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EFFECT OF SUPPLEMENTING YOGURT WITH ESSENTIAL OIL OF LEMON LEAVES ON PHYSICOCHEMICAL AND RHEOLOGICAL PROPERTIES

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ABSTRACT

Citrus limon is a flowering plant belonging to the Rutaceae family. Citrus fruits constitute one of the main valuable sources of essential oil used in foods and medicinal purposes. This study was designed to investigate the effect of adding the essential oil of lemon leaves in yogurt on its rheological and physicochemical parameters in comparison with potassium sorbate. Five batches of yogurt were prepared with different concentrations of the essential oil (0, 1250, 2500, 3750 µg/ml), one batch was dedicated to potassium sorbate (0.1%). Essential oil of lemon leaves was analyzed by gas chromatography/mass spectrometry (GC/MS), yogurts formulated were analysed by the rheometer. The GC/MS analysis allowed the identification of 27 compounds accounted for total percentage of 99.76%. The two dominant compounds were Limonene (57.96%) and β-pinene (16.29%). The essential oil exhibited an excellent DPPH scavenging activity with a half maximal inhibitory concentration an (IC50) of 2,41± 0,04 µg/ml comparable to that of ascorbic acid with an IC50 of 5,87± 0,75 µg/ml. pH values of yogurts with essential oil were significantly the highest after those of yogurts with potassium sorbate ($p<0.05$), which improves the acidity stability of yogurts during storage.The thixotropic index recorded for yogurts with essential oil was the greatest (3789 Pa/s for the highest concentration), while that with potassium sorbate was the lowest (2280 Pa/s) compared with the control (3329 Pa/s). The viscoelastic behaviour of the control yogurt and the essential oil yogurts were almost the same, the addition of essential oil did affect significantly the thixotropic behaviour of yogurts ($p<0.05$).

1.Introduction

Yogurt, is one of the most consumed dairy products by all age groups, rich in calcium, proteins, lipids, vitamins and minerals, it confers various health benefits along with pleasant taste (Tamime and Robinson, 2007). It is often used for weight management, lactose intolerance, immunological effects, gastro-intestinal or respiratory tract diseases and dental health (Das et *al*., 2019).

The contamination of yogurt and other dairy products with undesirable microorganisms, especially yeasts and moulds, during shelf life is a serious problem (Tamime and Robinson, 1985; Plockova et al*.,* 1997) . Some methods are used in order to increase the shelf life of yogurt. These strategies include addition of gas, restriction of oxygen, cold storage, heat treatment and the use of antimicrobial agents (Karagül-Yüceer et *al*., 2001). Food preservatives are specific additives used to prevent deterioration from enzymes, microorganisms, and exposure to oxygen. All chemical preservatives must be nontoxic and readily soluble, not impart off-flavors, exhibit antimicrobial properties over the pH range of the food, and be economical and practical (Mac Donald and Reitmeier, 2017). Sorbic acid and its potassium salt are commonly used as preservatives for food (Akbari-adergani et *al.,* 2013). In the code of federal regulation under the provision set forth by the FDA (US Food and Drug Administration) on potassium sorbate in 1999 is declared that if food additives are generally recognised as safe and declared on the label, they can be used in foods (JECFA, 1973) . Potassium sorbate prevents microbial growth and spoilage by inhibiting mould and yeast, slows changes in color, texture, and flavor (Mac Donald and Reitmeier, 2017).

During the last years, consumers are more aware and lean more and more towards a healthy and organic food, the trend in the market for functional foods or nutraceuticals has increased, particularly products that include in their formulation functional and natural food additives like essential oils, vitamins, minerals, carotenoids, flavonoids, anthocyanins, etc. (Torres-Giner et *al*., 2010). Essential oils are aromatic compounds found in great quantities in oil sacs or oil glands present at different depths in the fruit peel, mainly flavedo part and cuticles (Mahato et *al*., 2019).

Citrus are the most important crops in the world in terms of production according to the Food and Agricultural Organisation (FAO). The essential oil of lemon has previously been reported to possess antibacterial, antioxidant and fungicidal properties and is on the « Generally Recognized As Safe» list fully approved by the Food and Drug Administration FDA (2018) (Yazgan et *al*., 2019). Thus, it is applied in food industries as a preservative or flavoring agent (Sharma and Tripathi, 2008). After extracting the juice from citrus fruits, residues from different parts as the peels, leaves and flowers constitute a very important source of EO (Viuda-Martos et *al*., 2009).

Food rheology is the study of deformation and flow of food materials. Milk gels are visco elastic, thus yogurt's rheological properties can be characterized using both the viscous and elastic components. Visco-elastic indicates that the material has some of the elastic properties of an ideal solid and some of the flow properties of an ideal (viscous) liquid (Lee and Lucey, 2010).

According to the literature, there is no study on rheology of yogurt supplemented with lemon leaves essential oil. For this, the objective of this work is to formulate a naturally flavoured yogurt by essential oil of Algerian lemon leaves and investigate the effect of this supplementation on the acidity stability and the rheological behaviour of the yogurt in comparison with synthetic food preservative.

2. Materials and methods

2.1. Materials

In this study, we used whole milk powder (28% fat, protein 34.0%, carbohydrate 53.2%). The essential oil of lemon leaves was purchased from a local producer and supplier. Yogurt culture, *Lactobacillus delbrueckii subsp bulgaricus* and *Streptococcus thermophilus* obtained from Chr. Hansen Denmark was used as starter culture for yogurt preparation.

2.2. Yogurt preparation

The yogurt was prepared according to the method of Mihoubi et *al*. (2017). Aliquots of 13.7 g of whole milk powder and 12.5 g of sugar were dissolved into 100 mL of distilled water by stirring for 5min. After that, the reconstituted milk was heated to 95°C for 5min in a thermostatically controlled boiling water bath followed by cooling to the incubation temperature (42 to 43°C) and inoculated with 0.03g of yogurt culture (*Lactobacillus delbrueckii subsp bulgaricus* and *Streptococcus thermophilus*). After stirring, the essential oil of lemon leaves was added at different concentrations: (YEO1) 1250µg/ml, (YEO2) 2500µg/ml, (YEO3) 3750µg/ml. In order to be able to study the stability of acidity of yogurts with EO we have studied in parallel the effect of a synthetic preservative, for this, another trial of yogurt with potassium sorbate at a rate of 0.1 g / 100 g (YPS) was prepared (Codex Alimentarius, 2019). YC corresponds to the sample control. The different yogurt samples were then incubated at 45°C until pH 4.6 (within 5h). After fermentation, yogurts were transferred to a refrigerator at 4 °C overnight to reduce post-acidification. After stabilization, each yogurt sample was stored for 0, 1,7, 14, 21 and 28 days at 4°C in a refrigerator to evaluate the physicochemical characteristics. Each batch of yogurt was prepared in triplicate.

2.3. Physicochemical analysis

2.3.1. Gas Chromatography - Mass Spectrometry Analysis of the essential oil

The chemical composition of the EOLL was investigated by a gas chromatograph (Hewlett–Packard Co, Model 6890) coupled with a mass spectrophotometer (Hewlett–Packard Co, Model 5973, Palo Alto, CA, USA), with HP-5Ms capillary column (30.0m×0.25mm×0.25 µm). The carrier gas, the Helium, was used at 1.2 ml/min flow rate. The initial temperature was 60 ºC for 8 minutes that subsequently elevated to 200 ºC at a rate of 2ºC/min. The chromatograph was equipped with a split/split less injector used in the split mode, and 2 µL of essential oil was always injected. The compounds of essential oils were identified on the basis of GC-MS retention times, their Kovats indices (calculated using a homologous series of C10-C22 n-alkanes injected at the same conditions), and mass spectra (authentic chemicals and NIST 05 spectral library collection).

2.3.2. Determination of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The free radical-scavenging activity of EOLL was measured using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay according to the method of Kirby and Schmidt with some modifications (Kirby and Schmidt, 1997). Briefly, 500 µl of sample solutions (various concentrations of the EOLL) were mixed with 1ml DPPH solution (4% (w/v) in methanol) (Sigma-Aldrich, Germany). The mixture was mixed and incubated at room temperature for 30 min in the dark. Scavenging capacity was read spectrophotometrically by monitoring the decrease of the absorbance at 517 nm. DPPH solution was served as negative control and ascorbic acid was used as a positive control. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. DPPH radical scavenging activity (%), expressed as percentage inhibition of DPPH, was calculated according to the formula:

$$\mathbf{DPPH\ RSA}(\%) = [(\mathbf{A0} - \mathbf{At})/\mathbf{A0}] \times \mathbf{100} \quad (1)$$

Where:
RSA: radical scavenging activity
A0: the absorbance of the control reaction (blank) containing all reagents except the tested compound,
At: the absorbance value of the tested sample.
Extract concentration providing 50% inhibition (IC50) was calculated from the graph plotting inhibition percentage against extract concentration. Tests were carried out in triplicate, and the results are expressed as the mean ± standard deviation.

2.3.3. pH and Titratable Acidity

The pH values of yogurt samples were measured using a digital pH meter (Nick, 776, Jena, Germany). The titratable acidity of yogurts was determined according to IDF standard (IDF/ISO/AOAC). According to which, 10g of yogurt with a few drops of phenolphthalein were titrated with a solution of NaOH (0.1N). It is expressed in grams of lactic acid per 100 g of product and is defined by the following equation:

$$\mathbf{At} = \frac{\mathbf{Vx0.9}}{\mathbf{M}} \quad (2)$$

Where:
At: titratable acidity
V: volume (ml) of 0.1 M sodium hydroxide;
M: mass (g) of the sample;
0.9: conversion factor of the lactic acid.
All samples were measured in triplicate.

The results are expressed in degree Dornic (°D).

2.4. Rheological measurements

The rheology tests were carried out using a constrained rotary rheometer (AR2000, TA Instruments). It is fitted with a coaxial cylinder module (standard size DIN) with a cylindrical rotor: height of the immersion cylinder (42 mm), diameter of the external cylinder (14 mm), diameter of the internal cylinder (15 mm).

A fixed protocol, established during previous work on yogurt (Koskoy and Kilic, 2004), was applied for sample preparation.

Yogurts were stored before analysis in a refrigerator at 4°C for15 days (Cayot et *al*., 2003). In order to homogenize the content and eliminate the possible presence of serum on the surface, each pot of yogurt was gently mixed three times from bottom to top using a small spoon, while turning the pot from left to right. The temperature of the samples is adjusted to 10°C and is kept constant throughout the analysis. The yogurt sample (10 ml) is placed in the space between the inner cylinder and the outer cylinder. The samples remained in the system for 10 to 15 min until the temperature stabilized (10° C).

The rheological measurements are carried out by stress scanning and under harmonic conditions. A ramp of shear stresses between 0.1 Pa and 100Pa, with a frequency of 1 Hz was exerted. The stress distribution was logarithmic, with a step of 100.6 Pa (50 points). A controlled shear speed test was also performed to determine the curves and the type of flow of the four yogurts.

Thixotropic index refers to the area between the upward and downward shear stress curves. The Herschell- Bulkley model was used for fitting data (Karsheva et *al*., 2013):

$$\sigma=\sigma_0+k\dot{\gamma}^n \quad (3)$$

Where:

σ is the shear stress (Pa),

σ_0 is the yieldstress (Pa),

k is the consistency index (Pa.sn),

γ is the shear rate (s−1), and n is the flow index (dimensionless).

The apparent viscosity, η, was described as the ratio of shear stress, σ, to shear rate, γ.

The speed was varied linearly from 0 to 300 1/s for 5 min (increasing curve), then in the opposite direction from 300 to 0 1/s (decreasing curve) for 5 min. The results of the shear stress and viscosity are measured every 6 s. The viscosity in the flow curve corresponding to the shear rate of 50 1/s is taken as the apparent viscosity of the yogurt. This value is taken as the viscosity in the mouth: the value of the shear speed in the mouth is approximately 50 1/s (Bourne, 2002).

Dynamic oscillation tests were carried out following the evaluation of the flow behavior to characterize the viscoelastic properties of the yogurt.

The stress scans were increasing from 0.1 to 15 Pa to determine the linear viscoelastic range (LVR) of the yogurt samples at a constant frequency of 1 Hz (6.28 rad / s). Frequency sweeps of 0.05 to 100 Hz were then caused in the LVR with a constant shear stress of 1 Pa to obtain the storage module G', the loss module G" and the angle Delta δ (Delta degrees= Tanδ), which represents the ratio of G" to G'. When the material is more like a solid, the G' dominates and therefore Tanδ becomes <1.0. The analysis was repeated in triplicate.

2.5. Statistical analysis

The physiochemical and rheological properties were expressed as mean ± standard deviation (SD) of three replicates using SPSS (version 25.0, SPSS Inc.). Analysis of variance test and Duncan's multiple range tests were used to examine the significant difference. A *P-value* of less than 0.05 was considered as highly significant.

3.Results and discussions

3.1. Chemical composition of the essential oil

The components of EO are important, as their qualitative and quantitative composition determines the characteristics of the oils and subsequent effect on its biological activities.

The analysis by (GC /MS) of EOLL identified 27 compounds accounted for total percentage of 99.76%. The identified components, with their relative percentages and the retention time, are given in Table 1.

Table 1. Chemical composition of Lemon leaf EO analyzed by GC/MS.

N°	**Rt[a] (min)**	**KI[b]**	**Components**	**%**
1	10.35	939	α-Pinene	4.773
2	11.22	953	Camphene	0.020
3	13.19	994	β-Pinene	16.29
4	14.12	999	Mycrene	0.405
5	17.52	1038	Limonene	57.96
6	18.29	1051	β-Ocimene	0.030
7	18.97	1062	γ-terpinene	0.511
8	21.97	1075	Cis-sabinene hydrate	0.016
9	22.26	1088	Terpinolene	0.054
10	23.70	1103	Linalool	0.070
11	24.21	1184	Terpinen-4-ol	0.086
12	25.77	1162	Citronellal	1.027
13	31.85	1223	(Z)-carveol	0.036
14	32.10	1248	Cis Citral	0.152
15	34.22	1272	Neral	0.252
16	40.40	1362	Neryl acetate	0.478
17	41.64	1381	Geranyl acetate	0.232
18	43.42	1399	(E)-β-Farnesene	0.030
19	43.81	1410	(Z)-α-Bergamotene	0.291
20	46.15	1415	β-Caryophyllene	0.067
21	47.78	1481	(Z)-β-Farnesene	0.020
22	48.30	1490	Viridiforene	0.025
23	48.98	1498	(Z)-α-Bisabolene	0.024
24	49.35	1504	β-Bisabolene	0.375
25	53.68	1575	(−)-Caryophyllene oxide	0.175
26	55.95	1642	δ-Cadinene	0.019
27	75.29	1685	α-Bisabolol	0.032
			Monoterpenes	83.068
			Sesquiterpenes	2.156
			Aldehydes	7.463
			Alcohols	3.192
			Ester	3.710
			Others	0.175
			Total	99,764

[a]*Rt: retention time*
[b]*KI :Kovats Indices on HP-5MS Capillary Column in reference to C10-C22 n-alkanes injected in the same conditions*

The volatile compounds of EOLL are classified into 6 groups including monoterpenes, sesquiterpenes, alcohols, aldehydes, esters and others. Monoterpenes are the major components, accounting for 83.06% of the total oil. Limonene (57.96%) is the predominant component of monoterpenes, followed by β-pinene (16.29%), and α-pinene (4.77%). Aldehydes are the second group with Neral (6.25%) and citronellal (1.02%) as principal components. In addition, two esters: neryl acetate (1.47 %) and geranyl acetate (2.23%) were found in EOLL. The sesquiterpenes (2.15%) included β-caryophyllene (1.36%), β-bisabolene (0.38%) and (Z)-α-bergamotene (0.29%). the only sesquiterpene oxide detected was caryophyllene oxide (0.17%). The main alcohol detected was linalool (3.07%). Previous studies conducted on *C. grandis* leaf EO; it has been reported that the main compounds of the EO were sabinene (5.2%), β-pinene (8.0%), limonene (35.9%), β-Ocimene (7.4%), linalool (22.1%), and citronellal (15.6%) (Rowshan and Najafan, 2013).

In this study, limonene (57.96%) represents the most important component of the EOLL, it has been reported that EOLL of various origin is characterized by high concentrations of limonene (Kirbaslar and Kirbaslar, 2004). In previous study, limonene represented (3.2–75.2%) and (1.5 to 63.4%) of the total components of leaf EO of 43 taxa of lemons and limes respectively (Lota et *al*., 2001) this component represents (62%) of the composition of EO of *Citrus aurantium L.* leaves **(**Gholivand and Piryaei, 2013) .Previous studies also reported that the various EOLL had different volatile compound compositions and the main volatile compounds of the EOLL were found including limonene, β-pinene, sabinene and β-ocimene (Lan-Chi et *al*., 2019). The concentration of citral (Neral (6.25%) and citronellal (1.02%)) is the most important factor in determining the commercial value of the EOLL. This component contributes significantly to the quality of lemon flavor and aroma (Gramshaw and Sharpe, 1980).

3.2. DPPH radical scavenging activity

The major process of food deterioration is initiated by auto-oxidation process of lipids (Tepe et *al*., 2005). The sensory and nutritional qualities of fat-based foods (dairy products and meat or fried foods) are lost during this process. The use of synthetic and natural antioxidants reduces the oxidative degradation of foods. For this reason, the importance of antioxidants for human health and food industry is evident**.** The toxicological studies performed in the past have shown that some synthetic antioxidant compounds were involved in liver damage and carcinogenesis (Gülcin et *al*., 2004). Therefore, the antioxidant activity of natural products is of increasing interest to researchers and consumers**.** For this reason, the scavenging activity of EOLL was estimated using the DPPH assay by comparing with the activity of the ascorbic acid as a known antioxidant. The Radical scavenging activity of EOLL is shown in Fig 1.

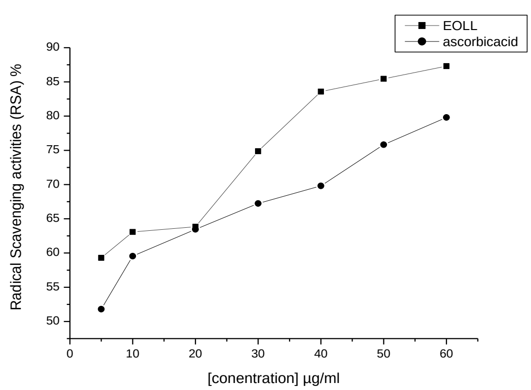


Figure 1. Scavenger effect of EOLL at different concentrations on the stable 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH)

The radical-scavenging activity (RSA) of the EOLL and positive controls ascorbic acid increased with increasing concentration. The EOLL showed 73.91% ± 11.82 inhibition of

DPPH while ascorbic acid (as positive control) exhibited 66.78% ± 9.56 activity, showing their significant difference ($p<0.05$).

EOLL exhibited an excellent DPPH scavenging ability with a IC50 of 2,41±0,04 µg/ml comparable to that of ascorbic acid with a IC50 of 5, 87± 0,75 µg/ml (Fig 2).

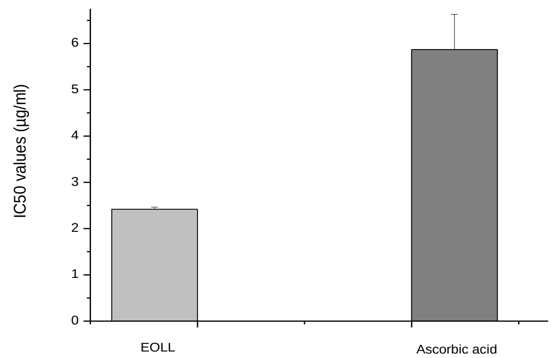


Figure 2. Antioxidant capacity (IC50) of EOLL by DPPH method

Table 2. Variation of pH and titratable acidity values of yogurts samples during storage at 4°C

Yogurt samples	Storage period (days)					
	0	1	7	14	21	28
pH						
YEO1	$4.66{\pm}0.02^{b}$	$4.63{\pm}0.02^{b}$	$4.48{\pm}0.01^{b}$	$4.44{\pm}0.01^{b}$	$4.44{\pm}0.04^{a}$	$4.36{\pm}0.01^{a}$
YEO2	$4.64{\pm}0.02^{b}$	$4.59{\pm}0.02^{c}$	$4.43{\pm}0.02^{bc}$	$4.41{\pm}0.02^{bc}$	$4.35{\pm}0.02^{b}$	$4.32{\pm}0.02^{b}$
YEO3	$4.51{\pm}0.01^{d}$	$4.44{\pm}0.02^{d}$	$4.39{\pm}0.06^{c}$	$4.36{\pm}0.01^{cd}$	$4.31{\pm}0.01^{c}$	$4.21{\pm}0.02^{c}$
YPS	$5.13{\pm}0.01^{a}$	$5.07{\pm}0.02^{a}$	$4.67{\pm}0.01^{a}$	$4.57{\pm}0.02^{a}$	$4.48{\pm}0.01^{a}$	$4.35{\pm}0.02^{a}$
YC	$4.55{\pm}0.03^{c}$	$4.45{\pm}0.01^{d}$	$4.38{\pm}0.01^{c}$	$4.34{\pm}0.06^{d}$	$4.21{\pm}0.02^{d}$	$4.14{\pm}0.01^{d}$
Titratrable acidity(D°)						
YEO1	$90.00{\pm}1.00^{c}$	$90.33{\pm}0.57^{c}$	$94.00{\pm}1.00^{b}$	$96.00{\pm}1.00^{bc}$	$97.00{\pm}1.73^{d}$	$102.00{\pm}2.00^{c}$
YEO2	$91.33{\pm}0.57^{bc}$	$92.00{\pm}1.00^{b}$	$95.66{\pm}0.57^{b}$	$97.00{\pm}1.73^{b}$	$101.00{\pm}1.00^{c}$	$102.66{\pm}0.57^{c}$
YEO3	$92.66{\pm}0.57^{ab}$	$96.00{\pm}1.00^{a}$	$98.66{\pm}1.15^{a}$	$101.66{\pm}1.52^{a}$	$104.33{\pm}0.57^{b}$	$107.66{\pm}0.57^{b}$
YPS	$72.00{\pm}1.73^{d}$	$87.00{\pm}1.00^{d}$	$90.00{\pm}1.00^{c}$	$93.66{\pm}1.52^{c}$	$94.66{\pm}1.52^{e}$	$100.66{\pm}0.57^{c}$
YC	$93.33{\pm}0.57^{a}$	$95.33{\pm}0.57^{a}$	$100.33{\pm}1.52^{a}$	$100.66{\pm}0.57^{a}$	$108.00{\pm}1.00^{a}$	$112{,}00{\pm}1.00^{a}$
ANOVA	**<0.001*****	**<0.001*****	**<0.001*****	**<0.001*****	**<0.001*****	**<0.001*****

The same superscript letter in each column shows no significant difference between values (p < .05). Data was provided as mean ± standard deviation (n=3).
YEO1= yogurt with1250µg/ml of essential oil; YEO2= yogurt with 2500µg/ml of essential oil; YEO3= yogurt with 3750µg/ml of essential oil; YPS= yogurt with 0.1 g / 100 g of potassium sorbate; YC= yogurt control with no additive.

This result is in agreement with those obtained by Frassinetti et *al*. (2011), demonstrating the scavenging abilities ranging from 20 to 70% of Citrus spp. EOs. The important antioxidant activity of the EOLL can be explained by the presence of monoterpenes, particularly limonene and γ-terpinene, which have been reported to have a good antioxidant activity (Conforti et *al*., 2007).The other components of the EOLL (caryophyllene, citral) are also responsible of the antioxidant properties **(**Dawidowicz and Olszowy, 2014). It is so difficult to explain the antioxidant activity pattern of EOs due to their complex mixtures constituted by several components. That is why; many reports on the antioxidant aptitude of the essential oils often refer to concepts such as synergism, antagonism and additivity **(**Ben Hsouna et *al*., 2017). In general, citrus leaf oil had higher antioxidant activity than peel (El-hawary et *al*., 2013).

3.3 Evolution of yogurts acidity during storage

According to the findings of the current research, acidity enhanced in the control, lemon EO and potassium sorbate yogurt samples during refrigerated storage over 28 days ($p<0.05$). On the other hand, pH decreased in all the samples within 28 days of storage, at the temperature of 4ºC ($p<0.05$) (Table 2).

The results below are in line with the findings of Wolfschoon (1983), Yeganehzad et *al*. (2007), Ahari et *al*. (2020) and Massoud and Sharifan (2020), they reported a decrease in pH of yogurt during refrigerated storage. For each day of storage, a significant difference was recorded for the pH of the five yogurt samples. The pH values of control YC yogurt were the lowest followed by those of yogurts with the essential oil YEO1, YEO2, YEO3, while the yogurt with potassium sorbate YPS, marked the highest pH during almost the 28 days of storage. This suggests a higher rate of organic acid production in the lemon leaves essential oil samples than those with potassium sorbate.

The reason the pH decreased was the acidity, which increased during the storage period following the conversion of lactose to lactic acid (Tamime and Robinson, 1985). During fermentation time, lactic acid production increases with the growth of the starter culture *S. thermophilus and L. bulgaricus.*

In this study, the change from an EO of lemon leaves concentration from 1250µg/ml to 3750µg/ml promoted the production of lactic acid by yogurt starter. Conflicting results have being reported by Massoud and Sharifan (2020), who observed a prevention of the growth of starter cultures and the production of lactic acid, by the *Rosmarinus Officinalis* essential oil.

According to table 2, the pH values obtained with essential oil yogurts at a concentration of 1250µg/ml are the closest to those obtained with potassium sorbate. However, the lemon aroma is not perceived at this concentration, for this reason a higher concentration of EO, which is 2500µg/ml, is necessary to flavour the yogurt and ensure a stability of acidity close to that of the synthetic preservative. From this, it can be assumed that the essential oil of lemon leaves slightly affects the development of yogurt cultures, unlike potassium sorbate, this synthetic preservative significantly reduced the acidity of yogurt throughout storage ($p<0.05$).

3.4. Rheological properties

3.4.1. Flow behaviour

The flow behaviors of the five yogurts produced are represented by the flow curves illustrated in fig 3. For all yogurts, the viscosity decreases as the shear rate is increasing. Massoud and Sharifan (2020) found the same viscosity trend in improved yogurt with *Rosmarinus officinalis* essential oil. Wang et *al*. (2020) have reported similar results in a study on stirred yogurt with apple pomace. The strength and the number of bonds between casein micelles in yogurt affect apparent viscosity, as well as their structure and spatial distribution (Lucey and Singh, 1998). According to Horne (1998), this can occur due to the physical destruction of the weak bonds between the molecules of the product and the decrease in the energy of interaction between them. This is the behaviour of a non-

Newtonianfluid. It appears that the addition of essential oil slightly increased the apparent viscosity of yogurts, in particular YEO3 in comparison with the control, however, at yogurt YPS the lowest apparent viscosities were recorded.

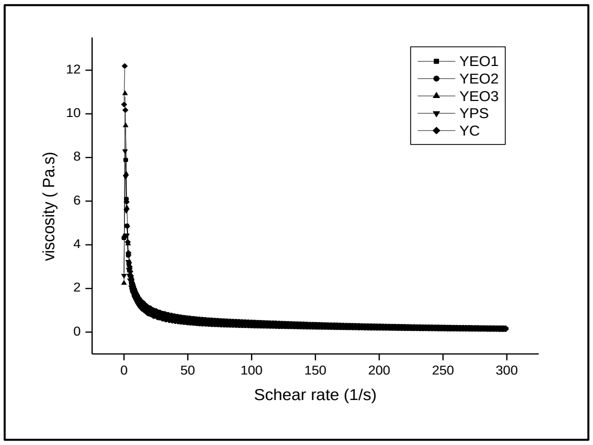


Figure 3. Flow curves of yogurt samples. Squares: YEO1, Circles: YEO2, up triangles: YEO3, down triangles: YPS, diamonds : YC. YEO1= yogurt with1250µg/ml of essential oil; YEO2= yogurt with 2500µg/ml of essential oil; YEO3= yogurt with 3750µg/ml of essential oil; YPS= yogurt with 0.1 g / 100 g of potassium sorbate; YC= yogurt control with no additive.

With the aim of objective characterization of the behaviour of the various yogurts, the experimental values were adjusted to the Herschel–Bulkley rheological model. This model is suitable for characterizing rheological behaviour in the pseudoplastic zone, for intervals of shear rate neither very high nor very low, and presented good determination coefficients ($R^2 \geq 0.99$).

$$\tau = K\gamma^n + \tau_0 \qquad (4)$$

With:
τ: shear rate (Pa);
τ_0: initial shear rate (yield stress) (Pa);
K: consistency coefficient (Pa .s^n);
γ: shear rate (1/s);
n: flow index.

Table 3. Herschel Bulkley model parameters.

Yogurts	**Apparent vicosity (Pa.s)**	**Thixotropy (Pa/s)**	**Yiel stress τ_0 (Pa)**	**Index (n)**
YEO1	20.21 ^{d}	3088 ^{d}	-16.64 ^{a}	0.2134 ^{b}
YEO2	22.43 ^{c}	3245 ^{c}	-19.38 ^{c}	0.2008 ^{c}
YEO3	30.89 ^{a}	3789 ^{a}	-26.16 ^{d}	0.1688 ^{e}
YPS4	6.997 ^{e}	2280 ^{e}	-32.052 ^{e}	0.3374 ^{a}
YC	23.22 ^{b}	3329 ^{b}	-18.96 ^{b}	0.1933 ^{d}

The same superscript letter in each column shows no significant difference between values ($p < .05$). Data was provided as mean ± standard deviation (n=3).
YEO1= yogurt with1250µg/ml of essential oil; YEO2= yogurt with 2500µg/ml of essential oil; YEO3= yogurt with 3750µg/ml of essential oil; YPS= yogurt with 0.1 g / 100 g of potassium sorbate; YC= yogurt control with no additive.

Table 3 shows the parameters of the Herschel Bulkley model. This model is designed to characterize the behaviour of fluids with a fluency threshold. The model incorporates an independent term, $\boldsymbol{\tau_0}$, to the Ostwald de Waele model, which represents the value of the minimum force that must be applied to the sample in order for it to begin to flow.

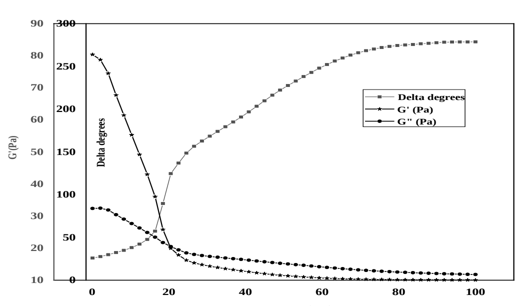
Since milk composition was kept constant in the present study, the differences observed were mainly due to change in the food preservative type. The addition of EO of lemon leaves in the yogurt significantly influences the flow parameters (table 3). All values of n (flow index), which measures the deviation degree from a Newtonian fluid, are less than 1, confirming the pseudoplastic behavior of yogurts, as already reported (Teles Et Flôres, 2007; Fischer et *al.*, 2009).The addition of lemon leaves EO has significatly increased the apparent viscosity in yogurt YEO3 with (30.89 Pa.s), however the use of the synthetic preservative, potassium sorbate, considerably decreased the viscosity (YPS: 6.99 Pa.s) in comparison with the control YC: 23.22 Pa.s. These results are in disagreement with those obtained by Ahari et al. (2020), who found the lowest viscosity of yogurt with the highest content of cumin essential oil. Increasing the concentration of essential oil in yogurts significantly reduced the initial shear rate τ0 (yield stress) compared to the control. YPS, showed the lowest τ0, this indicates that a higher shear stress is needed to initiate the flow in this yogurt.

According to the table 3, all samples exhibited thixotropic characteristics, due to the differences in tension and viscosity between the ascending and descending curves. This phenomenon results from the breakage of the gel when a shear force is used (Bourne, 2002).

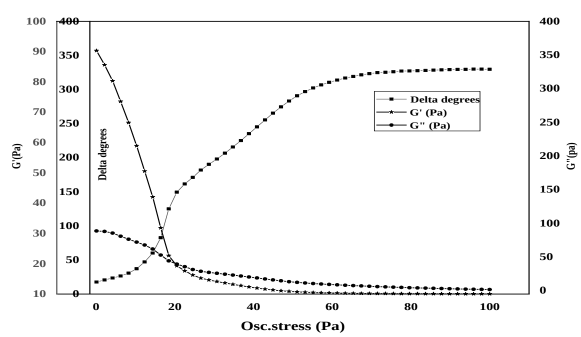
The thixotropic index recorded for YEO3 yogurt (3789 Pa/s) was the greatest, while that of YPS yogurt was the lowest (2280 Pa/s) compared with the control (3329 Pa/s). The addition of the EOLL or potassium sorbate, visibly affected the surface values of the hysteresis. These measures indicate that the yogurt YEO3 was more susceptible to structural breakdown under external force and less capacity to recover to its original structure. (Ahari et *al*., 2020) found a higher thixotropy in yogurt control compared to yogurt with cumin essential oil.

3.4.2. Viscoelastic properties of yogurts

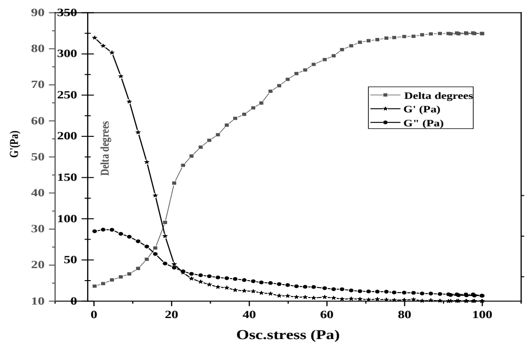
The understanding of the rheology and the microstructure of food gels is an important tool for improving consumer satisfaction (Ahmed et *al*., 2017). The storage modulus, G′, represents the elastic behavior of the network and strength of the structure contributing to the 3D network. The loss modulus, G″, represents the viscous behavior of a sample and characterizes interactions not contributing to the 3D network (Tabilo-Munizaga and Barbosa-C´anovas, 2005). Therefore, if G′ is higher than G″, the solid-like properties are dominant over the liquid-like ones, as it is the case of gel-like materials.

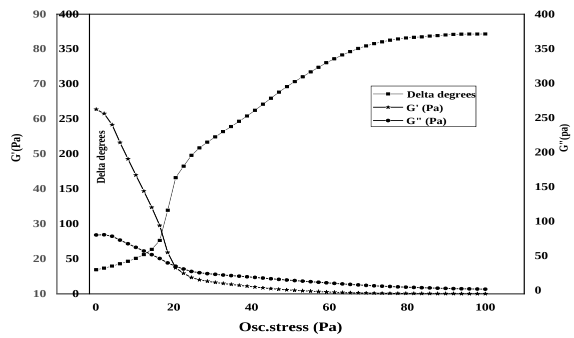


YC

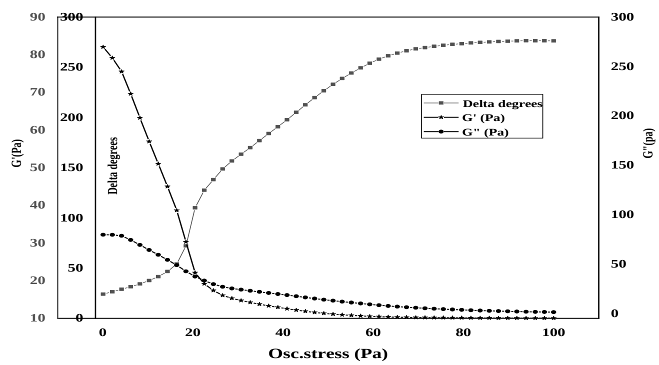


YEO1





YEO2 **YEO3**



YPS

Figure 4. Viscoelasticity curves of yogurt samples. Squares: Delta degrees, Circles: G′, stars: G″.YEO1= yogurt with1250µg/ml of essential oil; YEO2= yogurt with 2500µg/ml of essential oil; YEO3= yogurt with 3750µg/ml of essential oil; YPS= yogurt with 0.1 g / 100 g of potassium sorbate; YC= yogurt control with no additive

As shown in Fig 4, storage modulus (G′) and loss modulus (G″) for all the yogurts samples are frequency dependent. All yogurts exhibit viscoelastic characteristics. Higher G′ and lower tan δ values were observed at oscillation stress, less or equal to 22.53 Pa, 24.56 Pa, for (YEO1, YEO2, YEO3, YC) and YPS respectively, indicating a predominant solid-like behavior of the yogurt gel. Nevertheless, at higher oscillation stress, a cross-over between these parameters occurred, with behavior shifting to a predominant liquid-like one, indicating breakdown of the yogurt structure. The viscoelastic behavior of the control yogurt and the essential oil yogurts were almost the same.

4.Conclusions

This study demonstrated the effect of adding the essential oil of lemon leaves to low-fat yogurt on its physicochemical and rheological properties. The highest pH and lowest acidity rates belonged to groups with the potassium sorbate followed by the essential oil at a rate of 1250µg/ml, a concentration of 2500µg/ml of EO is maintained to improve the stability of acidity of yogurts during storage and to benefit from the natural aroma of the essential oil. The addition of EO did not significantly affect the viscoelastic behaviour of low-fat yogurts; the maximum viscosity was obtained with a rate of 3250µg / ml of EO. The addition of EO did affect significantly the thixotropic behaviour of low-fat yogurts ($p<0.05$).

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