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**ORIGINAL RESEARCH PAPER**

# DEVELOPMENT AND CHARACTERIZATION OF A NOVEL FOOD PRODUCT: YOGURT WITH GRAPEFRUIT ESSENTIAL OIL CAPSULES

**Roxana E. Gheorghita[1*], Oana C. Iatcu[1], Lidia Petcu[2], Adriana Dabija[3]**

*1"Stefan cel Mare" University of Suceava, College of Medicine and Biological Sciences, 720229, Suceava, Romania*
*[2] SC. Enzyme & Derivates SA, 617512, Neamt, Romania*
*3"Stefan cel Mare" University of Suceava, Faculty of Food Engineering, 720229, Suceava, Romania*

*Corresponding author: roxana.puscaselu@usm.ro

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**Abstract:** Biopolymers have many properties important for food industry. Due to their high biocompatibility and targeted release, they can be used for development of novel, functional food. Grapefruit is a citrus with benefits for consumer' health, especially as anti-obesity or anti-diabetic agent. The aim of this paper was to develop a new functional product: yogurt with capsules of grapefruit essential oil. Thus, yoghurt with 10 %, respectively 20 % capsules were obtained by simulating the industrial process. In order to establish the optimal stage of the process for capsules' addition, they were added before and after fermentation. According to the results, products with good sensorial properties was developed, no matter the addition time, but better results in order of syneresis, water holding capacity and acidity were observed for samples with capsules added before fermentation process. The yogurt with grapefruit essential oil capsules represents a good idea for a novel functional food product.

**Keywords:** *biopolymers, citrus, essential oil, functional food, metabolic disorders*

## INTRODUCTION

In recent years, consumer preference for functional foods has grown considerably. They preferred to choose healthy foods and a diet with benefits for the health of the body. In this way, functional or fortified foods with various biologically active compounds were developed. The most frequently used food products are the traditional ones and those with probiotics [1]. Dairy products are excellent vectors for innovative functional foods. Of these, yogurt is one of the foods preferred by the entire category of consumers and can be found in various forms: natural or with additions, drinking or frozen, etc.

In addition to the effect of probiotics naturally present in the product, yogurt represents a good food matrix for substances that have the ability to potentiate the beneficial effects of consumption. Thus, new products with additions of vitamin C [2], phenolic extracts [3], lactoferrin [4], natural powders [5] or essential oils [6] were obtained. Due to their numerous benefits, essential oils (EOs) have been intensively used for the development of nutraceutical products. According to literature, essential oils are considered therapeutic remedies for a wide range of ailments and complaints (metabolic and neurological disorders, allergies, depression) [7, 8] and are Generally Recognized as Safe (GRAS) [9].

Poor stability of volatile compounds can be improved by essential oils' encapsulation. The microencapsulation can prolong the active ingredients beneficial components, maintain physiochemical and microbiological characteristics, prolong sensorial properties, and even extend the shelf life [10 – 12]. The choice of microencapsulation process' type and substances used as coating or wall material, as well as food matrix is very important [13].

In the last few years, biopolymers, mainly sodium alginate, chitosan or carrageenan have been used as encapsulation material due to their higher digestibility, biocompatibility, regenerability, intestinal resistance, and ease of manufacturing [14].

Dairy products, especially yogurt, represent a good matrix for biopolymer-based microcapsules. Capsules with natural bioactive compounds were developed and greatly influenced the quality and sensorial properties of the product. For example, when added into yogurt, EOs microcapsules did not interfere into product fermentation process, and even improved the specific bacteria proliferation [15].

Citrus EOs are well-known in the food industry for their high nutritional content, abundance in bioactive components, especially vitamin C, good aroma, flavor, being a major component in many dishes. Citrus EOs are used for their antioxidant, antidiabetic, antimicrobial, anti-inflammatory, being added even into active food packaging formulations [16, 17]. Among citrus EOs, grapefruit (*Citrus paradisi L.*) is effective in reducing the proliferation and spoilage of pathogens, being considered a good alternative to synthetic additives used in food industry [18]. It is well-known as anti-obesity and weight control agent due to its capacity to mediate through mechanisms of anti-lipase activity, antihyperlipidemia, by down-regulating adipogenetic transcription, by increasing the plasma glycerol concentration, or by suppressing fat accumulation and intracellular triglyceride [19]. When tested on the Wistar and Sprague-Dawley rats, the grapefruit EO down regulated the appetite, reduced food consumption and weight [20]. Due to its amount of bioactive components, the consumption can interact with microbiota and influence the weight control process [21, 22].

Other protective effects are related to cardiovascular diseases due to the anti-ischemic, antioxidant, vasorelaxant and antithrombotic protective effects of flavonoids and polyphenols [23]. Various antioxidants, such as carvone, limonene, linalool, terpinene, lycopene, naringin, hesperidin, kaempferol, and quercetin present anti-inflammatory properties and have ability to protect against heart disease, prostate or lung cancer, leukemia cells' apoptosis, and kidney stone formation inhibition [24, 25].
The aim of present study was to develop a new yogurt formula, a functional product with grapefruit essential oil biopolymers-based capsules and to determine at what stage of the technological process EO can be added in order to not disintegrate and do not alter the properties of the yogurt.

## MATERIALS AND METODS

For yogurt preparation, fresh raw milk, with 3.5 % fats, 4.5 % carbohydrates, and 3 % proteins and lactic bacteria (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) supplied by SC Enzyme & Derivates SA Romania were used. Sodium alginate, glycerol, and calcium chloride were purchased from Sigma Aldrich Company Romania branch. Grapefruit essential oil was purchased from Ingen Laboratories, Romanian branch.
For capsule preparation, 5 % sodium alginate solution was prepared. The mixture was heat at 90 ± 1.5 °C under stirring (500 rpm) and maintained for 20 minutes, cooled until reached 40 °C, and 10 % / 20 % grapefruit EO added; the mixture was stirred at 500 rpm for other 10 minutes. Capsules were developed using a Caviar box through extrusion method. Thus, capsule forming solution was poured through Caviar box and the capsules were released into 2 % calcium chloride solution. After 3 minutes, the capsules were washed into water in order to eliminate the excess of calcium chloride solution. The capsules were kept in refrigeration conditions for future testing and addition into milk/yogurt.
Yogurt preparation was carried out following the steps used in food industry: milk pasteurization at 75 °C for 15 minutes, cooling at 41 °C, 0.02 (*w/v*) starter culture inoculation, 10 %, respectively 20 % grapefruit EO capsules (S1, S2) addition, dosing in 100 mL glass jars, and incubation at the same temperature until the 4.6 *p*H value [26]. After that, for other two samples (S3, S4), 10 %, respectively 20 % (*w/v*) grapefruit EO capsules were added and the mixtures were homogenized. Yogurt without capsules represents the control sample (C). Yogurt samples were cooled and stored at 4 °C for future evaluation.

### *Capsules testing*

After development, capsules were tested in order to evaluate the size, color, transmittance, opacity, and antioxidant activity. Capsule size was measured with Yato electronic micrometer (Shanghai, China), with a precision of 0.002 mm and the result was established after 30 readings of different capsules. Transmittance was read spectrophotometrically, at 660 nm, using an Epoch (BioTek Instruments Winooski, VT, USA) equipment. Color was evaluated through CIELAB system, using a Konica Minolta CR 400 colorimeter.

The antioxidant activity of capsules was evaluated using ABTS radical scavenging capacity method according to modified method [27]. ABTS radical solution was prepared by gently mixing 10 mL of 7 mM ABTS solution and 10 mL of 2.45 mM potassium persulfate solution, and incubation for 16 h in the dark at room temperature. Before use, the reagent was brought to an absorbance of 1 by diluting with ethanol. 0.1 g capsules and 1 mL ABTS solution was mixed and incubated in the dark for 30 minutes, room temperature. The absorbance at 734 nm was read using the same equipment used for transmittance evaluation. The inhibition was calculated according to the following formula, where A0 represent the control sample absorbance and A1 the absorbance of capsules.

$$Inhibition, \% = \frac{A0-A1}{A0} * 100 \quad (1)$$

***Yogurt evaluation***

After development, the yogurt samples were evaluated. Color evaluation was performed using CIELAB system and CR400 Chroma Meter equipment. *p*H was measured with *p*H-meter (Mettler Toledo, Germany) and acidity by titration with NaOH 0.1 N and expressed in Thorner degrees. For syneresis' susceptibility (S), 100 mL of sample was placed into a funnel, filtered for 6 h using filter paper. After 6 h, the whey volume was measured and susceptibility to syneresis was calculated using formula (2):

$$S, \% = \frac{V1}{V2} * 100 \quad (2)$$

where: $V_1$ is the volume of the whey after drainage,
$V_2$ is the volume of yogurt sample.

Water holding capacity (WHC) was determinate by 5 g samples centrifugation at 4500 x g for 15 minutes (4 °C). The WHC was calculated according to the formula (3), where W1 is the weight of whey after centrifugation and W2 the weight of yogurt.

$$WHC, \% = \left(1 - \frac{W1}{W2}\right) * 100 \quad (3)$$

## RESULTS AND DISCUSSIONS

The capsules were tested after development. The results are presented in Table 1.

***Table 1.*** *Physicochemical characteristics and antioxidant activity of grapefruit EO capsules*

| Transmittance [%] | Capsule size [mm] | Inhibition [%] | Color | | |
|---|---|---|---|---|---|
| | | | ***L**** | ***a**** | ***b**** |
| 77.30 ± 0.03 | 2.01 ± 0.01 | 20.66 ± 0.05 | 40.19 ± 0.43 | -0.46 ± 0.02 | 5.74 ± 0.13 |

The grapefruit EO capsules were homogenous, with regular edges and color and smell specific to grapefruit. The capsule sizes are smaller than those presented by Chang *et al*. In their study, they emphasize that the capsules size depends by calcium chloride concentration and at 2 % concentration the size was 2.5 mm [28]. The antioxidant activity is much higher than those observed by Crespo *et al*. for *Apium graveolens L*. and *Coriandrum sativum L.,* but not for *Thymus vulgaris L.* EOs. For example, after

evaluation using ABTS method, the *Apium graveolens L.* has 3.2 % inhibition, *Coriandrum sativum L.* 1.6 %, and for *Thymus vulgaris L.* was 96.5 % [29].
One of the possible changes of product with capsules addition is the change of color due to disintegration of the added substances and release of components. According to the results presented in Table 2, the color of the control sample (C) did not change due to capsules, no matter in what stage of the development process they were added. Furthermore, the results indicated that capsules, whose luminosity was 40.19, did not change the final products' parameters: luminosity was 78.79 for control and 76.78, respectively 76.32 for samples with 20 % addition. The results conclude that sodium alginate is good as coating material and did not permit the release of incorporated substance.
The color parameters difference between 10 and 20 % addition is very small, and these modifications cannot be noticed to the naked eye.

***Table 2.*** *Color evaluation of yogurt samples*

| ***Sample*** | ***Color*** | | |
|---|---|---|---|
| | ***L**** | ***a**** | ***b**** |
| **C** | 78.79 ± 0.01 | -2.61 ± 0.01 | 7.98 ± 0.03 |
| **S1** | 76.78 ± 0.83 | -2.26 ± 0.04 | 8.13 ± 0.73 |
| **S2** | 76.52 ± 0.96 | -2.10 ± 0.14 | 8.66 ± 0.23 |
| **S3** | 77.29 ± 0.21 | -2.28 ± 0.03 | 8.38 ± 0.02 |
| **S4** | 76.32 ± 0.05 | -2.12 ± 0.07 | 8.62 ± 0.30 |

The physicochemical properties of yogurt have not been influenced by capsules. The *p*H values were slightly lower (almost 0.12/0.15 units for 10 % / 20 % addition before fermentation process). These results may indicate that the additions could interfere with the fermentation process and during storage; it may lead to changes in acidity. The future research perspectives aim to assess the product during storage throughout its shelf life. The acidity of the control sample was higher (104 °T) than those of samples. Regarding samples with addition, according to the results presented in Table 3, the acidity presented lower values for product with 20 % capsules addition, no matter when these were added into yogurt. *p*H values were higher than those presented by Abedi *et al*. (4.01 for yogurt with fennel EO microcapsules and 4.06 for control) [30].

***Table 3.*** *Physicochemical characteristics of yogurt samples*

| **Sample** | **S [%]** | **WHC [%]** | ***p*H** | **Acidity [°T]** |
|---|---|---|---|---|
| **C** | 36.00 ± 0.06 | 41.98 ± 0.15 | 4.57 ± 0.02 | 104.00 ± 0.01 |
| **S1** | 27.00 ± 0.03 | 33.88 ± 0.48 | 4.43 ± 0.01 | 101.00 ± 0.03 |
| **S2** | 31.50 ± 0.02 | 38.11 ± 0.45 | 4.42 ± 0.02 | 103.00 ± 0.01 |
| **S3** | 21.50 ± 0.06 | 35.24 ± 0.33 | 4.49 ± 0.01 | 101.00 ± 0.01 |
| **S4** | 31.00 ± 0.01 | 34.28 ± 0.21 | 4.50 ± 0.02 | 102.00 ± 0.02 |

The same pattern of decreasing *p*H values after additions was observed by Lee *et al*. In the study regarding the development of a yogurt with microencapsulated peanut sprout extract, they stated that the increasing the concentration of capsules led to decreasing of *p*H value [31]. In our study we must take into account the acidic character of citrus

extract which may influence the final product acidity. Even so, the differences are small and did not affect the yogurt properties. Furthermore, according to studies that tested the samples with EO microcapsules during storage period, the changes in acidity and *p*H were higher in control sample than those observed at sample with additions, due to encapsulation structure protective effect on the system [32].
Syneresis and water holding capacity are very important for industry [33]. The syneresis of all samples with grapefruit EO encapsulated was lower than the control samples. For yogurt with 10 % addition (S1, S2), the differences were higher than control (C): 9, respectively 14.5 %. The results can be correlated with WHC results; the lowest value was obtained for sample 1, with 10 % grapefruit EO capsules added before fermentation process (33.88 % instead of 41.98 % of control). In our case, the 10 %, respectively 20 % of capsules, regardless of the moment of addition in the product, presented better values of syneresis and water holding capacity than the control sample.
These findings are of great interest for obtaining the product on an industrial scale and are favorable for the development of yogurt with capsules.

## CONCLUSIONS

The aim of this paper was to develop a new functional food product. Due to the ease in capsules manufacturing process, the yogurt with grapefruit EO encapsulated can be develop at industrial scale. According to results, 10 %, respectively 20 % capsules did not affect the physicochemical properties of the control sample. The grapefruit EO can be added into food product before or after fermentation process but, according to our results, the capsules added in the same time with culture led to the development of a better products in terms of acidity, syneresis and water holding capacity. Future research is based on product's testing over time and focuses on the changes that may occur during storage.

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