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CONTENTS

ORIGINAL PAPERS

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DETERMINATION OF VISCOELASTIC PROPERTIES OF MINOR CONCENTRATIONS OF ZEIN SOLUTIONS WITH DYNAMIC LIGHT SCATTERING (DLS) MICRORHEOLOGY

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ABSTRACT

In the present study, it was aimed to determinate the rheological properties of zein solutions using DLS microrheology. For this purpose, zein solutions at different concentrations range between 0,08 and 0,32% were produced in aqueous ethanol and optimized. Rheological properties, namely G' (storage modulus), G'' (loss modulus), G* (complex modulus), η* (complex viscosity) and tan δ values, were determined. Regarding results of rheological measurements, both G' and G'' values were found to the highest at 0,08% zein sample. Except 0,16% zein, there was a crossover point between G' and G'' values of the other samples. Complex viscosity nonlinearly decreased at all angular velocity values analyzed. Additionally, K' values of 0,08% zein sample were found to higher than those of 0,32 and 0,16% zein samples. No significant differences was observed between n', K'' and n* values of all samples. The findings indicated that DLS microrheology could be used for the measurements of rheological properties of small amount-zein solutions.

KEYWORDS:
microrheology, dynamic light scattering (DLS), zein, viscoelastic properties

INTRODUCTION

Zein, the major (40% of total) storage protein of maize, is a water-insoluble protein consists of more than 12 amino acid signal peptides and extracted from corn endosperm cells [1;2-3]. Zein, is a mixture of lipophilic amino acids, dominantly alanine, glutamine, proline, leucine [4] and has four fractions, these are α- zein, β- zein, γ- zein, and δ- zein [5]. Out of all of fractions, α- zein, approximately 80% of total zein, is the most abundant one [5]. Zein, considered GRAS as food additive [6], can solve in 55-90% aqueous-alcohol solution, due to its special amino acid composition, which is more than 50% nonpolar. Regarding amphiphilic properties of zein, these molecules have the unique ability to self-assembly into nanoparticles upon reducing solubility using an anti-solvent like water [7]. Zein nanoparticles can increase the stability, functionality and controlled release of the ingredient by making core-shell formation with the encapsulated material [8]. Zein nanoparticles has been used for different purposes in many areas such as drug delivery [9;10], tissue engineering [11] and nutrient delivery [12]. In addition to food applications, there are some studies about using zein for formation of films and coatings [13]. Zein molecules exhibit high thermal resistance and are known for great oxygen barrier, which may allow for the encapsulation of sensitive materials that can be affected by temperature or oxidation [2]. In the recent years using zein as carriers have studied in different studies such as vitamin D3 [14], curcumin [15] and thymol [16]. In addition, some studies showed that zein nanoparticles as carrier increased the oral bioavailability of folic acid [5], resveratrol [17]. Many methods for manufacturing nanoparticles have been reported; these are emulsion/solvent evaporation, supercritical fluid technology, electrohydrodynamic atomization, nanoprecipitation etc. [18]. Zein nanoparticles are commonly manufactured by liquid-liquid anti-solvent precipitation technique and these nanoparticles are reported to take shape a solid internal core with the dimension of 200-300 nm [19]. Zein solutions produced can be directly used as coatings and are stable against gelation 90% of protein extracted [20].

Food Rheology is the response of flow or deformation characteristics of food materials against mechanical stress at a macroscopic scale, while microrheology measures local rheological properties of a material at a microscopic scale [21;22]. Microrheology is a number of approaches try to eliminate some limitations of traditional rheology [23] in many ways such as the sample size, the range of frequency, heterogeneity etc. [24]. In microrheology, an embedded micron sized probe is used to locally deform the material, which allows carrying out rheological measurement on small volumes [23]. When comparing microrheology to traditional rheology, micro-rheology has many advantages such as higher range of frequencies independent from time-temperature superposition

[25], fast thermal and chemical homogenization, which allows changing systems' transient rheology and the ability of measuring in-homogeneities of materials that are not accessible to traditional methods [26]. Microrheology can be subcategorized as passive microrheology (PM) and active microrheology (AM). The former one, measures mechanical properties of materials by associating the diffusive fluctuations of probe with the shear modulus of the matrix. The latter, measures the mechanical properties of the matrix from the movements of microscopic particles by dragged by external force [21]. Because of the lower thermal energy that required fluctuating the particles, passive microrheology is suitable for soft food materials. On the other hand, due to this technique does not need an external force, simple instruments can be used to measure motion of the particles such as dynamic light scattering (DLS), laser deflection tracking, direct visualization or diffusing wave spectroscopy (DWS) [21]. Out of four methods, DLS and DWS are considered the most common PM methods [27].

In the present work, it was aimed to determinate the mechanical properties of zein solutions by DLS, which is one of the most common microrheological methods that allows measuring small amount of samples in a short scale. For this purpose, zein solutions were prepared at different percentages ranged 0,02-0,32% by using 85% alcohol and optimized. Mechanical properties of optimized samples were measured by DLS.

MATERIAL AND METHOD

Material. Zein was purchased from Acros Organics. Carboxylated melamine with the diameter of 615 nm, used as a tracer for DLS microrheology measurements, was procured from Microparticle GmbH.

Methods. Preparation of Zein Nanoparticles. Zein solutions were prepared in aqueous ethanol and the preparation steps explained as follows. Briefly, zein was dissolved in 100 ml 85% ethanol and diluted to different concentrations ranged between 0,02 and 0,32%. After optimization of the formulations, 0,08, 0,16 and 0,32% zein solutions were found to be the most appropriate to perform microrheological experiments. All measurements were conducted at 25°C. Optimized zein samples at different concentrations were homogenized for 15 min at % 100 amplitude in ultrasonic water bath (VWR,50-60Hz, USA). 2 µl tracer was added into each 10-ml sample to ensure that the dominant scattering over zein. Tracer molecules is desired to have bigger size than zein molecules to monitor the rheological properties from the motions of tracer molecules. Firstly, zeta potential value of tracer particle was measured. Zeta potential was measured by adding sample onto tracer particles to figure out the concordance (chemically) between zein and the tracer. After no chemical interaction between zein and the probe was observed, microrheological measurements of zein samples was carried out.

Zeta potential measurements. DLS (Dynamic Light Scattering) measurements were carried out using a Phase Analysis Light Scattering (PALS) (Malvern Zeta Sizer, UK). Laser Doppler electrophorese) electrophorese, which measured mobility of particles was used in the present study. The zeta potential measurements were carried out by diffusion barrier technique Corbett et al. [28] reported. 10 ml previously prepared zein sample was put into a special cuvette and determined the rheological properties. Passive microrheology, which provides to measure rheological properties of samples by detecting motions of particles undergoing thermal fluctuations, was used. DLS records the correlation function of tracer particles, evaluate the means square replacement (MSR) of samples and calculates the rheological data of sample using Stokes-Einstein equation (1).

$$D = \frac{k_B T}{3\pi\eta a} \qquad (1)$$

Storage moduli (G') and loss (G") moduli and the other parameters calculated using these values, complex modulus (G*) and complex viscosity (η*) were obtained from a thermal energy balance and the measured mean square displacement of zeta potential measurements [29]. Following models (Equations 2-4) were fitted to the viscoelastic parameters mentioned above to calculate the model parameters which are intercepts (K′, K″ and K*), and slopes (n′, n″ and n*) according to the following equations [30;31]

$$G' = K'(\omega)n' \qquad (2)$$

$$G''=K''(\omega)n'' \qquad (3)$$

$$\eta^* = K^*(\omega)^{n^*-1} \qquad (a)$$

RESULTS AND DISCUSSION

Viscoelastic properties of the various zein samples were analyzed by DLS microrheology. Different rheological properties of the zein solutions of varying concentrations were shown in Table 1, Figure 1, 2 and 3. Viscoelastic parameters, namely, storage moduli (G'), loss moduli (G''), complex moduli (G*) and complex viscosity (η*) values as a function of angular velocity were presented. G' implies the solid-like (elastic) behavior of the material and G'' represents liquid-like (viscous) behavior [32].

As seen in Fig. 1, both elastic and the viscos moduli of 0,32% zein-samples were in the range between 0,39 Pa and 1,5 Pa. Viscoelastic results

demonstrated that while G'' values tended to monotonically increase, G' values increased until a certain point and then started to decrease with the steadily increasing angular frequency. On the other hand, a crossover point of G' and G'' values was observed at a certain point. At this particular point, zein solution started to lose its gel strength and become more viscous. The gel point can be identified as the crossover point where the elastic moduli and viscous moduli are equal (G'=G") [33].

In a cross-linking polymerization, when the material is in the liquid form, which means viscous behavior is dominant and less energy is stored than dissipated (G''>G') [34]. Another parameter of rheological properties, complex viscosity, exhibited a drastically decreasing at all angular frequency values performed.

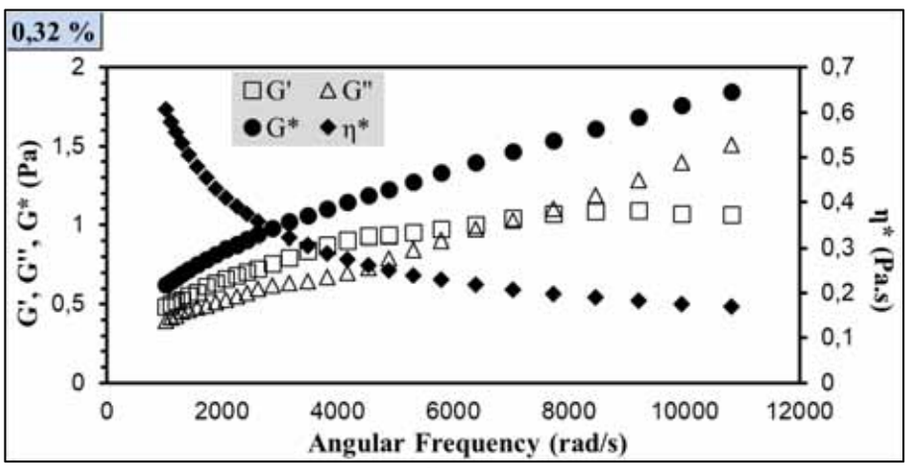

FIGURE 1
Viscoelastic Properties of 0,32% zein solutions

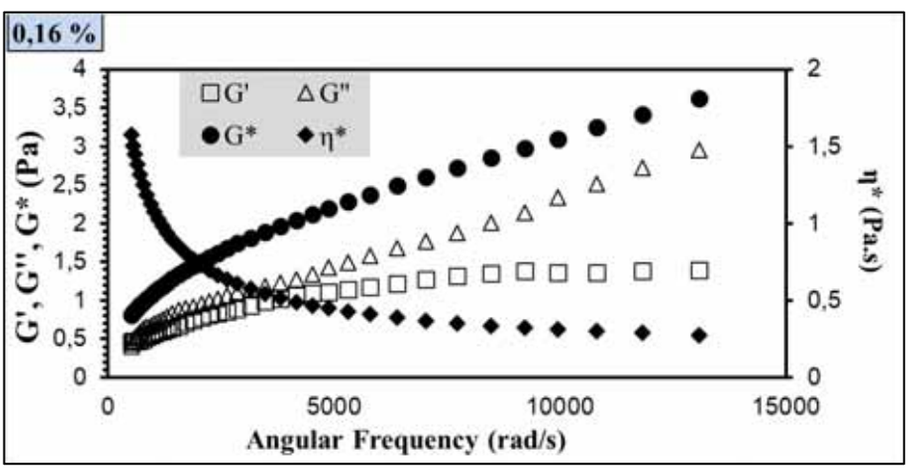

FIGURE 2
Viscoelastic Properties of 0,16% zein solutions

TABLE 1
Viscoelastic Properties of Zein Nanoparticles

	Dynamic Parameters								
	$G' = K'(\omega)n'$			$G'' = K''(\omega)n''$			$\eta^* = K^*(\omega)n^*-1$		
Samples	K' (Pa)	n'	$R2$	K'' (Pa)	n''	$R2$	K^* (Pa)	n^*	$R2$
0.32	0.049 ± 0.000b	0.342 ± 0.002a	0.980 ± 0.003	0.005 ± 0.000a	0.626 ± 0.029a	0.985± 0.000	29,0901± 1,1009b	0,442± 0,0024 a	0,999± 0.001
0.16	0.071 ± 0.003b	0.364 ± 0.035a	0.989 ± 0.003	0.012 ± 0.006a	0.577 ± 0.009b	0.989 ± 0.004	50,814±7, 9475ab	0,441± 0,0262 a	0,999± 0,0001
0.08	0.138 ± 0.028a	0.326 ± 0.006a	0.990 ± 0.002	0.016 ± 0.006a	0.565 ± 0.020b	0.985 ± 0.0001	102,737± 25,978a	0,392± 0,004a	0,999± 0,0001

*Different letters show significant differences between the zein samples (P<0.05)

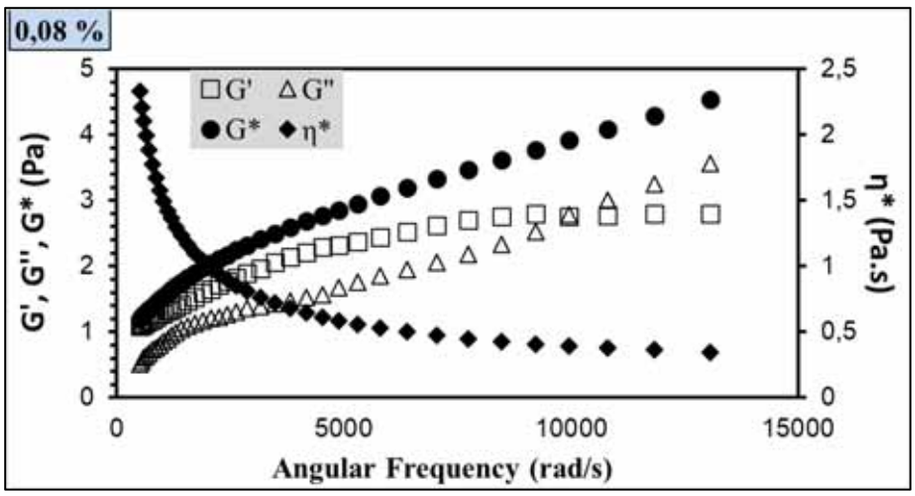

FIGURE 3
Viscoelastic properties of zein 0,08% zein solutions

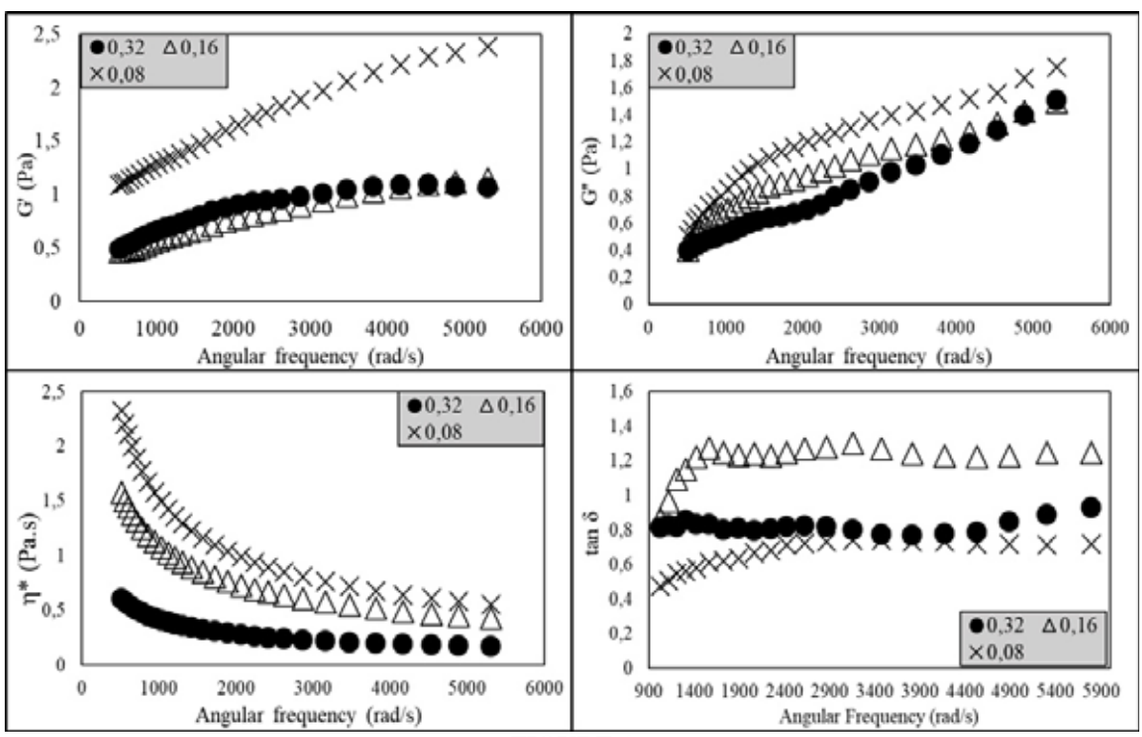

FIGURE 4
Comparison of different rheological properties of zein samples

As clearly seen in Fig. 2, while G' values tended to increase non-linearly, G'' values showed an increase constantly. Regarding viscoelastic properties of 0,16% zein solution, there was no crossover point at any angular frequency values. Complex viscosity displayed a decreasing tendency at all angular frequency values like 0,32% zein solution.

As seen in Fig. 3, both elastic and the viscos moduli of 0,32% zein-samples were in the range between 0,51 Pa and 3,6 Pa. Viscoelastic results exhibited that while G'' values tended to increase non-linearly, G' values increased until a specific point and then started to decrease. The decrease in the elastic moduli occurs from the connection loss between particles caused by shear rate [35]. Like 0,32% zein, crossover point was observed and complex viscosity decreased at all angular frequency applied. Slightly increase in G' and G" values versus to angular velocity, representing characteristics of solid-like gels [36]. When compared G' values of all samples, 008% zein solution had the higher G' than those of 0,32 and 0,16 zein samples. Decreasing amount of zein in solution resulted an increase at the G' value. Similarly, 0,08% zein sample had the highest G'' values among all samples analyzed. Madeka & Kokini [37] reported that n value of zein at 50°C was 0,59, both G' and G'' values increased and there was no cross over point. In another study, Zhang et al. [38] found that viscosity of 10% zein solution decreased and samples

had weak shear thinning behavior.

Experimental viscoelastic parameters versus ω (angular frequency) were fitted to power law, which describes the relation between ω and the corresponding parameter. R^2 values calculated for K', K'' and K* values were found to between 0.980-9.990, 0.985-0.989 and 0,99-0,99 respectively.

As seen in Table 1, K' values were higher than those of K'' values in all samples analyzed implying that zein samples had solid-like behavior rather than viscous character. K' values increased by lowering the amount of zein in solution. While the K' value of 0,08 zein sample was the highest (0,138), 0,32% zein had the lowest (0,049) and was equal to that of 0,16% zein statistically. Regarding flow behavior index (n), 0,16 zein sample was found to be higher than the other zein samples. At K'' values, there was no significant differences between all samples analyzed. Another parameter, K*, was found to the higher at 0,08% zein than those of other samples.

CONCLUSION

In the present study, rheological properties of different concentrations of zein solutions in aqueous ethanol were determined by DLS microrheolgy. A new method for measuring mechanical properties of materials was successfully performed. Both G' and G'' values of all samples increased at all angular frequency. 0,08% zein sample had the highest G' and G'' values among all three samples. On the other hand, except 0,16 zein sample; two cross over point were observed at a certain point. K' values of all samples were found to higher than those of K'' values representing that the elastic character was dominant over viscous character.

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IS PRICE SIGNIFICANT IN PLANNING FOR SUGAR BEET PRODUCTION? AN EXAMPLE FROM TURKEY

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ABSTRACT

Sugar beet is a major staple for Turkish agriculture and farmers in central and central eastern Turkey as it is the major natural sugar provider for Turkey. The sector has been controlled and supported until the WTO negotiations and privatization of the price setter public authorities. The supports so forth have been transferred to farmers via prices announced before the alterations by the public. But contracted farming has been maintained by the private sector as well in order to secure domestic sugar need. On this essence, it is intended to measure the impact of price changes on the contracted farming schemes and farmers decisions within an aggregate perspective. The price response of the sector was measured through time series analysis between 1960 and 2015 using secondary data. The traditional characteristics of the sector and attachments to the contracted farming were confirmed at the end of the analysis with 6 % response of price to short run and 13 % response of price to long run production.

KEYWORDS:
sugar beet, supply response, contracted farming, price, ECM

INTRODUCTION

Agriculture, being a vital sector for Turkey contributes to overall GDP by around 10 % and occupies 15 % of total exports. More significantly, around 30 % of the population is being involved in agricultural and food production activities. Considering the needs of the population as well as the needs of the food industry, one of the main staples essential for daily lives is sugar. Natural sugar can be supplied either from sugar cane or from sugar beet, when we exclude iso-glucose retrieved from corn. It is important to note that corn based sugar is not welcome in Turkish market due to its import-orientation and health considerations that take place around the world [1]. It is well known that sugar cane farming is more cost efficient. However, following some initial experiments, sugar cane farming did not produce desired output in Turkey [2]. Therefore, it was confirmed that sugar beet, which is around 25 % more efficient than sugar cane in provision of sugar content is more appropriate for Turkish ecological conditions and domestic market meets their sugar needs from sugar beet [3, 4].

Turkey, being one of the major sugar beet based sugar producers, takes the fourth place in the world with 8 % of the whole production while the third in the Europe with 10 %. The country occupies 65 % of Middle Eastern sugar market [4, 5]. Sugar beet farming is continued under irrigated conditions in central and south eastern parts of Turkey. Contracted farming has been pursued through input subsidies and market price supports in order to secure domestic demand and compete with the declining world prices [6]. Sugar beet has been supported for its sugar content only and pulp remedies of the industry are used to be delivered back to the farmers.

Public authorities was mainly responsible in market arrangements and contraction processes with 80 % market coverage until the issue of Sugar Law in 2001 [5]. However, the privatization process had started in 2001 and was completed in 2014 [7]. With the major law change in 2001, it was intended to assure domestic demand and supply equilibrium. The main orientation was both to limit iso-glucose and similar sugar content use in the food industry and sugar imports and to disable stock generation leading lower market prices as well [6]. Therefore, the policies were designed neither to support exports nor to accept imports of sugar on 2001.

It is also important to note that the market prices were used to be determined on yearly basis and announced for the consecutive production period. Specific quota implementations were issued for excess supply in order to cope with off-price exports at the lower world prices. 1996 was also a critical year for policy challenges as the rising stocks were translated to rising compulsory exports on the expense of main public authority's loss as sugar was stocked by the authority after all supports were transferred to the farmers. This is also the year just after Turkey became a member of the World Trade Organisation in 1995 [8, 9]. All export supports were converted to subsidies afterwards and the privatization of the sector was put on the agenda.

With these market control mechanisms, it is still a question for the researchers whether the pric-

es had an effect on encouragement or discouragement of sugar beet farmers because there were significantly lower price periods as well. Accordingly, it was intended to analytically search the price impact and policy changes in sugar beet market using time series supply response analysis between 1960 and 2015. The main objective was to understand the effect of price on the quantity supplied and search effects of policies implemented. Specifically, the impacts of rising supplies and WTO membership in 1995 and the new legal base set forward on 2001 were also searched in the scope of the analysis. The analysis is also expected to set forward the traditional characteristics of sugar beet farming as well.

MATERIALS AND METHODS

Material. Being a major staple, production of sugar beet is common in Turkey. Both the climatic conditions and traditional production knowledge and demand of powerful food and beverages industry leaded extensive production of sugar beet in Turkey. Looking at the main figures regarding production, we found out significant improvements. While the amount of land devoted to sugar beet cultivation was 202.917 hectares in 1960, it rose to 272.272 hectares in 2015 due to official Turkish Statistical Institute records. This almost stable amount of cultivation area, which rose by 36 %, can be attributed to traditional production attitudes and increasing attention on food and beverages industries. The cultivation area was at its peak on 1998 with more than 500 thousand hectares, which started to decline afterwards with strict contracts as demonstrated in Figure 1.

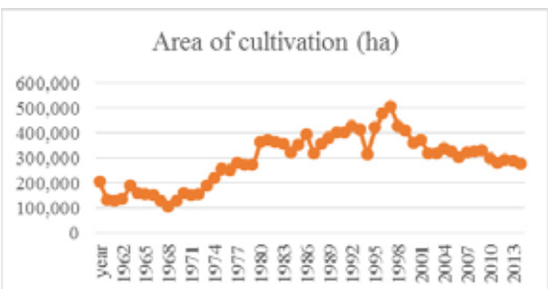

FIGURE 1
Amount of land devoted to sugar beet farming in hectares

The total production on the other hand was 4,4 million tonnes in 1960 which rose to 16,5 million tonnes in 2015 with 2,75 times increase as can be understood from Figure 2. This signs to a rise in the yield as expected, when the declination of land is considered with the rise in production. The yield per hectare rose from 21,61 tonnes per hectare in 1960 to 59,8 tonnes per hectare with 1,76 times in 2015.

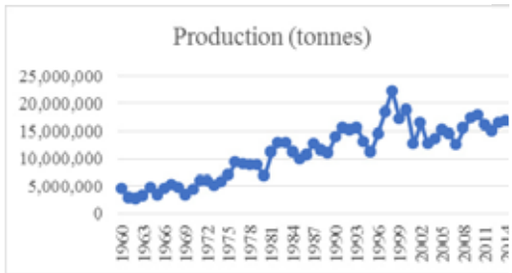

FIGURE 2
Amount of sugar beet production in tonnes

Therefore, it was intended to analyse the reasoning behind the change in amount of production and to understand the impact of price and non-price factors on decision making process of the sugar beet producers. Accordingly, the secondary data withdrawn from Turkish Statistical Institute was analysed to estimate the price impact and the term structure of Turkish sugar beet farming. The data used refers to amount of sugar beet production, unit price and amount of land devoted to sugar beet cultivation between 1960 and 2015.

Methodology. Planning for production in mostly competitive market settings depends on evaluation of price expectations and relevant market situations. As evaluation becomes eligible for the producer, he/she also starts to think over production alternatives and change his/her future plans. The supply response modelling with reference to price expectations of the product, as well as relevant non-price factors providing market information, is one of the mostly utilised methodologies. This Nerlovian supply function is specific for market planning as it uses output related factors apart from direct structural analysis conducted with reference to input market equilibrium. The initial form of the supply equation refers to estimation of the impact of price and non-price factors on the quantity produced based on the past data [10, 11].

$$Q_t^* = a + bP_t^* + cZ_t \quad (1)$$

$$Q_t^* = level\ of\ output\ for\ time\ t$$

$$P_t^* = expected\ real\ price\ for\ time\ t$$

$$Z_t = non-price\ exogenous\ variables$$

However, we need to consider unique features of agriculture and agricultural products in analysis and decision making. Agricultural producers, in fact like in many other sectors, cannot decide on the amount of production and the amount that they will bring to the market considering present prices. Because, the producer should have started planting the crop or even have received the harvest when they learn the market price. This is also valid for non-price factors. Producers cannot revise their production decisions considering a seasonal price shock, a climatic shift or a legal change. Accordingly, they need to observe the previous price levels and market movements to decide for current year's production. This calls for an adaptive expectations

framework depending on the past information [11].

Besides, not a lot of producers decide to shift between products year after year as every crop needs different approaches and knowledge. Therefore, the decision is also related with the amount produced in the recent periods. Our main question is 'by how much?' This query refers to the elasticity interpretation of the production. Therefore, the following final form of the supply equation needs to be estimated and analysed with respect to adaptive expectations.

$$Q_t = a_0 + a_1 P_{t-1} + a_2 Q_{t-1} + a_3 Z_t \quad (2)$$

Here, the subscripts t-1 refer to the previous term's price and quantity information and the parameters to be estimated are important for elasticity interpretation of the production. While parameter of lagged price variable, α_1 is read as the short-run price elasticity, $\alpha_1/1-\alpha_2$ refers to the long-run price elasticity [11, 12].

However, it is important to briefly explain the single ordinary least squares estimation of the supply response function and propose purification methods to potential impediments of the estimation procedure. When the data is used in the level form, the data is expected to have a time information itself. This means using non-stationary data for elasticity estimation and the relationship set forward would mostly probably be statistically meaningless [13, 14]. Accordingly, an error correction model (ECM) adjustment for the data is needed [15, 16].

ECM estimation enables using stationary variables which are adjusted for time and this modification does not lead any change in the interpretation of short-term response of quantity to price [3, 15]. When the economic relationship between quantity supplied and price are defined as following equation 3 and dependent and independent variables are considered to be co-integrated even when they are non-stationary on level, there is a possibility of estimation of the system. ECM methodology refers to estimation of the short run supply relationship within a linear combination of the variables and incorporation of error terms to the equation [17]. The error terms are directly expected to include past data to the system which in the end is expected to purify the time information in the supply functions of products that carry over past relationships to present decisions. Finally, the ECM approach with inclusion of the lagged dependent variable is an autoregressive distributed lag model augmented and it is a modified version of a stable long-run relationship of the variables [11, 18, 19].

$$Q_t = a + bP_t + u_t \quad (3)$$

$$\Delta Q_t = a + b\Delta P_t + cu_{t-1} + u_t \quad (4)$$

$$\Delta Q_t = \alpha_0 + b\Delta P_t - c(Q_{t-1} - a - bP_{t-1}) + u_t \quad (5)$$

$$Q_t = (\alpha_0 + ca) + bP_t - bP_{t-1} + (1-c)Q_{t-1} + cbP_{t-1} + u_t \quad (6)$$

$$Q_t = (\alpha_0 + ca) + bP_t + (1-c)Q_{t-1} + (cb - b)P_{t-1} + u_t \quad (7)$$

Depending on the set forward methodology, the static long-run supply function of the sugar beet is defined as following:

When Q_t is million tonnes of sugar beet production in Turkey from 1960 to 2015, P_t is price per kilogram and A_t is cultivation lands in million hectares respectively.

$$Q_t = a + bP_t + cA_t + u_t \quad (8)$$

Respecting adaptive expectations framework the static equation is shaped as following.

$$Q_t = a + bQ_{t-1} + cP_{t-1} + dT_t + \varepsilon_t \quad (9)$$

The relevant variables are:

$Q_t =$ *Sugar beet production in year t in million tonnes*

$Q_{t-1} =$ *Sugar beet production in year* $t-1$ *in million tonnes*

$P_{t-1} =$ *Real producer price for sugar beet per kg in year* $t-1$

$T_t =$ *Time trend from* 1 *to* 55

Here the price variable, which was considered in real terms, was taken in TL per kg terms for the ease of the interpretation. The impacts of stock rise and attributed domestic price change in 1996 and two consecutive years, which are in relation with the WTO membership attained and the Sugar Law issued on 2001, was measured by two structural dummy variables initially. However, no significant relationship was detected between the amount of production and policy changes, which is attributed to the stability of the production market. Therefore, the data between 1960 and 2015 was estimated and analysed using E-Views 5 statistical program.

Stationarity Testing and Integration. First the time character of the data was visually checked by correlograms and Q-statistics attached and the findings are shown in Table 1. The probability of estimated Q-statistics and partial correlation coefficients that die directly after the first lag are interpreted as a preliminary proof of the first order autocorrelation for the static variables.

TABLE 1
Q-statistics

Variable	Q-stat	p(Q)
Q_t	46.25	0.00
Q_{t-1}	45.122	0.00
P_{t-1}	49.478	0.00

In addition, all variables were tested for their levels and first differences in order to determine the degree of integration and the test results are demonstrated in Table 2. The quantitative dependent variables of the dataset were tested for their stationarity using ADF unit root tests [17].

$$\Delta X_t = \alpha_0 + \delta X_{t-1} + \sum \beta \Delta X_{t-1} I + e_t \quad (10)$$

Here ΔX_t is the first difference of the variable and δ is the test coefficient.

TABLE 2
ADF Stationarity Testing Results

Variable	Estimated ADF	ADF – 1 %	ADF – 5 %	p-value
Q_t	-1.69	-3.57	-2.93	0.43
Q_{t-1}	-1.66	-3.57	-2.93	0.44
P_{t-1}	-1.28	-3.57	-2.93	0.64
$D(Q_t)$	-6.89*	-2.62	-1.95	0.00
$D(Q_{t-1})$	-6.83*	-2.62	-1.95	0.00
$D(P_{t-1})$	-5.87*	-2.62	-1.95	0.00

Critical value of ADF tests are based on Mackinnon (1996) one sided p-values referred by E-Views 5 automatically. *, Significant at 1 %

Checking out the unit roots and cointegration level of the variables, the short-run equilibrium of the supply response was estimated through difference estimation. This procedure is called as Vector Error Correction (VEC). As well as the price effects, VEC modelling provides inferences with regards to the non-price time data of production. In other words, with analysis of the short-term dynamics, it becomes possible to consider how much of the production is attributed to the traditional character of agricultural production.

Therefore, as the dependent and independent variables of the static equation were found non-stationary on level and stationary when their difference were taken, it is important to check whether these non-stationary variables were co-integrated. The error terms of the static equation were checked with reference to Johansen Cointegration test [20] and findings are indicated in Table 3.

TABLE 3
Outputs of Cointegration Test

Dependent variable: D(e)	
e(-1)	-0.99
t(p(t))	-7.22 (0.00)

Therefore, the non-stationary static equation variables seemed to be integrated of order 1, which means that the first difference estimation would make it possible to comment over short term dynamics of the supply equation. The short-run supply function accordingly is as following.

$$\boldsymbol{D(Q_t) = \beta_0 + \beta_1 D(Q_{t-1}) + \beta_2 D(P_{t-1}) + \beta_3 ECM + u_t} \quad (11)$$

Here, the variables were estimated in their first difference and the error correction coefficient retrieved from the static long-run relationship was included in the model as an estimator.

RESULTS AND DISCUSSIONS

The estimated long run relationship is as following, of which the parameter statistics are demonstrated in Table 4.

$$Q_t = 1.77 + 0.55*Q_{t-1} + 0.06*P_{t-1} + 0.12*T_t$$

TABLE 4
Long-run relationship estimates

Variable	Parameter Estimate	Standard Error	t-Statistic	p-value
Q_{t-1}	0.55	0.12	4.57	0.00
P_{t-1}	0.06	0.21	0.29	0.77
T_t	0.12	0.03	3.07	0.01
α_0	1.77	0.72	2.46	0.02
R^2	0.86	F-statistic		102.76 (0.00)
D-W	1.97	Mean dependent v.		11.26

Therefore, more than 50 % of the production is related with traditional producers' efforts. This means that more than 50 % of producers prefer to continue producing sugar beet irrespective of any price alterations or policy changes. This is mostly related with characteristics of sugar beet production and contracted farming structure. In addition, the rise observed year after year is referred with the time trend and around 10 % of the rise is related with both population changes, rising interest in food and beverages industries with specific reference to export orientation and corresponding yield improvements which also means developing farming methodologies. Besides, increasing demand of food industries also leaded to extension of sugar beet farming in accordance with privatization of the industry. The trend parameter also covered the impacts of structural changes of 1995, WTO membership and 2001 Sugar Law issue. Therefore, there appeared no need to indicate the insignificant dummy variables and they were changed with the time trend as mentioned previously.

In addition, even when we take the unit of price as per kilograms, it was understood that the short-term impact of price changes in production is 6 %, while long-term impact is 13 % retrieved through calculations. This is an understandable figure as it is not so easy to shift from sugar beet to substitutes due to irrigation characteristics of sugar beet and it is the only natural sugar source produced in Turkey that can be used as input for the industrial purposes. The contracted farming implemented in the industry with prepaid supports seemed to act as a stabilizer even after transformation of the sector.

In addition, it is also important to consider short-term dynamics of sugar beet supply. This means underlying traditional characteristics of producers and how they insist on sugar beet farming regardless of price signals or policy changes referred. The short run relationship mostly refers to the difference of previous term's production and unexplained traditional structure of sugar beet farming. The estimates are as following as referred in Table 5.

$$D(Q_t) = 0.16 + 0.51*D(Q_{t-1}) + 0.95*ECM$$

TABLE 5
Short-run relationship estimates

Variable	Parameter Estimate	Standard Error	t-Statistic	P-value
$D(Q_{t-1})$	0.51	0.27	1.92	0.06
ECM(-1)	-0.95	0.30	-3.12	0.01
α_0	0.16	0.27	0.57	0.57
R^2	0.36	F-statistic		6.51 (0.01)
D-W	1.99	Mean dependent v.		0.25

Firstly, the relationship explains around 40 % of the variation in the quantity produced. This seems to be low, yet it still brings up information about the production characteristics. The price seemed not to affect short term production differences and also it was found to distort the statistical relationship. Therefore, the short-run relationship was built upon the spill over between periods.

Firstly, the consecutive production periods affect each other by 50 %. This refers to the traditional characteristics and the finding is compatible with the long-run. Accordingly, it can be inferred that sugar beet producers have to take long term decisions depending on the product characteristics and the contracts they have with either the public authority or the private sugar provider of today.

The estimate of the error correction coefficient was 0.95. The estimated value indicates the speed of adjustment from short-run to long-run equilibrium and it is significant at 1 %. The disequilibrium encountered in sugar beet production resulting from non-price factors like climatic factors rarely or contracting policy differences of the legal changes as appeared in the beginning of 2000s were offset in one production period by more than 90 % and the disequilibrium is purified towards the long-run equilibrium. Being a main staple and being essential for the productive food and beverages industries, sugar beet production is permanently secured and it is considered as a continuous activity by the farmers as well as the agricultural policy makers.

CONCLUSIONS

Sugar beet is mainly produced through contracted farming in Turkey. With pre-arrangements and market price adjustments in competition with the world, Turkey had proven to be one of the few countries that are close to self-sufficiency. The production system under coordination of public authorities until the mid of 1990s is needed to be released with the free trade arrangements. Following, non-quantity based supporting system requirements, privatization of the industry and increasing number of substitutes as corn based sugar had appeared as challenges of the sector. Accordingly, these challenges set forward the need to analyse the impact of price changes and policy alterations on production decisions of farmers. Main question behind is the market price, controlled for a long time, effective on farmers' production decisions.

Therefore, the aggregate supply response of sugar beet was analysed for Turkish sugar beet production with respect to 1960 and 2015 using a time series methodology with secondary data. The results had indicated that, the traditional structure of sugar beet farming is more effective than the price alterations announced or radical changes appeared in support systems. The contracted farming, once managed by public and is being maintained by private sector serving both to table sugar providers and food and beverages industry, is the traditional attachment of farmers to sugar beet production. The producers are bound their activities, respond to price by 6 % in the short run and 13 % in the long run and try to maintain their production activities. However, there is more need to analyse the response and assessment of farmers to the policy changes, specifically those who are involved in the sector for more than a decade, through face to face studies to measure the future potential of the industries attached to sugar production.

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CHEMICAL COMPOSITION AND INSECTICIDAL EFFECTS OF THE ESSENTIAL OIL OF THE PEPPERMINT, *MENTHA PIPERTIA* ON THE MELON APHID, *APHIS GOSSYPII*

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ABSTRACT

The melon aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), is a widely distributed pest of a variety of agricultural crops in the families Cucurbitaceae, Rutaceae and Malvaceae. To control the pest population, research on the use of the environmental and plant-based compounds has increased in recent decades. Essential oils due to volatility and very short-term persistence in the environment, as biocompatible pesticides can be considered as one of the best alternatives to chemical pesticides in aphid's control. In the current study, chemical composition and fumigant toxicity of the essential oil from Peppermint, *Mentha pipertia* L. (Lamiaceae) was evaluated against the melon aphid, *A. gossypii* in the laboratory conditions under 25±2°C, 60±5% RH and 16L:8D photoperiods. The essential oil was obtained by hydrodistillation method, using a modified Clevenger-type apparatus. Mortality was evaluated at different concentrations that rang from 0.1 to 0.9 µL/L air, and with three replications at the interim of 24 hours. Also, nymph production deterrent effect of the oil at sublethal concentration was studied against parthenogenesis form of aphid. Results indicated that essential oil of *M. pipertia* is toxic to *A. gossypii*. The major components in the oil were Menthol (30.09%), Menthone (20.38%), 1,8- Cineole (9.09%), Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1α,2β,5α (8.72%), 2-Cyclohexen-1-one, 2-methyl-5- (6.63%) and Carane, cis (4.53%). Probit analysis showed that the LC_{50} values for nymphs and adults of *A. gossypii* were 0.059 and 0.0081 µl. L-1 air, respectively. Also, the degree of nymph production deterrent effect was calculated for essential oil of *M. pipertia* as 48.51 ± 5.51%. The overall results showed that the Peppermint essential oil has high potential in controlling the melon aphid especially in protected areas such as greenhouses.

KEYWORDS:
chemical composition, *Mentha pipertia*, fumigant toxicity, nymph production deterrent, *Aphis gossypii*

INTRODUCTION

The melon and cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae) is extremely polyphagous and very damaging to many economically important crops, including cotton, aubergine, citrus, coffee, melon, okra, peppers, potato, squash, and sesame. It is a major pest of cotton and cucurbits [38]. *Aphis gossypii* has a worldwide distribution, although in arctic regions it is mostly confined to glasshouses [5]. It is particularly abundant in the tropics. Economic damage due to *A. gossypii* is by direct feeding, the excretion of honeydew and virus transmission. Damage to cotton, okra, and certain cucurbits occurs when large populations of aphids build up, feed on the crops and excrete honeydew [3]. However, its biggest overall economic impact is a vector of pathogenic plant viruses in over two dozen crops [28]. There is little quantitative information on exact crop losses. In cotton, for example, *A. gossypii* is only one of many crop pests. Monetary losses to this pest are substantial and are a result of crop loss and crop quality reduction, and the expense of pesticides.

The melon aphid, *A. gossypii* Glover is one of the basic pests in cucumber grown in greenhouses of Iran [27]. Frequently the use of chemical insecticides results in negative consequences. The botanical pesticides are an alternative for control of pests in modern ecological technologies. They do not carry the threat for the environment and human health. The spectrum of these products continuously expands that requires recognition of the mechanism of their action [13, 14]. The plant extracts contain alkaloids, esters, glycosides etc. and they have phytopesticide properties [23]. Some of the plant substances are used towards the pests as antifeedants and repellents [14].

Plant essential oils have a broad range and safe for the environment because the array of compounds they contain quickly biodegrade in the soil and can be used as a replacement for traditional pesticide [21]. Plant oils are able to penetrate by aromatic and aliphatic components in the waxy cuticle of pest and are impair activity neurotransmitters, growth hormones and digestive enzymes of pest [2]. Much research has been on the biological activity of plant essential oils. The results show that

plant essential oils have insecticidal, fungicidal, bactericidal and miticidal effects [21,4,15,16,20,10].

Many plants essential oils show a broad spectrum of activity against pest insects ranging from insecticidal, antifeedant, repellent, oviposition deterrent, growth regulatory and anti-vector activities. Recent investigations indicate that some chemical constituents of these oils interfere with the octopaminergic nervous system in insects. They cover the criteria for "reduced risk" pesticides. These plant oils are well accepted in the agricultural practice as "green pesticides" that could be effective enough particularly for biological foods production. Further, while resistance development continues to be an issue for many synthetic pesticides, it is likely that resistance will develop more slowly to essential-oil-based pesticides owing to the complex mixtures of constituents that characterize many of these oils [19]. The Peppermint, *Mentha pipertia* L. (Lamiaceae) is a native plant of the Mediterranean region, which is now grown all over the world. The plant is a perennial glabrous herb with a strong, pepper-like, pungent odor and hence the specific name "piperita". Peppermint oil is a colorless, pale yellow liquid with a strong agreeable odor and a powerful aromatic taste. The essential oil of peppermint is between 1 to 2.5% in the leaves dried. Menthol is the major constituent of this oil. Peppermint oil is the most popular and widely used essential oil employed in flavoring, pharmaceuticals, confectionery, and medicines [34,39].

The plant products possess a range of priorities that make them preferable in modern biological agriculture. It is studied the effect of different essential oils and water extracts towards *A. gossypii* [40,6]. Gorski and Tomczak [7] have examined the efficacy of natural essential oils, such as basil, citronella, eucalyptus, juniper, and patchouli in the control of foxglove aphid, *Aulacorthum solani* Kalt. Insecticidal effects of 23 essential oils against adults of turnip aphid, *Lipaphis pseudobrassicae* Davis. have been studied by Sampson et al. [31]. Hori and Komatsu [12] studied repellence effects of rosemary oil against onion aphid, *Neotoxoptera formosana* Takahashi.

In spite of considerable research efforts in many laboratories around the world and the increase of scientific articles regarding the pesticides property of herbal essential oils and their compounds, unfortunately, a few pests of agricultural products are controlled on the basis of herbal oils. Since application of plant essential oils in greenhouses and fields is not possible in pure form, therefore, it is necessary to prepare commercial formulations of these compounds [18]. As part of future strategies for aphid control, essential oils with deterrence and/or insecticidal properties should be studied. Therefore, the aim of the present work was to study the fumigant toxicity of the Peppermint, *M. pipertia* essential oil against nymphs and adults of *A. gossypii*.

MATERIALS AND METHODS

Plant material and extraction of essential oil. Peppermint (*Mentha piperita* L.) plant material was collected from the field in Khoramabad, Iran during summer 2016. The collected plant material was authenticated by a plant taxonomist of the Department of Horticultural Sciences, Lorestan University of Iran. Areal parts were separated and dried under shade at room temperature, after drying; the plant material was ground by the grinder. 100 g of dried plant material and 1000 mL of water was subjected to hydro distillation for three h by using Clevenger apparatus. Extracted oils were dried over anhydrous sodium sulphate and in microtubes of 2 ml which were covered with aluminium coating were kept in a conventional refrigerator in +4°C and away from light until used in experiments.

Gas Chromatography–Mass Spectrometry Analysis. The oil was analysed utilizing a GC model: 7890B- MS model: 5977A Agilent HP-5973 chromatograph (Agilent Technologies, www.agilent.com). Gas chromatographic (GC) analysis was carried out using a Shimadzu (www.shimadzu.com) GC-9A with helium as a carrier gas, with a linear velocity of 1.1 ml/s on HP-5 Agilent Column (30m × 250 nm i.d, 0.25 µm film thickness). Injection mode was split (100:1). The oven was programmed to rise at 60°C (10 min) isotherm, and then to 250°C at a rate of 5°C/min. Injector and detector temperatures were 250 and 280°C, respectively. The GC mass analysis was performed on a Varian 3400 equipped with an HP-5 column with similar characteristics as the one employed in GC. The transfer line temperature was 260°C. The ionization energy was 70 ev with a scan time of 1 s and mass range of 50-500 amu. Unknown essential oil was identified by comparing its GC retention time to that of known compounds and by comparison of its mass spectra, either with known compounds or published spectra. Quantitative analysis is performed by normalizing the level of the spectrum (the concentration of each component is equal to the peak level associated with that composition divided by the sum of the levels corresponding to other compounds).

Insect Rearing. Cucumber plant (*Cucumis sativus*) for rearing aphids and preparing leaf discs for testing was planted in pots containing soil, peat moss and perlite (two thirds of soil and one third of peat moss and perlite) in a research greenhouse of the Khorramabad Technical and Professional Training Center, Iran was planted in pots containing soil,

petite mas and perlite (two thirds of soil and one third of pit moss and perlite). The pots were then transported under a Grid Scaffolding to prevent the transmission of any infestation. The pots were kept in greenhouse conditions at 25±5°C and relative humidity of 50±20%. Irrigation was carried out every day, one month later, the plants were used for the rearing of aphids and from leaves for bioassay experiments.

To create an initial population of aphid, infested cucumber leaves with the melon aphid were collected from cucumber fields. The aphids were transferred to the cucumber pots. The pots were placed in a cage with net cover and kept in a greenhouse at 25±5°C, 50± 5% relative humidity, and a light period of 16 L: 8D hours of photoperiods until the colony reached an acceptable level. In order to maintain the population of aphids and having a high population, three healthy plants entered the colony of the aphid in the cage every three days. This method was continued until 6 to 7 generations of aphid.

Bioassay experiments. For fumigant toxicity experiment, Tripathi et al. [35] method was used with a little change. Investigation of toxicity of essential oil of Peppermint on adult and nymphal stages were carried out. To run experiments, glass bottles of 250 ml and plastic containers of 6 cm in diameter were used. The leaves were placed on a thin layer of 1.5% agar in Petri dishes. Ten aphids (adult/nymph) were separately transferred to the leaves by a soft brush. The experiments were performed in three replications for each stage of the insect. Then, they the Petri dishes were covered with a thin layer of the net to prevent possible direct contact of insects with essential oils. The Petri dishes were attached to the inside lid of the 250 ml glass bottles. A specific amount of essential oil (0.1, 0.3, 0.5, 0.7 and 0.9 µl/l) was placed on a piece of cotton by a micropipette sampler (http://www.htl.pl) and it was placed on the bottom of glass containers. The control treatment included the same conditions, but without essential oil application. To prevent evaporation of essential oil to the outside, the lid of the cap was tightly sealed with parafilm tape. In the preliminary experiments, there was no difference in the mortality rate after 24 and 48 hours. Therefore, the appropriate time of 24 hours was determined and mortality was counted. The insects were considered dead if the antennae and legs were stimulated by a needle, no reaction was seen.

Nymph Production Deterrent. In this research, deterrent effect was tested at the sublethal LC_{50} concentration of essential oil. In each of tested containers, one parthenogenesis (fundatrix) adult aphid of 12 hours old was placed on the cucumber leaf. The desired essential oil was released on the filter paper in the inner surface of containers. Data were recorded daily until 72 hours. After counting, produced nymphs were removed from test containers. 10 replications were used in each experiment. The rate of nymph production deterrent was calculated from the following formula [30]:

$$\text{Nymph production deterrent} = \left(1 - \frac{NNt}{NNc}\right) \times 100$$

NN_t= Number of nymph on the treatment

NN_c= Number of nymph on the control

TABLE 1

Volatile compounds in steam-distilled oil of the leaf from the Peppermint, *Mentha pipertia* identified by gas chromatography–mass spectrometry.

	Compound	RT	Start Time (min)	End Time (min)	% Area
1	1R-α-Pinene	4.76	4.69	4.823	0.58
2	β-Pinene	5.611	5.572	5.673	0.94
3	Cineole- 1,8	6.843	6.672	6.937	9.09
4	1,4-Cyclohexadiene, 1-methyl-4-(	7.421	7.374	7.483	0.25
5	Terpineol, cis-β	7.639	7.569	7.717	1.15
6	Menthone	10.05	9.792	10.112	20.38
7	Cyclohexanol,5-methyl-2-(1-methylethyl)-, (1α,2β,5α-(	10.276	10.112	10.339	8.72
8	Menthol	10.69	10.339	10.744	30.09
9	Cyclohexanol, 2-methyl-5-(1-meth	11.01	10.986	11.041	0.16
10	Estragole	11.088	11.041	11.127	0.64
11	cis-Carveol	11.618	11.556	11.704	0.20
12	Cyclohexanone, 5-methyl-2-(1-metylendehid)	12.18	12.055	12.219	2.05
13	2-Cyclohexen-1-one, 2-methyl-5-(	12.367	12.219	12.414	6.63
14	2-Cyclohexen-1-one, 3-methyl-6-(	12.562	12.492	12.617	0.81
15	2,6-Octadienal, 3,7-dimethyl-, (E)-	12.898	12.843	12.952	0.18
16	Carane, cis	13.592	13.459	13.631	4.53
17	yclobuta[1,2:3,4]dicyclopentene	15.933	15.823	15.972	0.34
18	Caryophyllene	16.822	16.713	16.931	1.56
19	1,6,10-Dodecatriene, 7,11-dimeth	17.626	17.571	17.75	0.25
20	1,6-Cyclodecadiene, 1-methyl-5-m	18.328	18.242	18.429	0.98
21	1H-Cycloprop[e]azulen-7-ol, deca	20.606	20.512	20.661	0.40
22	Caryophyllene oxide	20.754	20.661	20.801	0.55
23	Ledol	20.949	20.871	21.035	0.53
24	α-Cadinol	22.322	22.252	22.377	0.17

TABLE 2
LC_{50} and LC_{90} values of essential oil of the Peppermint, *Mentha pipertia* against the adults and nymphs of the melon aphid, *A. gossypii*.

Growth stage	LC_{50} (µl/l)	LC_{90} (µl/l)	Slope ± SE	Intercept ± SE	χ^2 (df)	Sig.
Nymph	0.056	0.357	1.59±0.41	1.99±0.27	1.43 (3)	0.70
Adult	0.023	0.699	0.87±0.36	1.42±0.21	3.98 (3)	0.26

PROBIT model: PROBIT (p) = Intercept + BX (Covariates X are transformed using the base 10 logarithm).

TABLE 3
Mean (±SE) of nymph production and nymph production deterrent of the melon aphid, *Aphis gossypii* caused by essential oil of the Peppermint, *Mentha pipertia*.

Time (h)	Control	Essential oil	
		Nymph production	Nymph production deterrent
24	5.0 ± 0.0 a	4.5 ± 0.58 a	26.0 ± 6.00 a
48	7.0 ± 0.0 b	2.6 ± 0.48 b	62.86 ± 6.80 b
72	3.0 ± 0.0 c	1.8 ± 0.55 c	56.67 ± 11.17 c
Total	5.0 ± 0.32	2.97 ± 0.37	48.51 ± 5.51

* Comparison was performed at each column, the means with the same letters based on Duncan's test did not have a significant difference at 5% level.

Statistical Analysis. The LC_{50} and LC_{90} values with their fiducial limits were calculated by probit analysis using the SAS software [32]. The experiments were arranged in a completely randomized design and the data from nymph production deterrent were subjected to (PROC GLM) ANOVA (P<0.05) after checking for normality. The means were separated using the Duncan Multiple Range test at the 5% level.

RESULTS AND DISCUSSION

Chemical Compositions of Essential Oil. Chemical constituents of the Peppermint, *M. pipertia* from the results of the component analyzes by gas chromatography–mass spectrometry (GC-MS) are summarized in Table 1. In total, 43 compounds were identified by GC-MS, representing 100% of the essential oil that 24 of them which were important and representing 91.18% of composed ingredients are presented in Table 1. The major components include Menthol (30.09%), Menthone (20.38%), 1,8- Cineole (9.09%), Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1α,2β,5α (8.72%), 2-Cyclohexen-1-one, 2-methyl-5-((6.63%) and Carane, cis (4.53%).

Fumigant Toxicity. The insecticidal activity of seed essential oil from the Peppermint, *M. pipertia* was investigated. The essential oil had a remarkable insecticidal activity, and the results are shown in Table 2. The nymph and adult mortality were observed against the melon aphid, *A. gossypii* after an exposure period of 24 h. The nymphicidal activity was LC_{50}=0.056 and LC_{90}=0.357 µl. L^{-1} air and the adulticidal activity were LC_{50}=0.023 and LC_{90}=0.699 µl. L^{-1} air, respectively.

Results revealed that the essential oil of *M. pipertia* had high mortality on the different stage of *A. gossypii* after 24 h exposure. The results showed that by increasing dose and time, the mortality rate of nymphs (F= 8.16, df= 4, P<0.01) and adults (F= 4.11, df= 4, P<0.01) was significantly increased (Fig. 1). The mortality rate of the nymphs and adults of *A. gossypii* at the highest concentration of the Peppermint was 100 and 96.66%, respectively. At the lowest concentration of oil, the mortality of *A. gossypii* was recorded as 66.67% for nymphs and 70% for adults, respectively.

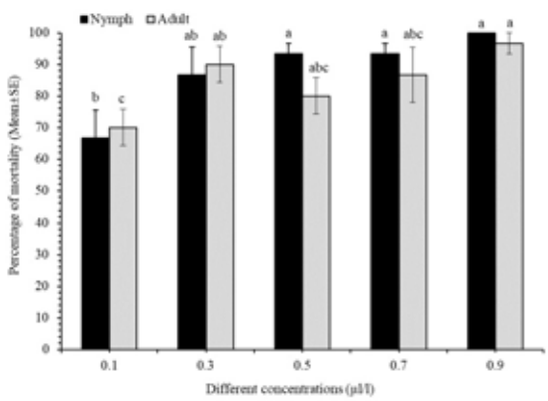

FIGURE 1
Percentage of mortality (±SE) of nymphal and adult stages of the melon aphid, *Aphis gossypii* in different essential oil concentrations of the Peppermint, *Mentha pipertia*. The similar letters indicate no significant difference.

Effect of Essential Oils on Nymph Production Deterrent. The effect of the Peppermint essential oil on nymph production and nymph production deterrent of the melon aphid, *A. gossypii* is shown in Table 3. The results of the analysis of variance showed that there is a significant difference between nymph production in 24, 48 and 72 hours (F= 13.61, df= 2, P<0.01). Also, with increasing time after treatment, the number of produced nymphs was decreased. Mean comparison of produced nymphs of aphid (Table 3) compared to the

control showed a significant difference.

Based on the analysis of variance (Table 3), there was a significant difference between treatments for the percentage of nymph production deterrent, at 24, 48 and 72 hours, respectively (F= 14.36, df= 2, P<0.01). Also, with increasing time after treatment, the percentage of deterrence was also increased. Mean comparison of the nymph production deterrent of the aphid (Table 3) compared to the control showed a significant difference. The degree of nymph production deterrent effect was calculated for the Peppermint, *M. pipertia* as 48.51 ± 5.51.

According to GC-Mass analysis in this study, it was found that most of the volatile compounds identified in plant essential oils are monoterpenes and have insecticidal properties, fumigant toxicity, repellency, and anti-feeding activity for insects. Consequently, monotropic compounds are suitable alternatives for chemical insecticides due to their insecticidal property and have the lowest risk for human health and the environment. Khan and Abourashed [17] reported that peppermint yields 0.1-1.0% of the volatile oil that is composed mainly of menthol (29-48%), menthone (20-31%), and menthyl acetate (3-10%). Studies by Tyagi and Malik [37] indicated that 18 monopropene compounds were identified in the peppermint oil. The most important of these compounds were α-pinene (17.3%), limonene (18.4%), β-pinene (13.9%), menthol (4.8%), myrcene (3.8%), iso-menthanol (1.8%), isopulegol (1.8%) and piperitone (2.1%). Based on GC/MS analysis of Tsai et al. [36], the major components of peppermint essential oil were menthol (30.35 %), menthone (21.12 %), and transcarane (10.99 %). Also, Taherpour et al. [33] showed that the main identified components (higher than 5%) by hydro distillation method were menthol (45.34%), menthone (16.04%), menthofuran (8.91%), and *cis*-carane (8.70%). The results obtained by the above-mentioned researchers are consistent with the results obtained in this study. In our study, nearly the same compounds were identified in different quantities. This observed difference in the chemical composition may be attributed to the occurrence of chemotypes, geographical locations, season at the time of collection, stage of development, culture climate, and other culture conditions, which may affect biological activities [29].

In this study, the fumigant toxicity effect of essential oils of the Peppermint on the melon aphid was different in relation to the life stage of the insect, and with increasing concentrations, mortality also increased. Previous experiments conducted on nymph and adult stages of aphids showed that adults are the most tolerant stage against plant essential oils. Due to the application of very low concentrations of the essential oil of the above plant, the mortality rate was found to be comparable to the concentrations used for the adult stage. Many studies have assessed the insecticidal activities of peppermints. Leaf extract of peppermint (*M. piperita*) has showed promising results against the cabbage aphid, *Brevicoryne brassicae* L. [24]. *Mentha piperita* essential oil was evaluated for larvicidal activity against 3rd instar larvae of different mosquito species including *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* [1]. Of the three-species tested *C. quinquefasciatus* was most susceptible followed by *A. aegypti* and *A. stephensi*. The oil showed strong repellent action against adult mosquitoes when applied on the human skin.

In addition, Hasanshahi et al. [10] studies showed that the peppermint essential oil in very low amounts on the black bean aphid, *Aphis fabae*, is very toxic, which is similar to the results of this study. In the study of Riazi et al. (2015), the 50% lethal concentration (LC_{50}) of the Spearmint, *Mentha spicata* L. essential oil on the different stages of 1st, 3rd nymphal instars and adults of the melon aphid, *A. gossypii* were 2.70, 3.41 and 5.24 µl. L^{-1} air. However, in the present study, the essential oil of the Peppermint plant in much lower amounts and with the lethal concentration (LC_{50}) on nymph and adult of the melon aphid, *A. gossypii* were 0.056 and 0.023 µl. L^{-1} air, respectively.

In a study by Mahmoudi [22] on the melon aphid, *A. gossypii*, the LC_{50} values of essential oil of parsley (*Petroselinum crispum* L.) and ajowan (*Carum copticum* L.) (Apiaceae) on the adult insects of aphid was calculated as 2.71 and 21.2 µl/l, respectively. Also, in the study of Mousavi [25], the fumigant toxicity of tarragon (*Artemisia dracunculus* L.) and dill (*Anethum graveolens* L.) plants on the adult insects of the melon aphid was investigated. Based on the results, the calculated LC_{50} values after 24 hours of essential oil exposure was 18.63 and 28.84 µl/l, respectively. Razmjou et al. [26] studies on the insecticidal activity of essential oil of two species of eucalyptus (*Eucalyptus microtheca* Muell.) and (*E. spathulata* Hook) on the cotton aphid, *A. gossypii* showed that the aphid had a high susceptibility to these essential oils, and the LC_{50} values were 366.12 and 15.952 µl/l, respectively. The results related to the LC_{50} value of studied essential oil in our study showed that oil toxicity effect of this essential oil on *A. gossypii* in low quantities. Jahan et al. [15] were studied insecticidal effects of *Artemisia dracunculus* L. and *Satureja isophylla* Rech against the cabbage aphid, *Brevicoryne brassicae* were concluded that *A. dracunculus* (LC_{50}=6.25 µL/L air) possesses the highest lethal activity whereas *S. isophylla* the lowest (LC_{50}=45.60 µL/L air). Insecticidal effect of essential oils of *Thymus carmanicus* Jalas and *Elettaria cardamomum* L. were tested against the 3rd nymphal instars of *B. brassicae* by Jahan et al. [16] and it was found that the highest toxic effect was recorded for *E. cardamomum.*

Hasanshahi et al. [9] were studied fumigant toxicity of five essential oils on *B. brassicae* and found that the highest toxicity was related to *Artemisia dracunculus* L. essential oil. LC_{50} value for *A. dracunculus* oil was calculated equal to 6.25 µL/L air in the laboratory conditions. Hori [11] was studied toxic activities of 10 essential oils against *Myzus persicae* Sulzer and it was found that spearmint, thyme, pennyrol, mint and peppermint oils have high activity. Effect of essential oils of *Thymus vulgaris*, *Veronica officinalis* L. and *Agrimonia eupatoria* L. on the cabbage aphid, *B. brassicae* showed that essential oil of *T. vulgaris* caused about 85% mortality in aphid population [8]. Based on the results obtained from the current research, it can be stated that studied essential oil had the good effect in very small quantities in comparison with other plant essential oils on the melon aphid, *A. gossypii*.

These results suggest that *M. piperita* oil has potential to be used for sustainable pest management in the greenhouse. It will purely safeguard the environment and health of the user especially when the application of synthetic insecticides give rise to the development of resistance and pollution of the environment.

CONCLUSION

The overall results showed that the Peppermint essential oil has high potential in controlling the melon aphid especially in protected areas such as greenhouses.

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A COMPARATIVE STUDY ON ANTIDIABETIC EFFECT OF BUFFALO AND CAMEL FERMENTED MILK IN INDUCED DIABETIC RATS

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ABSTRACT

Diabetes mellitus is a metabolic disorder in which the carbohydrate and lipid metabolism is improperly regulated by insulin. This study aimed to evaluate the therapeutic efficiency of substitution of buffalo milk with camel milk in the form of fermented milk with substitution ratios of 0, 25, 50, 75 and 100 %. With addition to study their effect on physiochemical, synersis, viscosity and sensorial attributes of fermented milk. The results showed that moisture, ash, pH and synersis increased. While, fat, protein and viscosity decreased with substitution ratios of buffalo with camel milk. Sensory evaluation revealed that stirred fermented milk till substitution ratios of buffalo with 50% camel milk was acceptable compared to other treatments.

Diabetes and hyperlipidemia were induced by the injection of alloxan (150mg/ kg body weight). Thirty male albino rats were divided into five groups of six rats each and treated as following: G1 was fed on normal basal diet (negative control), G2 diabetic rats (positive control), G3 diabetic rats fed with yogurt prepared from 100% camel milk, G4 diabetic rats fed with fermented milk prepared from 50% camel milk + 50% buffalo milk and G5 diabetic rats fed with fermented milk prepared from 100% buffalo milk. After eight weeks of feeding, results showed significant decrease $P < 0.05$ in levels of blood serum glucose as compared with diabetic rats. Data revealed significantly decrease $P < 0.05$ of TC, TG, LDL and VLDL as compared with diabetic rats. However, HDL-C was significantly increased $P< 0.05$ as compared with diabetic rats. GPT and GOT in treated rats were decreased significantly $P < 0.05$ as compared to the diabetic group. Fermented milk camel milk showed the significant highest efficiency in all parameters. These findings indicate that camel milk have a potential benefits in the treatment of diabetes and play a role in its management as well as reduces the risk of diabetic complications.

KEYWORDS:
Camel milk, Buffalo milk, Fermented milk, Alloxan, Hypoglycemia, Diabetic, Hypolipidemic, Liver function.

INTRODUCTION

Diabetes mellitus is one of the most prevalent and serious chronic diseases to humans in nearly all countries. Study of the diabetes is important in order to allocate community and health resources and encourage measures to counteract trends for increasing prevalence [1] [2]. In 2011, the disease affected 366 million people worldwide, of which 4.6 million lost their lives. The number is expected to have risen to 552 million by 2030 [3] [4]. The prevalence of diabetes mellitus is particularly higher in low- and middle-income countries, which constitutes 80% of the people living with diabetes [5]. Diabetes mellitus is a group of metabolic disorders characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. There are two major types of diabetes mellitus: type 1 diabetes mellitus or insulin dependent diabetes mellitus and type 2 diabetes mellitus or non-insulin dependent diabetes mellitus [6].

The use of camel milk as medicine has been known since ancient times. The milk has been shown to possess hypoallergenic [7] and antidiabetic properties [8]. In addition, Agrawal *et al.* [9] reported that camel milk has an adjuvant effect to insulin therapy in controlling diabetes due to the presence of high levels of insulin or insulin like protein.

Camel milk is used in hot and arid regions as an essential nutritional source, and its high energy and vitamin contents are known to help immune-deficient patients as well as those recovering from diseases [10] [11]. Oral camel milk is well tolerated by lactase-deficient children who are allergic to cow milk [12], and it shows protective effects against heavy metal toxicity [13] and viral and bacterial infections [14]. Additionally, Indians used camel milk for the treatment of multiple acute and chronic health problems, including asthma, anemia, jaundice, and spleen problems [8].

Camel's milk is pure white as the fats are finely homogenized throughout the milk. Camel's milk chemical composition is different from that of other mammals. Camel milk has high percentage of water content, which ranges from 86-91% [15], and it is inversely proportioned to the availability of drink-

ing water to camels. This makes camel milk a valuable source of water for suckling young camels and the camel herdsmen who are normally live in scarce water areas. Fresh camel milk has a pH of 6.4±0.18 [16]. This pH of the milk allows enhanced absorption of milk constituents from the duodenum, especially the iron. When camel milk is left to stand, the acidity rapidly increases [17]. The lactic acid content increases from 0.03 percent after standing 2 hours to 0.14 percent after 6 hours [17]. Camel milk contains high concentration of insulin i.e. 52 U/L [18] to 59 U/L [19]. The milk also contains one protein that possesses many characteristics similar to human insulin [20]. Camel milk is not affected by acidic environment and does not form coagulum in acidic environment such of the stomach [21]. This lack of coagulum formation allows the camel milk to pass rapidly through stomach together with the specific like protein/insulin and remains available for absorption in intestine. It was proposed that, this unique property of camel milk gives it the advantage to serve as a vehicle and a protector that facilitates the absorption of intact molecules of insulin by the small intestine [22]. Camel milk contains little fat, in average about 2%; and this fat consists mainly of polyunsaturated fatty acids that are completely homogenized and gives the milk its smooth white appearance [17]. Camel milk has high concentrations of volatile acids especially the essential fatty acid, linoleic acid and other polyunsaturated fatty acids, which are essential for human nutrition [23]. Fats in camel milk dispersed as small micelles instead of a layer that are non-reactive to acid [24]. Camel milk is low in cholesterol 40-folds lesser than cholesterol concentration in caw milk [17]. Camel milk has high content of ascorbic acid of 5.7-9.8%, which is 3 times greater than of other mammalian milks [25]. Camel milk is also rich in other vitamins such as B12, E, B1, B2 and A. Camel milk has high concentrations of minerals such as calcium, iron, magnesium, cupper, manganese, sodium, phosphorus, zinc and potassium [17]. Lactose in camel milk, presents in average concentrations of 4.8%, but this milk sugar is easily metabolized by persons suffering from lactose intolerance [17]. The proteins of camel milk do not cause food allergies, because camel milk contains no beta-lactoglobulin [26] and a different beta-casein [20], the two proteins in cow milk that are responsible for allergies. Immunoglobulins IgM, IgG, IgA and IgD have been detected in camel sera on the basis of cross-reactivity with human immunoglobulins [27].

Therefore, the present study was carried out to evaluate the therapeutic efficiency of stirred yogurt camel milk on alloxan-induced diabetic rats.

MATERIAL AND METHODS

Material. Camel milk samples. Daily milk samples were collected early in the morning from camel farm in Bilbis desert area (Sharkia governorate). The samples were collected in sterile screw bottles and kept in cool boxes until transported to the laboratory.

Buffalo milk. Fresh buffalo's milk used in this study was obtained from the heard of Faculty of Agriculture, Cairo University, Egypt.

Starter culture. Yoghurt starter culture consists of *Streptococcus Salvarius sub sp. thermophilus* and *Lactobacillus delbreuckii sub sp. bulgaricus* were obtained from Cairo MIRCEN culture collection center, Faculty of Agriculture, Ain Shams University.

Chemicals. Alloxan was purchased from Sigma Chemical Company (St Louis Mo, USA). All of kits were purchased from Biodiagnostic, Dokki, Giza, Egypt. All other chemicals were of analytical grade.

Manufacture of fermented milk. The buffalo and camel milk were heated to 90 °C for 10 min, then they were divided into 5 portions, (T1: 100% buffalo milk; T2 : 75% buffalo milk + 25 % camel milk; T3: 50% buffalo milk + 50% camel milk; T4: 25% buffalo milk + 75 % camel milk; T5: 100 % camel milk) and cooled to (42 °C). Then 3% starter culture (*streptococcus thermopllius and lactobacilli bulguricus* (1:1)) was added and the mixtures were incubated at 42 °C until the gel structure was formed. The gel was stirred and stored at refrigerator (5 ± 2 °C).

Experimental animals. A total of thirty male albino rats weighing (100-115 g) were obtained from El- Salam Farm Giza, Egypt and used in this study.

Methods. Physicochemical analysis. Stirred yoghurt samples were analyzed for moisture, total protein, fat, lactose, and ash content were determined according to AOAC [28]. The pH values of cheese samples were measured using electric pH meter (HANNA instrument pH 213 microprocessor). While, viscosity of stirred yoghurt samples were determined according to Petersen *et al.* [29] using a Brookfield viscometer (Brookfield DVIII Ultra Programmable Rheometer equipped with a spindle No.

Viscosity. Viscosity of different stirred yoghurt fortified with PPE was measured using Brookfield Viscometer (Brookfield Engineering Laboratories, USA), equipped with SC4-21 spindle

running at 20, 30, 50, 60 and 100. Measurements were taken at room temperature.

Syneresis indexes. The syneresis indexes were determined by centrifugation method. The samples (15 g) were centrifuged at 1500 rpm in a refrigerated centrifuge (5 ± 1 °C) (Jaetzki K24, Jena, Germany) for 10 min. The supernatant was collected and weighed, and the syneresis index was calculated through Eq.

$$\text{Syneresis}(\%) = \frac{\text{Supernatant(g)}}{\text{Fermented milk(g)}} \times 100$$

Sensory evaluation. Sensory evaluation of stirred yoghurt was evaluated by a 10 trained panelists from the staff members of the Dairy Department of food technology research institute Egypt according to the following scores: appearance (10) points, body and texture (40) points and flavour (50) points. Results were expressed as mean value of judge's scores as described by Nelson and Trout [30].

Induction of diabetes. The animals were fast overnight, and received a single intraperitoneal injection of freshly prepared alloxan using citrate buffer 0.1M (pH = 4.5) as vehicle, at a dose of 150 mg alloxan/kg body weight [31]. Control rats were injected with citrate buffer alone. The animals were considered as diabetic, if their blood glucose values were above 240 mg/dl on third day after alloxan injection.

Experimental design. Thirty male albino rats were housed in stainless steel cages under standard conditions of humidity, temperature and light (12 h light/12 h dark) and give free access to food and water at all time. After one week of acclimatization, animals were divided randomly into five groups of six rats in each group.

The rats were fed a standard diet according to Reeves *et al.*, [32]. It contained 15% casein, 5% cellulose, 4% salt mixture, 1% vitamin mixture, 0.25% choline chloride, 10% corn oil and 65% starch. Different diabetic rats groups (G3, G4 and G5) were given different prepared yogurt samples orally at a dose of 10 ml /rat/day every day for eight weeks [33]. The groups were treated as follows:

Group (G1) control rats (negative control group).
Group (G2) diabetic rats (positive control group).
Group (G3) diabetic rats fed with yogurt prepared from 100% camel milk.
Group (G4) diabetic rats fed with yogurt prepared from 50% camel milk + 50% buffalo milk.
Group (G5) diabetic rats fed with yogurt prepared from 100% buffalo milk.

Biochemical analysis. Blood samples were taken at the start of the experiment, after two weeks and at the end of the experimental period. The blood samples were obtained from orbital plexus venous by means of fine heparinized capillary glass tubes. Each sample was placed into a dry clean centrifuge tubes and centrifuged at 3000 rpm for 10 min to separate blood serum, which subjected to the biochemical analyses. Samples were analyzed for the following biochemical parameter: Blood glucose [34]. Lipids profile including total cholesterol (TC) [35], triglycerides (TG) [36] and high-density lipoprotein (HDL-C) [37]. Calculation of LDL-C and V LDL-C [38]. Liver functions including GPT and GOT [39].

Statistical analysis. Data were analyzed by Analysis of Variance using General Liner Model (GLM) procedure according to the procedure reported by Snedecor and Cochran [40]. Means were separated using Duncan's test at a degree of significance ($P \leq 0.05$). Statistical analyses were made using the producer of the SAS software system program [41].

RESULTS AND DISCUSSION

Physiochemical of fermented milk. Data presented in Table 1 shows the physicochemical composition of fermented milk made from camel and Buffalo's milk in fresh. It was noticed that an increasing in moisture and synersis values, while increasing the substitution ratio of Buffalo's milk

TABLE 1
Physicochemical composition of fermented milk made from camel and Buffalo's milk in fresh

Treatments	Moisture	Fat	protein	pH	Ash	Synersis
T1	84.25	6.30	4.40	4.40	0.87	6.92
T2	85.42	6.00	4.02	4.47	0.92	9.49
T3	85.94	4.50	3.90	4.51	0.95	10.09
T4	86.59	4.10	3.60	4.63	0.99	11.15
T5	87.94	3.00	3.40	4.79	1.08	15.38

Treatment(1): Fermented milk made from Buffalo's milk (100%)
Treatment(2): Fermented milk made from camel milk and Buffalo's milk (25 % : 75%)
Treatment(3): Fermented milk made from camel milk and Buffalo's milk (50 % : 50%)
Treatment(4): Fermented milk made from camel milk and Buffalo's milk (75%: 25%)
Treatment(5): Fermented milk made from camel milk (100%)

with 0, 25, 50, 75 and 100% camel's milk leads to protein and fat contents were decreased. Substitution with camel milk is known to decrease the curd formation leading to increasing in moisture and synersis, indicating a weaker water holding capacity [42]. Presence antibacterial activity of camel milk led to render lactic acid bacterial growth which cause of acid production in fermented milk [43].

Viscosity. Viscosity defined as the internal resistance of a substance to flow when a shear stress is applied. Viscosity behavior is influenced by the complex hydrodynamic properties (*i.e.,* size, shape, and hydration potential) and independent on the shear rate and time, while, resistance to flow is caused essentially by molecular or ionic cohesion. The viscosity of a fluid may increase in a linear or non linear fashion with a transition from Newtonian to non newtonian behavior as the total solids concentration is increased. Data in Fig 1 revealed that viscosity was affected by substitution of buffalo's milk with camel's milk (0, 25,50,75 and 1 nnnn00 %),which led to a decrease in viscosity. This could be due to the higher protein, fat and total solids content of buffalo's milk [44] [45] [46] [47].

Sensory evaluation. Table 2 shows the sensory evaluation of fermented milk made from camel and Buffalo's milk in fresh, data revealed that there was no significance difference with the substitution ratio of buffalo milk with camel milk until 50% substitution ratio and the product was acceptable, however increasing the substation ratios above 50% leads to lower appearance, flavor, body and texture and finally the total score values. fermented dairy product made with camel milk can be marketed if the product rheology can be improved .Among the reasons that camel milk alone cannot be used for production of dairy products is the abrupt disappearance of the casein micelle state in addition to the higher concentrations of the antimicrobial components lysozyme, lactoferrin, and immune-globulins in camel milk than buffalo milk [43].

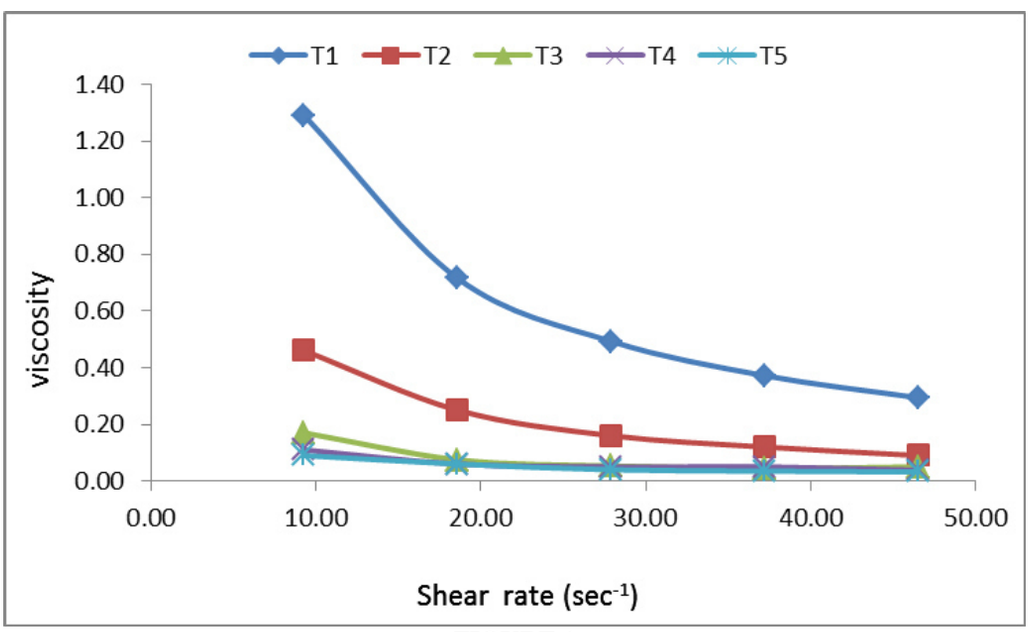

FIGURE 1
Show the effect of substitution of buffalo's milk with camel's milk with ratios of (0, 25, 50, 75 and 100%) on viscosity of stirred yoghurt

TABLE 2
Sensory evaluation of fermented milk made from camel and Buffalo's milk in fresh.

Treatments	Appearance 10	Flavor 50	Body and texture (40)	Total
T1	10	49	39	98[a]
T2	9.8	48.7	38.8	97.3[a]
T3	9.6	48.5	38.4	96.5[a]
T4	8	44	35	87[C]
T5	7.5	40	32	79.5[d]

* Means followed by different letters in the same column are significantly different by Duncan's multiple test ($p<0.05$).
Treatment(1):Fermented milk made from Buffalo's milk (100%)
Treatment(2):Fermented milk made from camel milk and Buffalo's milk (25 % : 75%)
Treatment(3):Fermented milk made from camel milk and Buffalo's milk (50 % : 50%)
Treatment(4):Fermented milk made from camel milk and Buffalo's milk (75%: 25%)
Treatment(5):Fermented milk made from camel milk (100%)

TABLE 3
Effect of feeding camel milk of different treatments on blood glucose level (mg/dl) of diabetic rats

Experimental Groups	Serum glucose (mg/dl)		
	Initial period	After injection with alloxan	Final period
G1	**90.10±0.88^{a}**	**93.26±0.62^{b}**	**98.30±0.43^{c}**
G2	**91.03±0.58^{a}**	**321.12±0.48^{a}**	**361.33±0.62^{a}**
G3	**90.23±0.33^{a}**	**315.36±0.50^{a}**	**117.45±0.67^{c}**
G4	**89.96±0.19^{a}**	**322.11±0.33^{a}**	**136.03±0.22^{c}**
G5	**91.30±0.45^{a}**	**319.33±0.17^{a}**	**224.58±0.84^{b}**

* Means followed by different letters in the same column are significantly different by Duncan's multiple test (p<0.05).
(G1) control rats (negative control group).
(G2) diabetic rats (positive control group).
(G3) diabetic rats fed with yogurt prepared from 100% camel milk.
(G4) diabetic rats fed with yogurt prepared from 50% camel milk + 50% buffalo milk.
(G5) diabetic rats fed with yogurt prepared from 100% buffalo milk.

TABLE 4
Effect of feeding camel milk of different treatments on lipids profile (TG, TC, HDL-C, LDL-C and VLDL-C) of diabetic rats mg/dl

Experimental Groups	Triglycerides (mg/dl)	Total cholesterol (mg/dl)	HDL- cholesterol (mg/dl)	LDL-cholesterol (mg/dl)	VLDL- cholesterol (mg/dl)
G1	82.43±1.15^{c}	77.28±1.32^{c}	39.21±0.33^{a}	15.14±0.27^{d}	16.49±0.28^{c}
G2	130.80±0.58^{a}	100.49±1.63^{a}	26.74±0.71^{c}	47.58±1.21^{a}	26.16±0.33^{a}
G3	84.82±0.37^{c}	78.00±1.42^{c}	39.09±0.82^{a}	22.34±0.58^{c}	16.96±1.02^{c}
G4	107.44±0.84^{b}	84.43±0.88^{b}	36.77±0.44^{b}	26.19±0.76^{c}	21.49±0.17^{bc}
G5	115.13±0.29^{b}	86.38±1.22^{b}	35.30±0.60^{b}	34.11±0.45^{b}	23.03±0.58^{b}

* Means followed by different letters in the same column are significantly different by Duncan's multiple test (p<0.05).
(G1) control rats (negative control group).
(G2) diabetic rats (positive control group).
(G3) diabetic rats fed with yogurt prepared from 100% camel milk.
(G4) diabetic rats fed with yogurt prepared from 50% camel milk + 50% buffalo milk.
(G5) diabetic rats fed with yogurt prepared from 100% buffalo milk.

Blood glucose level. According to the sensory evaluation, selected fermented milk samples were biologically evaluated as present in Table 3. From the obtained data, it could be observed that, in the blood serum glucose levels, there were non-significant difference between all groups in the beginning of the experimental; the initial level of glucose in blood serum recorded in average (89.96 – 91.30) mg/dl blood serum. While, after injection with alloxan a significant increase (P <0.05), in the level of glucose of diabetic groups (positive control group) compared to negative control group (93.26mg/dl). This increase can be explained by that the single dose of alloxan injected to rats was able to produce a reproducible model of diabetes mellitus that had minimal beta cell activity and elevated glucose. Similar results obtained by Akpan, [48] and Radhika *et al*., [49] who demonstrated that alloxan administration was associated with hyperglycemia.

After eight weeks of administration of selected fermented milk samples (G3: prepared from 100% camel milk, G4: prepared from 50% camel milk + 50% buffalo milk and G5: prepared from 100% buffalo milk) the level of glucose in blood serum were decreased. Furthermore, the highest reduction in glucose level was in G3 (diabetic rats fed with yogurt prepared from 100% camel milk) followed by G4 (diabetic rats fed with yogurt prepared from 50% camel milk + 50% buffalo milk). While, the glucose level remained high in G5 (diabetic rats fed with yogurt prepared from 100% buffalo milk) compared to the negative control group (G1).

Results were in agreement with Singh, [50] who reported that the camel milk contains a high concentration (52 units/liter) of insulin. It should be noted that camel milk does not form coagulum in the stomach or the acidic media, thereby it prevents degradation of insulin in the stomach [21]. Amino acid sequences of some camel milk proteins are rich in half- cystine, which has superficial similarity with insulin family of peptides [20]. High mineral content (sodium, potassium, zinc, copper and magnesium) as well as a high vitamin C intake may act as antioxidant thereby removing free radicals [51]. All these factors may contribute to the observed hypoglycemic effect of camel milk in the present study.

Lipid profile. The results in Table 4 showed that a significant increase P <0.05 in total cholesterol (TC), total triacylglycerol (TG), low density lipoprotein (LDL-C) and very low density lipoprotein (VLDL-C) of diabetic group compared to negative control group. While, HDL-C decreased significantly (P <0.05) as compared to negative control group. A significant increase in TG (G2) may be due to the lack of insulin under diabetic condition.

TABLE 5
Effect of feeding camel milk of different treatments on liver functions (GPT and GOT) of diabetic rats

Experimental Groups	Glutamate pyruvate aminotransferase (GPT) (IU/L)	Glutamate oxaloacetate aminotransferase (GOT) (IU/L)
G1	12.41±1.20[b]	37.00±0.98[b]
G2	80.95±0.88[a]	92.01±0.54[a]
G3	12.50±0.65[b]	35.50±0.75[b]
G4	12.33±0.48[b]	39.67±0.91[b]
G5	10.75±0.88[b]	44.17±0.45[b]

* Means followed by different letters in the same column are significantly different by Duncan's multiple test ($p<0.05$).
(G1) control rats (negative control group).
(G2) diabetic rats (positive control group).
(G3) diabetic rats fed with yogurt prepared from 100% camel milk.
(G4) diabetic rats fed with yogurt prepared from 50% camel milk + 50% buffalo milk.
(G5) diabetic rats fed with yogurt prepared from 100% buffalo milk.

This result agreed with that of Arkkila *et al*., [52] who found that the abnormalities in the lipid metabolism may be due to insulin deficiency. While, the groups fed with selected fermented milk samples (G3: prepared from 100% camel milk, G4: prepared from 50% camel milk + 50% buffalo milk and G5: prepared from 100% buffalo milk) decreased significantly ($p < 0.05$), the levels of (TC), (TG), (LDL-C) and (VLDL-C) as compared with diabetic group and this was associated with a significant increase ($p < 0.05$) in HDL-C in these groups. These results are supported with those of Hull, [53] and Agrawal *et al*., [54] who show that a high insulin concentration of camel milk can cause the activation of lipoprotein lipase enzyme. Also, the above mentioned results were agreed with Makni *et al.,* [55] who stated that the increase in HDL-C ratio is one of the most important criteria of anti-hypercholesterolemic agent.

Liver function. A comparison of the liver functions parameters data for the negative control group (control rats), positive control group (diabetic rats), diabetic rats fed with yogurt prepared from 100% camel milk, diabetic rats fed with fermented milk prepared from 50% camel milk + 50% buffalo milk and diabetic rats fed with yogurt prepared from 100% buffalo milk administration is shown in Table 5. A significant increase ($p<0.05$) in the levels of liver enzymes (GPT and GOT) appeared in diabetic rats. These results are accordance with data reported by Sunil *et al*., [56] who indicated that the liver enzymes GPT and GOT levels were increased in alloxan diabetic rats. This elevation reflected the generally recognized detrimental effect of hepatocyte damage, which represented in the leakage of GPT and GOT from damaged hepatic cells. The results showed that a significant ($p <0.05$) overall improvements in liver functions parameters appeared within diabetic rat groups feed on selected yogurt samples (G3: prepared from 100% camel milk, G4: prepared from 50% camel milk + 50% buffalo milk and G5: prepared from 100% buffalo milk), with a particular respect to the highest refinement effect in yogurt camel milk group (G3). Accordingly, it is interested to note that giving fermented milk from camel milk led to an improvements in both GPT and GOT activities, respectively, as compared to diabetic rats. This finding is consistent with the observation of Magjeed, [57] and Khan and Alzohairy, [58] who found that giving camel milk improved the levels of GPT and GOT activities in intoxicated rats.

CONCLUSION

This study has demonstrated the therapeutic efficiency of camel milk for diabetic rats. From results it could be concluded that camel milk with different treatments possesses anti-diabetic, hepato-renal protective and hypolipidemic effect in alloxan-induced diabetic rats. These results may have important implication for the clinical management of diabetes mellitus in humans.

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AUTHOR INDEX

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