system is to investigate and establish the relationship of hepatomas and pivotal natural and synthetic compounds to prevent and eliminate this liver cancer, and comprises several aspects related to physiological, pathophysiological, and differentiation processes of specific cells. However, these investigations *in vivo* are inappropriate [18,19].

Thus, the objective of the present work was to know the achievements and limitations role of different omega fatty acids and their cytotoxicity effect on Huh-7 cells by using vegetable oils including sesame, canola, and peanut oil produce Ras cheese as KD model in the purpose of weight reduction. In addition, the chemical and sensory characteristics of Ras cheese were investigated during its ripening period.

2. Materials and Methods

2.1. Raw materials.

Different vegetable oil such as canola, sesame, and peanuts was obtained from the Agricultural Research Center, Giza, Egypt. Fresh cow's and buffalo's milk were obtained from the Faculty of Agricultureherd, Cairo University, Egypt.

2.2. Other materials.

Cell culture, Huh7: Liver Cancer was obtained from Nawah Scientific Inc. (Mokatam, Cairo, Egypt). Rennet (RENIPLUS) from *Mucormiehei* was purchased from Gaglio Star, Spain. Cheese starter cultures were obtained from the Egyptian Microbial Culture Collection (MIRCEN), Egypt, Ain Shams University. All other reagents and chemicals were used in analytical grade.

2.3. Methods.

2.3.1. Determination of Ras cheese samples fatty acid composition with gas chromatography.

The fatty acid composition of Ras cheese supplemented with canola, sesame, peanut, and hydrogenated oil was determined as described by the modified procedure of Zahran and Tawfeuk (2019) [20]. The FAMES were separated with an HP 6890 plus gas chromatography (Hewlett Packard, USA), using a capillary column Supelco™ SP-2380 (60m×0.25mm×0.20µm), (Sigma-Aldrich, USA), Detector (FID) and the injector and detector temperature were 250°C. The column temperature was 140°C/5 min and raised to 240°C, at a rate of 4°C/min, and held at 240°C/10 min. The carrier gas was helium at a 1.2 ml/min flow rate. Sample volume was 1µl (in n-hexane) and injected through a split injector at a splitting ratio of 100:20. FAMES were identified by comparing their relative and absolute retention times to those authentic standards of FAMES (Supelco TM 37 component FAME mix). The fatty acid composition was reported as a relative percentage of the total peak area.

2.3.2. Determination of total phenolic compounds of oils extracts.

Total phenol content was determined by the Folin-Ciocalteu method according to Lafka *et al.* (2007) [21]. Results were calculated as gallic acid equivalents (GAE) in mg/100 g of dry plant material using the equation:

$$C = A \times \gamma \times (V/M) \times 100,$$

where: C, the total amount of phenolic compounds, mg GAE/100g plant; A, dilution number; γ , concentration obtained from the calibration curve, mg/ml; V, the volume of aqueous ethanol used for extraction; M, the weight of dry plant material, g.

2.3.3. Identification of phenolic compounds of different oil using HPLC.

Phenolic compounds of different vegetable oil were identified and quantified by HPLC using an Agilent 1260 series. The separation was carried out using a C₁₈ column (4.6 mm x 250 mm i.d., 5 µm). The mobile phase consisted of water (A) and 0.02% trifluoroacetic acid in acetonitrile (B) at a flow rate of 1 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (80% A); 0–5 min (80% A); 5–8 min (40% A); 8–12 min (50% A); 12–14 min (80% A) and 14–16 min (80% A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 10 µl for each of the sample solutions. The column temperature was maintained at 35°C. Phenolic compounds of each sample were identified by comparing their relative retention time with those of the standard mixture chromatogram. The concentration of an individual phenolic compound was calculated on the peak area measurements, then converted to µg phenolic compound per ml of vegetable oil.

2.3.4. Determination of radical scavenging activity.

1,1-diphenyl-2-picrylhydrazine (DPPH) radical scavenging activity assay was performed as described by Stankevičius *et al.* (2010) [22]. Oil extracts of ethanol (400 µl) (blank) were added to 3600 µl of DPPH solution (100 µM) in ethanol and mixed. After 20 min, absorbance at 517 nm was measured using a spectrophotometer. All measurements were performed in duplicate. The DPPH radical scavenging activity (%) was calculated using the equation:

$$\text{Radical scavenging activity (\%)} = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100$$

Where: A control, the absorbance of blank; A sample, the absorbance of the oil extracts sample.

2.3.5. Cancer cells.

Hepatocellular carcinoma (Huh7); cells were maintained in DMEM media supplemented with 100 mg/ml of streptomycin, 100 units/ml of penicillin, and 10% of heat-inactivated fetal bovine serum in humidified, 5% (v/v) CO₂ atmosphere at 37°C [23].

2.3.6. Anti-cancer activity.

Anti-cancer activity of Ras cheese supplemented with JRP was tested using the cell line technique according to Allam *et al.* [24]. Cytotoxicity assay of hepatocellular carcinoma (Huh7) was assessed by sulforhodamine B (SRB) assay. Aliquots of 100µl cell suspension (5×10^3 cells) were in 96 well plates and incubated in complete media for 24h. Cells were treated with another aliquot of 100µl media containing drugs at various concentrations. After 72 h of drug exposure in hypoxic conditions using hypoxic chambers (Anaero Pack, Mitsubishi Gas Chemical Co., Tokyo, Japan), cells were fixed by replacing media with 150 µl of 10% TCA and incubated 4°C for 1h. The TCA solution was removed, and the cells were washed 5 times with distilled water. Aliquots of 70µl 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 air-dry overnight. Then, 150µl of TRIS (10mM) was added to dissolve the protein-bound SRB stain; the absorbance was measured at 540 nm using a BMGLABTECH®- FLUOstar Omega microplate reader (Ortenberg, Germany).

2.3.7. Ras cheese manufacture.

Ras cheese was produced as described by Hofiet *al.* (1970) [25]. The cheese milk was divided into 4 portions. The milk portions were supplemented with different vegetable oil (10%), including sesame (T1), peanut oil (T2), canola (T3), and hydrogenated oil (T4). Ras cheese samples were taken at monthly intervals for analysis during its ripening at $12 \pm 2^\circ\text{C}$ for 3 months.

2.3.7.1. Ras cheese chemical characterization.

Ras cheese samples were chemically analyzed according to AOAC (2000) [26] for total solids, fat, total protein, ash, and soluble nitrogen contents. The pH of the cheese samples was measured using a pH meter (Hanna Instruments Model 170300, Ingold, Knick, Germany). Acidity, as lactic acid, according to Ling (1963) [27]. According to Kosikowski (1982) [28], total volatile fatty acids were determined with 0.1 N NaOH.

2.3.7.2. Organoleptic evaluation of Ras cheese.

The sensory properties of Ras cheese samples during their ripening period at 12 ± 2 °C were evaluated according to Pappas *et al.* (1996) [29]. 10 panelists assessed ras cheese with maximum score points of 50 points for flavor, body, and texture (40 points) and 10 points for the cheese's appearance.

2.3.8. Statistical analysis.

The results were statistically analyzed by SAS software using ANOVA to analyze variance and the general linear model (GLM) procedure [30]. The results were expressed as mean \pm standard error, and the differences between means were tested for significance using Duncan's multiple range tests ($p\leq 0.05$).

3. Results and Discussion

3.1. Fatty acid content of different Ras cheese samples.

The total fat percentage of Ras cheese supplemented with 10% of sesame, peanut, canola, and hydrogenated oil was 45, 44, 46, and 43, respectively. Ras cheese supplemented with different oils was analyzed using gas chromatography (GC). Fatty acid composition of cheese samples was identified 25 different types of fatty acids the results presented in Figs.1, 2, 3, and 4. GC analysis result showed that Ras cheese supplemented with sesame oil (T1) has plentiful unsaturated fatty acids, the most precious of $\omega 6$ represented in linoleic acid with a percentage at (36.53%) of total fatty acids. However, the oleic was appointed with (24.62 %), linolenic acid recorded (13.74%), and the arachidic acid value was (5.62%) of total fat. Ras cheese was supplemented with peanut oil (T2) as a source of omega $\omega 3$, mainly linolenic acid, with a percentage (29.29%). Also, it consists of linoleic acid (17.58%), Oleic acid (20.34 %) of total fat. Ras cheese supplemented with canola oil (T3) showed an impressive provenance of omega $\omega 9$ enrich in oleic acid (34.15 %) of total fat. Moreover, it had linoleic acid with a percentage (18.71%), linolenic acid (23.01%), and arachidic acid (6.55%) of total fat. According to our results, Ion *et al.*(2011) [31] reported that canola oil mimetic compared to corn oil has a higher fraction of omega-3 fatty acids than the corn oil mimetic. In another point of view, the Ras cheese supplemented with hydrogenated oil (T4) aligns with the lowest value of omega fatty acids. The majority of fatty acids of T4 refer to palmitic acid, lauric acid, myristic acid, and stearic acid with value (29.21%, 19.55%, 11.78%, and 10.71%) respectively of total fat. Likewise, in another research, the obtained results show similar; the most prevalent fatty acids were palmitic, stearic, and myristic. Moreover, the ratios of the fatty acids may elicit divergent in percentage presented as (26.894%) for total unsaturated fatty acids, (71.903%) as saturated fatty acids, monounsaturated fatty acids as (25.811%), and polyunsaturated fatty acids were(1.083%) [32]. The result affirms using canola oil as a culinary oil and its dietary application. To, canola oil had zero trans-fat and the lowest amount of saturated fat of all common cooking oils. It is one of the healthiest oils available, agreeing with Song *et al.* [33]. Other authors observed similar results that canola oil was recognized as a high content of unsaturated fatty acid and had a good balance of omega-6/omega-3 [34]. Also, the results agree with Moon *et al.* (2021) [35], who analyzed the fatty acid compositions of imitation cheese mead of fat replacement with canola oil in addition of 9%, which leads to low in saturated fat otherwise high in unsaturated fat 95% of total fatty acids as compared to our results it appointed

86% of total fatty acid. The results conduct fatty acids particularly, oleic acid, linoleic acid, and linolenic acid, contributing to positive health and having anti-inflammatory effects and protection against certain types of cancer. In addition, canola, sesame, and peanut oil are rich in oleic content associated with their good oxidative and freezing stabilities [36,37].

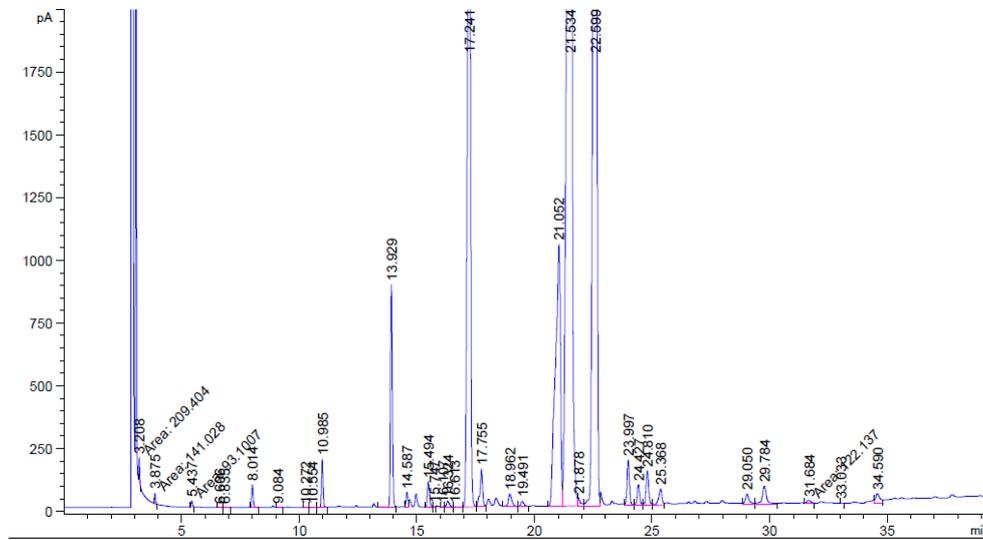


Figure 1. Fatty acids content of Ras cheesesupplemented with sesame oil.

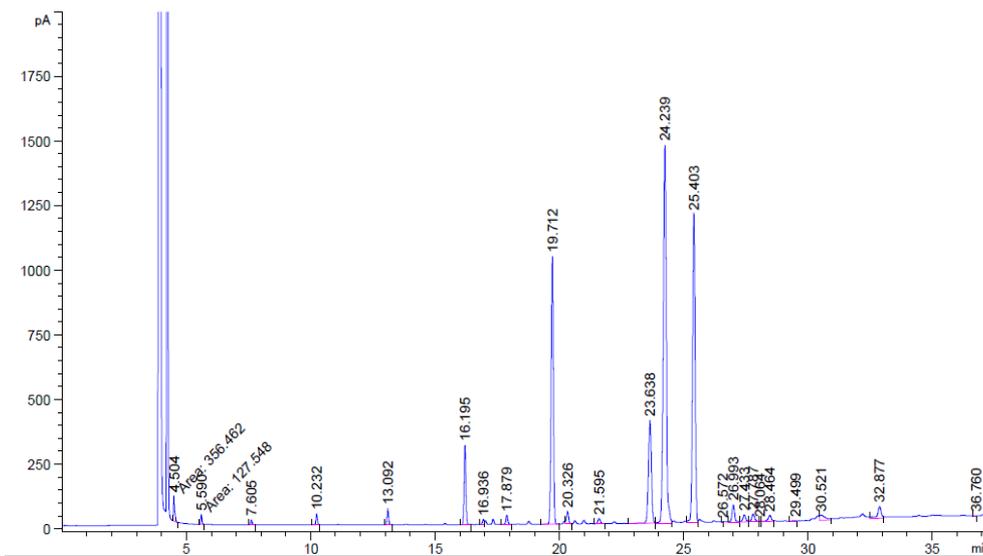


Figure 2. Fatty acids content of Ras cheesesupplemented with peanut oil.

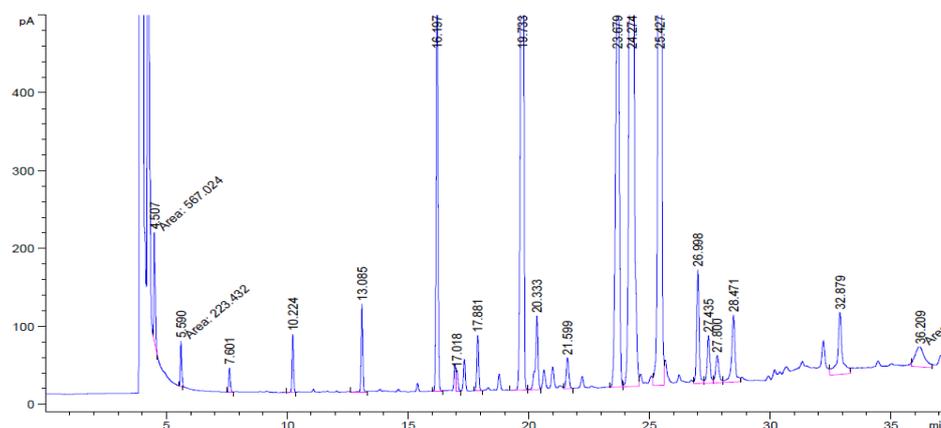


Figure 3. Fatty acids content of Ras cheesesupplemented with canola oil.

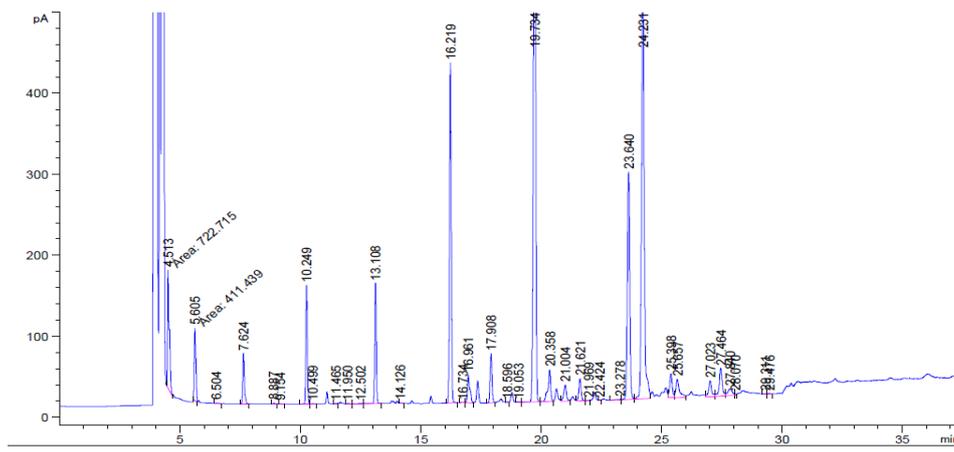


Figure 4. Fatty acids content of Ras cheese supplemented with hydrogenated oil.

3.2. Total phenolic content and antioxidant activity of different oil.

Phenolic components in vegetable oils possess antioxidants and contribute to oxidative stability and prevent oxidative degradation. They also imply taste properties such as odor, bitterness, color, astringency, and pungency. In addition, it emphasizes the stability of the oil itself and is considered a remarkable agent in various human diseases and health impairment [38]. So, they are relative to medicinal qualities and can reduce the risk of various diseases, including cardiovascular disease and various cancers [39]. The results of this study further showed TPC of ethanolic extracts oils, mainly sesame, peanut, and canola, were 140.42, 100.14, and 150.59 mg GAE/ml, respectively. On the contrary, with Bopitiya *et al.* (2013) [40], the results revealed that the TPC of the methanolic sesame oil extracts was 26 mg GAE/g of extract. The TPC of methanolic extract sunflower, corn, rapeseed, and soy oils are 12.0, 12.6, 13.1, and 14.8 mg GAE/g. Sesame oil extract contained higher TPC followed by canola oil compared to other available vegetable oils due to kaempferol and gallic acid with value for sesame oil (2478.44 and 143.89 $\mu\text{g/g}$) followed by canola oil (hesperetin, 793.44, Kaempferol 628.15, and gallic acid 157.88 $\mu\text{g/g}$), respectively. This result seems similar to Hoed (2010) [41], who confirmed the presence of gallic acid in canola oil. The antioxidant activity of sesame, peanut, and canola oil extracts was evaluated using DPPH assay, and the results revealed 80%, 72%, and 93%, respectively. The results obtained from another study showed sesame seed oil possessed a strong antioxidant activity compared to α -tocopherol; therefore, it can be categorized as an edible oil with a high potential for antioxidant activity [42]. However, another study examined the antioxidant activity of peanut oil; it was 42.02~52.34% [43]. The peroxide value is indicative of the rancidity of the oils sample and formation oxidation. The guidelines set by Codex for refined vegetable oils as peroxide values were below (10 mEqO₂/kg oil) [44]. The peroxide value of oil samples was (2.64, 3.89, 1.97, and 9.24 mEqO₂/kg oil) for sesame, peanut, canola, and hydrogenated oil, respectively. Similar findings of Tabasum *et al.* (2012) [45], who studied the peroxide value of various canola oils, further showed good quality and suitable for frying with valueless. Due to high oxidative stability and the presence of components with antioxidant and nutritional properties of, sesame, peanut, and canola oils seem to be good choices for cooking.

3.3. Cytotoxicity and cell line of different oil.

The present study investigates the role of selective oils on cell culture (Huh7) in the cytotoxicity model. The results are shown in Fig. 5. The results revealed that canola oil <https://nanobioletters.com/>

followed by sesame oil are shown an inhibitory effect on cancer cell lines Huh7, and they may be had a favorable anti-cancer effect. This could be referring to the canola plant, which belongs to the same genus of the cruciferous vegetable family, which contains glucosinolates. Other studies reveal that cruciferous vegetables are considered cancer prevention and many other biological processes [46]. The obtained results agree with Mekky *et al.* (2021) [47], who confirm that canola and sesame oil contains kaempferol and gallicacids, which have anti-cancer properties, and the ability to regulate oxidative stress and modify the oxidoreductive status of cancer cells. Similar results were observed by other authors who emphasize the role of hesperetin in canola oil gain anti-cancer effects such as kinase activity, inhibition of cell proliferation, and induction of apoptosis on cancer cells. [48,49]. On the other hand, the obtained results likewise to Giulitti *et al.* (2021) [50] to study the role of oleic acid in the modulation of neutral lipid accumulation in human hepatocellular carcinoma and hepatocyte cell lines; (THLE-2, Hep3B, and Huh7.5) the results showed that, cell prevalence inhibition and apoptosis induction after oleic acid administration in carcinoma cells. That implies oleic acid has valuable functions and can induce cell death through apoptotic and non-apoptotic pathways. That confirms that oleic acid had specific antitumor effects in HCC in an autophagy-dependent manner. In this respect, canola oil may have a more favorable fatty acid profile for decreasing the chance of inflammation that is promotional for developing chronic diseases. Therefore, canola had a potential role in the chemopreventive and chemotherapeutic effects of some types of cancer [51]. In further explanation, canola and sesame oil have shown an inhibitory effect on cancer cell (Huh-7) lines and maybe had a promising anti-cancer effect.

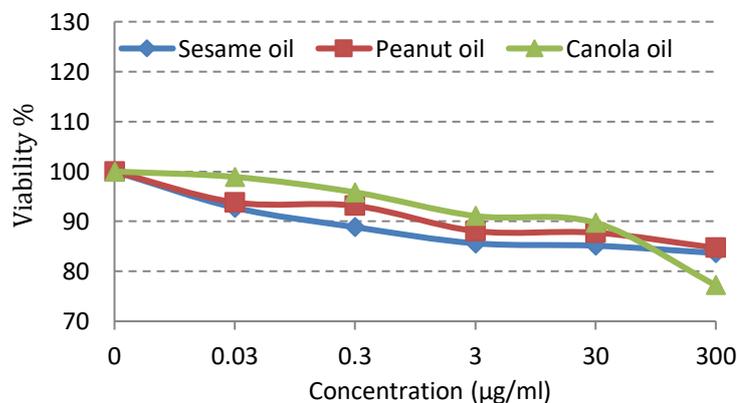


Figure 5. The survival curve of hepatocellular carcinoma cell line (Huh7)..

3.4. Ras cheese chemical characteristics.

Effect of different vegetable oil addition on dry matter (DM) content of Ras cheese during its ripening period was given in Table 1. Ras cheese with canola oil had the highest DM followed by sesame, hydrogenated, and peanut oil. Also, it could be noticed that all Ras cheese DM content increased throughout it's ripening mainly due to the loss of moisture content [52].

Protein and fat contents of all Ras cheese were increased with the ripening period progressed, as presented in Table 1, which confirmed the increase of DM with the ripening period progressed. However, the protein and fat contents of canola Ras cheese showed higher contents than other Ras cheese treatments [53].

Also, Table 1 showed that the highest ash content of Ras cheese was recorded in canola Ras cheese followed by peanut, sesame, and hydrogenated oil, as well as the ash content of all

produced Ras cheese, were significantly ($p \leq 0.05$) increased with the ripening period progressed mainly due to loss of moisture throughout ripening [52].

It could be noticed in Fig. 6 that canola Ras cheese's acidity values were higher than sesame, hydrogenated oil followed by peanut oil cheese with opposite trends in pH values, which might be due to its protein and fat contents. Similar results were also noted by Shan *et al.* (2011) [54], who demonstrated that the rich herbal extracts with phenolic compounds in the cheese decreased the pH throughout its storage period. However, the acidity of all Ras cheese increased with the ripening period, which could be due to the residual lactose fermentation and intermediates components of protein and fat degradation [55].

Table 1. Chemical changes in Ras cheese supplemented with different vegetable oil during the ripening period for 3 months.

Parameter	Ripening period (month)	Ras cheese treatments			
		T1	T2	T3	T4
Dry matter (%)	Fresh	64.88±0.55 ^{cdeA}	63.94±0.53 ^{eB}	64.60±0.30 ^{cdeBC}	64.75±0.37 ^{cdeA}
	1	65.33±0.62 ^{bcdA}	64.17±0.09 ^{deAB}	65.06±0.04 ^{cdeB}	65.30±0.53 ^{cdA}
	2	66.65±0.17 ^{abA}	65.28±0.05 ^{cdA}	65.92±0.42 ^{abcAB}	65.61±0.18 ^{abcA}
	3	65.86±0.77 ^{abcA}	65.57±0.25 ^{abcA}	66.83±0.40 ^{aA}	65.70±0.24 ^{abcA}
Total protein (%)	Fresh	19.40±0.13 ^{defB}	18.50±0.32 ^{fB}	19.55±0.16 ^{cdeB}	18.86±0.56 ^{efB}
	1	19.72±0.18 ^{bcdAB}	19.21±0.24 ^{defAB}	19.71±0.16 ^{bcdAB}	19.23±0.33 ^{defAB}
	2	20.10±0.09 ^{abcdAB}	19.53±0.21 ^{cdeAB}	20.50±0.32 ^{abcAB}	19.50±0.21 ^{deAB}
	3	20.56±0.48 ^{abA}	19.87±0.50 ^{abcdA}	20.72±0.09 ^{aA}	20.04±0.20 ^{abcdA}
Total fat (%)	Fresh	42.50±0.58 ^{efC}	41.33±0.60 ^{fC}	42.50±0.58 ^{efC}	41.17±0.73 ^{fB}
	1	43.33±0.60 ^{cdeBC}	42.83±0.33 ^{defBC}	43.17±0.73 ^{cdeBC}	42.67±0.44 ^{efAB}
	2	43.67±0.60 ^{bcdABC}	43.50±0.29 ^{cdeABC}	44.67±0.17 ^{bcABC}	42.50±0.50 ^{efAB}
	3	45.25±0.38 ^{abA}	44.50±0.58 ^{bcdA}	46.33±0.44 ^{aA}	43.50±0.58 ^{cdeA}
Ash (%)	Fresh	4.12±0.02 ^{abA}	4.20±0.04 ^{abA}	3.80±0.06 ^{bcB}	3.50±0.07 ^{cA}
	1	4.28±0.08 ^{abA}	4.22±0.02 ^{abA}	4.24±0.09 ^{abAB}	3.80±0.21 ^{bcA}
	2	4.30±0.08 ^{abA}	4.31±0.05 ^{abA}	4.32±0.17 ^{abAB}	3.85±0.42 ^{bcA}
	3	4.31±0.01 ^{abA}	4.48±0.30 ^{aA}	4.56±0.28 ^{aA}	4.04±0.03 ^{abA}

T1, Ras cheese supplemented with 10% of sesame oil; **T2**, Ras cheese supplemented with 10% of peanut oil; **T3**, Ras cheese supplemented with 10% of canola oil; **T4**, Ras cheese supplemented with 10% of hydrogenated oil. All parameters are represented as mean of replicates ± standard error. Means with different small superscript letters in the same row and different capital superscript letters in the same column are significantly different at $p \leq .05$.

3.5. Ripening indices of Ras cheese.

Total volatile fatty acids (TVFA) of Ras cheese, as presented in Fig. 7, showed that Ras cheese with canola oil was significantly ($p < 0.05$) higher than Ras cheese containing sesame oil, and hydrogenated oil), which might be attributed mainly to the fatty acids content of different oil as given in Fig. 7.

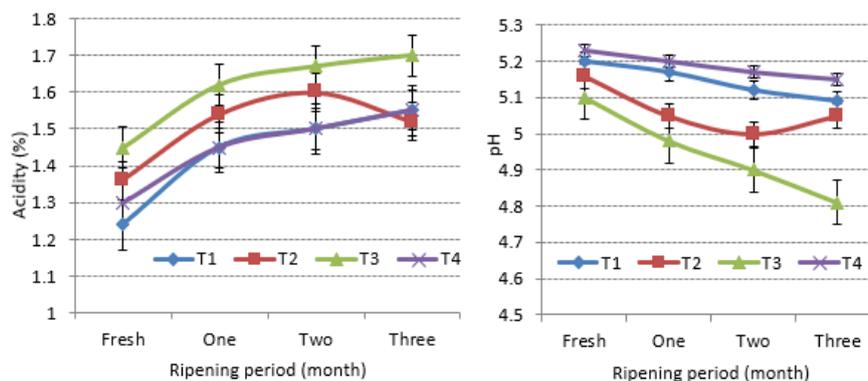


Figure 6. Acidity and pH changes of Ras cheese supplemented with different vegetable oil during the ripening period for 3 months. [T1, Ras cheese supplemented with 10% of sesame oil; T2, Ras cheese supplemented with 10% of peanut oil; T3, Ras cheese supplemented with 10% of canola oil; T4, Ras cheese supplemented with 10% of hydrogenated oil.]

This finding was similar to Fathi Achachlouei *et al.* (2015) [56], who demonstrated that adding hazelnut oils as a milk fat replacer increased the lipolysis of cheese during its ripening period. Moreover, the deviance from the relative between both moisture and fat contents in the progressive stages of cheese ripening can be recognized as the normal lipolysis occurring in Ras cheese during ripening [57].

Water-soluble nitrogen (WSN) is regarded as a ripening index for cheese, reflecting its proteolysis extent during ripening [58]. It could be observed from Fig. 7 that the highest WSN content was recorded in canola Ras cheese followed by Ras cheese containing sesame, peanut, and hydrogenated oil, which mainly due to the vegetable oils increased the cheese proteolysis during ripening by promoting their starter culture growth. This finding is in line with Fathi Achachlouei *et al.* (2013) [53], who reported that adding vegetable oils as milk fat replacers increased the proteolysis during ripening. However, WSN in cheese is primarily formed by enzymatic coagulants, plasmin, or cell-wall envelope proteases during the early stage of proteolysis. It is well recognized that protein degradation breakdown is an essential factor for equally flavors and texture of cheese throughout its ripening period [59-61].

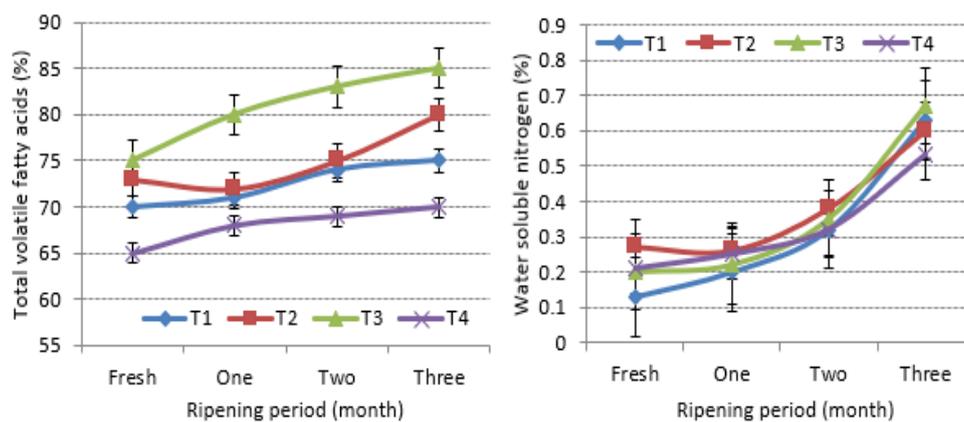


Figure 7. Ripening indices of Ras cheese supplemented with different vegetable oil during the ripening period for 3 months. [T1, Ras cheese supplemented with 10% of sesame oil; T2, Ras cheese supplemented with 10% of peanut oil; T3, Ras cheese supplemented with 10% of canola oil; T4, Ras cheese supplemented with 10% of hydrogenated oil.]

3.6. Organoleptic evaluation.

Odor and taste are organoleptic characteristics that have been used instinctively for food selection and increase its palatability [62]. Total scores of the organoleptic attributes of Ras cheese were given in Fig. 8. It could be observed that the total score of flavor, body & texture, and appearance of Ras cheeses contained different vegetable oil (sesame, peanut, canola) were significantly ($p \leq 0.05$) higher than hydrogenated oil Ras cheese.

Also, it could be observed from the organoleptic evaluation that no significantly ($p \leq 0.05$) differences of body & texture, and appearance of sesame and canola oil Ras cheese with higher values than Ras cheese supplemented by peanut and hydrogenated oil; while the flavor of Ras cheese supplemented with sesame oil recorded the highest scores followed by canola, peanut, and then hydrogenated oil (data not shown). Hence, the supplementation with sesame and canola oil followed by peanut oil showed a positive impact in Ras cheese flavor-compared to hydrogenated oil cheese. It could be due to the fatty acid contents of such oil, which mainly affect the cheese flavor and increase their acceptability [63-67]. These findings

were confirmed with their WSN and TVFA content as ripening indices of Ras cheese compared to hydrogenated oil cheese (Fig. 7).

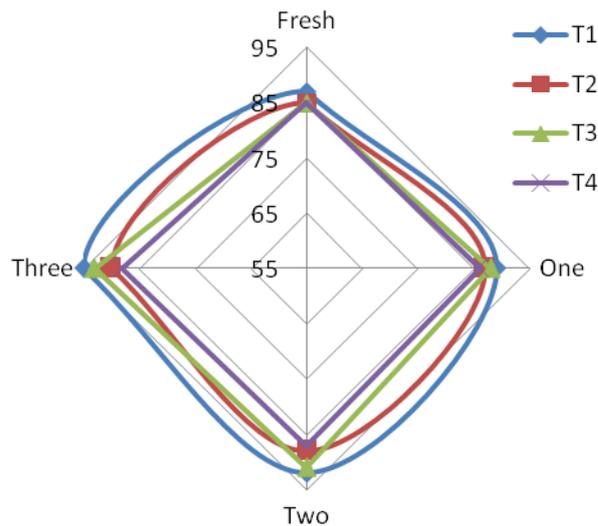


Figure 8. Total organoleptic properties of Ras cheese supplemented with different vegetable oil during the ripening period for 3 months. [T1, Ras cheese supplemented with 10% of sesame oil; T2, Ras cheese supplemented with 10% of peanut oil; T3, Ras cheese supplemented with 10% of canola oil; T4, Ras cheese supplemented with 10% of hydrogenated oil.] [Each point described the collective total scores of all organoleptic properties including flavor, body, and texture, and appearance.]

4. Conclusions

Ras cheese supplemented with a vegetable oil such as sesame canola and peanut improves the Ras cheese characteristics and overall acceptability. Also, Ras cheese supplemented with sesame and canola oil had a promising anti-cancer effect. Moreover, it considers a new functional dairy product suitable for the consumers who follow ketogenic with accepted tasty foods and healthful impact.

Ethical considerations

The present work was carried out according to the Medical Research Ethics Committee, National Research Centre (NRC), Cairo, Egypt, followed the recommendations of the Good medical and Laboratory Practice guidelines and the Institutional Animal Care and Use Committee (IACUC) guidelines and recommendation. World Health Organization (WHO) rules regarding the ethics of scientific research (Approval code 1493042021).

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Conflicts of Interest

The authors declare no conflict of interest.

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