

# Effect of lemon balm (*Melissa officinalis* L.) essential oil on some characteristics of oil/water food emulsions

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**Abstract.** Food emulsions are used on a daily basis by people, therefore it is of great importance to study the factors that can influence their stability. The aim of this work is to track the influence of lemon balm essential oil (*Melissa officinalis* L.) on the change of some physical and chemical indicators, kinetic (turbidity) and thermodynamic (Gibbs free energy, enthalpy and entropy) parameters, which are related to their stability. 24 food emulsions were developed with oleic type sunflower oil acting as an oil phase in concentrations of 20 and 30%, soybean protein emulsifier in concentrations of 1, 2, and 3% and lemon balm essential oil in concentrations of 0.1, 0.2 and 0.3%. The pH of the emulsions was determined, microscopic photographs were taken to measure the sizes of the colloidal particles, turbidity was observed for 15 days, their absorbance was determined, Gibbs energy, enthalpy and entropy were calculated. The most stable is the emulsion with 30% oil phase, 3% soybean protein emulsifier and 0.3% lemon balm essential oil.

## 1 Introduction

Food emulsions are widely used in human nutrition in today's modern times. They consist of two liquid immiscible phases – oil and water, which is why they are defined as oil/water and water/oil [1]. Oil/water emulsions are defined as thermodynamically unstable systems in which the oil phase is dispersed in the water phase [2-5].

The stability of the emulsion depends both on the composition and the amount of the oil phase and the emulsifier used, as well as on the surrounding conditions, temperature fluctuations, mechanical agitation, storage conditions, *etc.* Choosing an appropriate emulsifier that adsorbs at the interface between the two phases and reduces the interfacial tension is important to create a kinetically stable system [6].

Various liquid and solid components are included in the oil phase of food emulsions - glyceride oils, fats, wax-like substances. The most widely used are soybean [7-9], walnut

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[10], olive [11] oils, their chemical composition and properties also affect the stability of the emulsions.

As emulsifiers in food emulsions, proteins are used, most often soybean protein isolate, casein or a mixture of them, which have a positive effect on their physical and thermodynamic parameters, and thus improve their stability [12-15].

To improve the aroma-taste complex and increase the quality of food products, very often essential oils are included in their composition [16-18]. They are known to have antimicrobial and antioxidant properties, which also makes them a suitable and desirable ingredient for food emulsions [19-21].

Lemon balm (*Melissa officinalis* L.) is a very common essential oil plant that is processed by steam distillation to obtain its oil. It is an easily mobile liquid with a characteristic smell of lemon and a specific aroma [22, 23]. The oil has proven antimicrobial and antioxidant properties, which is why it is suitable for inclusion in various food products [24-26].

The aim of the present work is to develop food emulsions containing lemon balm essential oil, following its influence on their stability. The obtained data will be the basis for further research on the antimicrobial and antioxidant stability of the emulsions with the aim of their application.

## 2 Materials and methods

### 2.1 Materials

For the oil phase, oleic type sunflower oil was used, purchased from the commercial network of the city of Plovdiv, harvest 2023 [27].

Soybean protein isolate was used as an emulsifier, supplied from FILLAB Bulgaria.

Distilled water was used for the aqueous phase. The lemon balm essential oil was provided by a company producing essential oils from North-Eastern Bulgaria, harvest 2021. It is an easily mobile liquid with a specific smell of lemon and a specific aroma. Its main components are  $\beta$ -caryophyllene (20.45%), geranial (19.74%), neral (15.81%), germacrene D (11.50%), citronellal (3.07%), and geranyl acetate (3.04%) [23]. The oil has established antimicrobial [23, 24] and antioxidant properties [25, 26], making it a suitable additive in food emulsions.

Emulsions were developed with an oil phase content of 20 and 30% oleic type sunflower oil and an emulsifier of 1, 2 and 3% soybean protein, with the addition of 0.1, 0.2 and 0.3% lemon balm essential oil. The emulsifier is added with stirring to the aqueous phase, then the addition of the oil phase is slowly started. The emulsion was stirred with a homogenizer for 1 min and 30 s at a temperature of 25°C. The lemon balm essential oil was added after cooling. The amounts of the oil phase, the emulsifier and the lemon balm essential oil are according to literary data [28, 29].

### 2.2 pH measurement

The pH was measured using a pH meter Hanna HI 98127 with a replaceable electrode. The pH values of the prepared emulsions are determined immediately after their preparation.

### 2.3 Kinetic parameter turbidity

The investigations were carried out using a Camspec M107 UV/VIS spectrophotometer. The change in turbidity of the emulsions was measured at a maximum wavelength of 450 nm. The determination is according to equation 1 [8]:

$$\tau = \frac{A}{l} \quad (1)$$

where:  $\tau$  – turbidity,  $A$  – absorption,  $l$  – way of light equal to 1 cm.

## 2.4 Microscopic observations

All emulsions were tested immediately after preparation. Microscopic observations were performed with a Brassler digital microscope with a USB port on a computer.

## 2.5 Determination of absorption in emulsions

The absorbance of a series of standard solutions of exact concentration was determined. The studies were carried out with a Camspec M107 Spectrophotometer with a 1 cm cuvette path length at a maximum wavelength equal to 450 nm. Solutions of different concentrations prepared from the respective emulsion were used as standard solutions. Tests were conducted at room temperature (25°C).

## 2.6 Thermodynamic parameters

All parameters the Gibbs energy, enthalpy and entropy were calculated [30, 31].

## 2.7 Statistical analysis

All experiments were performed in triplicate, and the data presented in the tables are the arithmetic mean with their corresponding statistical error.

# 3 Results and discussion

The developed emulsions are white viscous masses, and those with essential oil have a specific lemon smell.

## 3.1 pH

The measured pH values of the controls were between 6.01 and 6.24. For the emulsions with added lemon balm essential oil, the values are lower (from 5.70 to 6.20), which can be explained by the influence of its acid number, which is 3.02. For all emulsions, the pH values are suitable for their application in food.

## 3.2 Kinetic parameter turbidity

The turbidity of the emulsions was measured for 15 days by spectrophotomeasuremens. All controls showed a trend of high turbidity from day 1 to day 4 of storage, then decreasing proportionally with time. The emulsions were stable at the beginning of the study until the 8th day. After that, the turbidity starts to decrease sharply. A higher turbidity was observed for the emulsion with 3% soybean protein and 30% oil phase, being 9.5 on the first day and decreasing to 4.5 at the end of follow-up.

Emulsions with 0.1% lemon balm essential oil showed a tendency for high turbidity from the 1st to the 6th day of storage (from 9.0 to 10.5), then decreasing proportionally with time. The emulsion with 3% soybean protein and 30% oil phase was the most stable, with the turbidity at the end of the storage period between the 13th and 15th day being 8.5 to 8.0.

For emulsions with 0.2% lemon balm essential oil, the turbidity was also high at the beginning of the storage period (from 8.3 to 11.5), and then decreased proportionally with time. The turbidity of the emulsion with 3% soybean protein and 30% oil phase was the highest, and at the end of the observation, its value decreased to 8.0.

Emulsions with 0.3% lemon balm essential oil showed a tendency for high turbidity up to the 6th day of storage, after which again decreasing proportionally with time. For the emulsion with 2% soybean protein and 30% oil phase, a slight destabilization was observed on day 6, where the turbidity reached 7.5, then increased again and the emulsion stabilized. The highest remained the turbidity of the emulsion with 3% soybean protein and 30% oil phase, which was 12 at the beginning of the storage period and reached 7 on the 15th day.

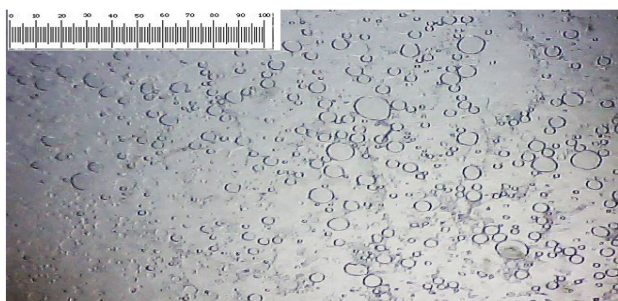
According to research by Linke and Drusch, [32], particle size is the most important factor affecting emulsion turbidity, with the maximum value being at an average diameter of 0.2  $\mu\text{m}$ .

In our research, the highest turbidity values were reported for the emulsion with 30% oil phase, 3% soybean protein and 0.3% lemon balm essential oil. This is probably related to a smaller size of the colloidal particles defining the emulsion as more stable [32, 33] and can be confirmed by the micrographic images.

### 3.3 Microscopic observations

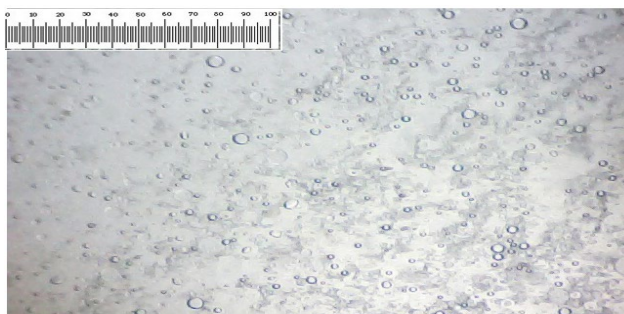
The micrographic image of each emulsion was taken 10 min after its preparation in order to determine the size of the colloidal particles. The images show polydisperse emulsions of different sizes – from small to large. Their size is a main characteristic of the stability of emulsions, and those with a large size of colloidal particles are more unstable, as this is related to the stronger interactions between them and the progress of the coalescence process. As the amount of the oil phase increases, colloidal particles with smaller sizes are observed, which define the emulsions as more stable.

The size of the colloidal particles in the controls vary (from 12 to 35  $\mu\text{m}$ ), the smallest being (Fig. 1) in the emulsion with 3% soybean protein and 30% oil phase (from 5  $\mu\text{m}$  to 15  $\mu\text{m}$ ).



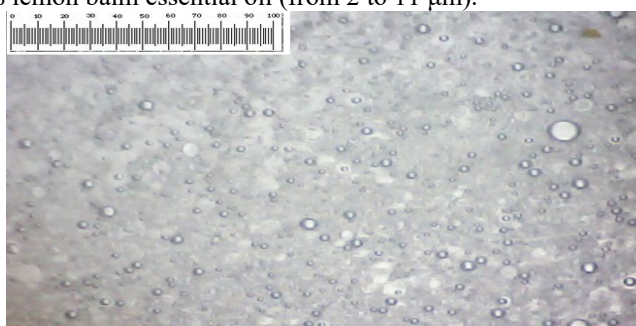
**Fig. 1** Control: 3% soybean protein and 30% oil phase.

The emulsions with 0.1% lemon balm essential oil, have a colloidal particle size ranging (from 5 to 27  $\mu\text{m}$ ), with the smallest being (Fig. 2) in the emulsion with 3% soybean protein, 30% oil phase and 0.1% lemon balm essential oil (6 to 12  $\mu\text{m}$ ).



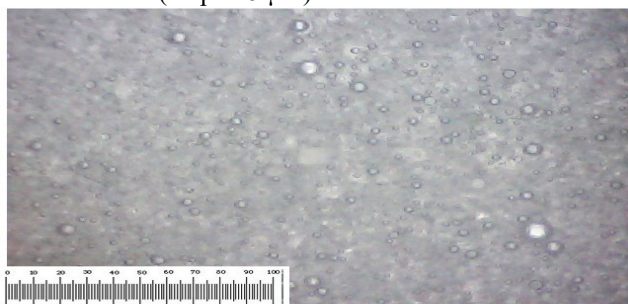
**Fig. 2** Emulsion: 3% soybean protein, 30% oil phase and 0.1% lemon balm essential oil.

For emulsions with 0.2% lemon balm essential oil, the size of the particles ranges (from 6 to 14  $\mu\text{m}$ ), the smallest being (Fig. 3) in the emulsion with 3% soybean protein, 30% oil phase and 0.2% lemon balm essential oil (from 2 to 11  $\mu\text{m}$ ).



**Fig. 3** Emulsion: 3% soybean protein, 30% oil phase and 0.2% lemon balm essential oil.

Those with 0.3% lemon balm essential oil, have a particle size that varies (from 1 to 9  $\mu\text{m}$ ), smallest being (Fig. 4) in the emulsion with 3% soybean protein, 30% oil phase and 0.3% lemon balm essential oil (1 up to 5  $\mu\text{m}$ ).



**Fig. 4** Emulsion: 3% soybean protein, 30% oil phase and 0.3% lemon balm essential oil.

The emulsion with the largest colloidal particle sizes (from 5  $\mu\text{m}$  to 15  $\mu\text{m}$ ) is thermodynamically unstable due to the relatively high interfacial tension associated with the contact between the oil and water phases [34]. In emulsions with a high content of oil phase and emulsifier, with the addition of lemon balm essential oil, the size of the particles is significantly reduced, which is an indication of obtaining more stable emulsions [35]. The studies for determining the turbidity are in confirmation with this claim, the values of which are the highest in the emulsion with 30% oil phase, 3% soybean protein and 0.3% lemon balm essential oil.

### 3.4 Thermodynamic parameters

The thermodynamic parameters Gibbs free energy, enthalpy and entropy were calculated for all emulsions. These are indicators that give information about the stability of a system and are related to the thermal effect and establishment of equilibrium in it. The equilibrium constant of the dispersion process of separation of the two phases was calculated. Positive values of this parameter were found for all emulsions. To determine the values of this constant, a linear regression model of the dependence of absorption on the concentration of a series of solutions prepared by the dilution method for all emulsions was used. The obtained equations are presented in Table 1.

**Table 1.** Linear equations of the dependence of absorption on the concentration of emulsions.

Emulsions	Equations	Coefficients
<b>Control</b>		
1% soybean protein, 20% oil phase	$y = 0.337 + 1.005*x$	$R^2 = 0.997$
1% soybean protein, 30% oil phase	$y = 0.099 + 1.150*x$	$R^2 = 0.993$
2% soybean protein, 20% oil phase	$y = -0.113 + 1.069*x$	$R^2 = 0.982$
2% soybean protein, 30% oil phase	$y = 0.081 + 0.903*x$	$R^2 = 0.976$
3% soybean protein, 20% oil phase	$y = -0.092 + 1.401*x$	$R^2 = 0.988$
3% soybean protein, 30% oil phase	$y = 0.119 + 1.125*x$	$R^2 = 0.999$
<b>With 0.1% lemon balm essential oil</b>		
1% soybean protein, 20% oil phase	$y = 0.089 + 6.597*x$	$R^2 = 0.994$
1% soybean protein, 30% oil phase	$y = 0.225 + 3.840*x$	$R^2 = 0.995$
2% soybean protein, 20% oil phase	$y = 0.256 + 5.090*x$	$R^2 = 0.993$
2% soybean protein, 30% oil phase	$y = 0.205 + 4.937*x$	$R^2 = 0.978$
3% soybean protein, 20% oil phase	$y = 0.218 + 5.187*x$	$R^2 = 0.977$
3% soybean protein, 30% oil phase	$y = 0.312 + 3.790*x$	$R^2 = 0.985$
<b>With 0.2% lemon balm essential oil</b>		
1% soybean protein, 20% oil phase	$y = -0.603 + 0.634*x$	$R^2 = 0.936$
1% soybean protein, 30% oil phase	$y = -0.399 + 0.729*x$	$R^2 = 0.963$
2% soybean protein, 20% oil phase	$y = 0.232 + 3.248*x$	$R^2 = 0.998$
2% soybean protein, 30% oil phase	$y = -0.483 + 0.809*x$	$R^2 = 0.975$
3% soybean protein, 20% oil phase	$y = 0.276 + 3.610*x$	$R^2 = 0.998$
3% soybean protein, 30% oil phase	$y = 0.135 + 4.218*x$	$R^2 = 0.982$
<b>With 0.3% lemon balm essential oil</b>		
1% soybean protein, 20% oil phase	$y = 0.108 + 4,230*x$	$R^2 = 0.996$
1% soybean protein, 30% oil phase	$y = 0.311 + 2,623*x$	$R^2 = 0.997$
2% soybean protein, 20% oil phase	$y = 0.412 + 1,807*x$	$R^2 = 0.972$
2% soybean protein, 30% oil phase	$y = 0.271 + 4,167*x$	$R^2 = 0.987$
3% soybean protein, 20% oil phase	$y = 0.451 + 1,875*x$	$R^2 = 0.997$
3% soybean protein, 30% oil phase	$y = 0.149 + 5,158*x$	$R^2 = 0.996$

The high values of the correlation coefficients ( $R^2$  from 0.999 to 0.972) indicate a good comparability of the values obtained as a result of a conducted experiment.

The values of the thermodynamic parameters are presented in Table 2.

Gibbs free energy can be used as criteria for emulsion stability. Large negative values were found in the high oil phase controls, being the highest in the emulsion with 3% soybean protein and 30% oil phase equal to -3.698 kJ/mol.

The data shows that as the amount of lemon balm essential oil increases, the Gibbs energy values also increase and are highest at 3% soybean protein and 30% oil phase, respectively at 0.1% lemon balm essential oil it is -4.736 kJ/mol, at 0.2%, -5.068 kJ/mol and at 0.3%, -5.281 kJ/mol.

**Table 2.** Thermodynamic parameters – Gibbs energy, enthalpy and entropy.

Emulsions	Gibbs energy, J/mol	Enthalpy, kJ/mol	Entropy, kJ/(mol.K)
<b>Control</b>			
1% soybean protein, 20% oil phase	-1.754 ± 0.011	-18.105 ± 0.084	-0.054 ± 0.001
1% soybean protein, 30% oil phase	-1.897 ± 0.009	-18.167 ± 0.107	-0.055 ± 0.005
2% soybean protein, 20% oil phase	-2.738 ± 0.013	-18.532 ± 0.092	-0.053 ± 0.002
2% soybean protein, 30% oil phase	-2.838 ± 0.012	-18.576 ± 0.117	-0.052 ± 0.004
3% soybean protein, 20% oil phase	-3.569 ± 0.019	-18.893 ± 0.113	-0.051 ± 0.001
3% soybean protein, 30% oil phase	-3.698 ± 0.016	-18.949 ± 0.124	-0.051 ± 0.003
<b>With 0.1% lemon balm essential oil</b>			
1% soybean protein, 20% oil phase	-2.938 ± 0.012	-18.619 ± 0.117	-0.052 ± 0.002
1% soybean protein, 30% oil phase	-3.858 ± 0.015	-19.018 ± 0.109	-0.051 ± 0.001
2% soybean protein, 20% oil phase	-3.919 ± 0.011	-19.045 ± 0.125	-0.051 ± 0.004
2% soybean protein, 30% oil phase	-3.982 ± 0.018	-19.072 ± 0.112	-0.050 ± 0.001
3% soybean protein, 20% oil phase	-4.004 ± 0.013	-19.082 ± 0.108	-0.050 ± 0.005
3% soybean protein, 30% oil phase	-4.736 ± 0.017	-19.402 ± 0.093	-0.049 ± 0.004
<b>With 0.2% lemon balm essential oil</b>			
1% soybean protein, 20% oil phase	-3.233 ± 0.009	-18.746 ± 0.106	-0.052 ± 0.009
1% soybean protein, 30% oil phase	-3.925 ± 0.015	-19.047 ± 0.117	-0.050 ± 0.001
2% soybean protein, 20% oil phase	-4.225 ± 0.012	-19.178 ± 0.098	-0.050 ± 0.003
2% soybean protein, 30% oil phase	-4.291 ± 0.011	-19.206 ± 0.122	-0.050 ± 0.007
3% soybean protein, 20% oil phase	-4.624 ± 0.016	-19.398 ± 0.087	-0.049 ± 0.004
3% soybean protein, 30% oil phase	-5.068 ± 0.008	-19.544 ± 0.124	-0.048 ± 0.006
<b>With 0.3% lemon balm essential oil</b>			
1% soybean protein, 20% oil phase	-3.915 ± 0.013	-19.043 ± 0.103	-0.051 ± 0.001
1% soybean protein, 30% oil phase	-4.357 ± 0.015	-19.235 ± 0.115	-0.049 ± 0.003
2% soybean protein, 20% oil phase	-4.587 ± 0.009	-19.335 ± 0.095	-0.049 ± 0.005
2% soybean protein, 30% oil phase	-4.763 ± 0.016	-19.411 ± 0.101	-0.049 ± 0.001
3% soybean protein, 20% oil phase	-5.146 ± 0.011	-19.578 ± 0.117	-0.048 ± 0.003
3% soybean protein, 30% oil phase	-5.281 ± 0.014	-19.636 ± 0.106	-0.048 ± 0.004

In the case of oil/water emulsions, the smaller the value of the surface energy (Gibbs free energy), the more stable they are [36]. Therefore, we consider the emulsion with 30% oil phase, 3% emulsifier soybean protein and 0.3% lemon balm essential oil to be the most stable. Negative enthalpy values and small negative entropy values are found. At a negative enthalpy value, the process is defined as exothermic. Entropy is related to the rapid phase separation of emulsions and this leads to difficulties in determining the direction of the process.

The inclusion of lemon balm essential oil, in our opinion, affects all parameters of the developed emulsions, contributing to an increase in their stability, also found for other natural products [37].

The influence of lemon balm essential oil on the stability of the developed food emulsions can be explained by its chemical composition – a high content of the aldehydes geranial (19.74%) and neral (15.81%). The mixture of the two isomers is known as citral, with which attempts have been made to track the influence on the stability of the same emulsions [26]. The author found that when adding 0.1, 0.2 and 0.3% citral to emulsions with 30% oil phase and 3% emulsifier soybean protein, compared to controls, the colloidal particle size decreased, the turbidity value was from 8.0 to 9.0, and Gibbs energy is -4.597, -4.666, and -4.809 kJ/mol, respectively. These values are lower compared to those obtained in the present study because the lemon balm essential oil contains other aromatic components that probably affect the stability of food emulsions.

The comparative analysis of the values of all analyzed indicators and parameters shows that the lemon balm essential oil has a significant effect on the stability of the developed food

emulsions. By increasing the amount of the oil phase, the emulsifier and the lemon balm essential oil, the stability of the food emulsion increases. The smallest colloidal particle sizes (1 to 5  $\mu\text{m}$ ), with high turbidity indices up to 15 days of storage (8.0) and with the highest Gibbs energy values (-5.281 kJ/mol) is the food emulsion prepared with 30% oil phase, 3% soybean protein and 0.3% lemon balm essential oil

## 4 Conclusion

Food emulsions with different concentrations of the oil phase have been developed – oleic type sunflower oil, emulsifier - soybean bean protein and a supplement of lemon balm (*Melissa officinalis* L.) essential oil. Some physical (pH, microscopic images), kinetic (turbidity) and thermodynamic parameters (Gibbs energy, enthalpy and entropy) were determined. The correlation between these parameter and storage period shows that the best behavior was recorded for the sample containing: 30% sunflower oil, oleic type, 3% emulsifier soybean protein and the higher concentration of lemon balm essential oil (0.3%), which is a guarantee of its high stability.

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