



# Sensory and Physicochemical Characterisation of Functional Yoghurts Containing Probiotic *Lactobacillus rhamnosus* MGRE Enriched by Six Plants and Extracts.

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**ABSTRACT:** Prebiotics are functional foods with health-promoting properties that are used in many health and nutrition aspects. Combined with appropriate probiotics, they produce synbiotic products with high nutritional value and health benefits. In Egypt, many traditional organic plants are reported to have prebiotic properties. In this study, the potential prebiotic effects of three different plants (*Lepidium sativum*, date palm pollen, and date kernel powder) and three plant extracts (Hibiscus, Hyphaene, and Hibiscus+Hyphaene 1:1) were investigated at five concentrations each (1.0, 1.5, 2.0, 2.5, and 3.0% w/w). The selected plants were used to evaluate the prebiotic properties of the probiotic bacteria *L. rhamnosus* MGRE. Eighteen enriched yoghurts were further used to assess the sensory and physicochemical characteristics. Some of these selected plants have a prebiotic effect. *Lepidium sativum* had a superior effect on bacterial growth during fermentation;  $\Delta OD_{600}$  increased significantly from 0.10 to 1.19-1.55 (in a concentration-dependent manner), while Hibiscus negatively influenced bacterial growth compared to control. The pH of the yoghurt trials ranged from 4.34 to 4.45, which was close to that of the control, with a difference of  $\pm 0.11$ . The viscosity of the trials ranged from 1319 to 2816 mPa.S, higher than that of the control (1395 mPa.S), except for the treatment containing 2% *Lepidium sativum* (1319 mPa.S). The yoghurt prepared with *Lepidium sativum*, and Hyphaene showed the best organoleptic properties with synbiotic potential. It seems promising that *Lepidium sativum*, Hyphaene, and date kernel could be used as prebiotics.

**Keywords:** prebiotics; synbiotics; fortification; organoleptic properties; pH; viscosity

## INTRODUCTION

Food fortification is regarded as a very effective and cost-efficient public health strategy currently available. It is defined as “the addition of one or more essential nutrients to food, whether or not they are normally present in the food, to prevent/correcting a demonstrated deficiency of a nutrient(s) in the population or specific population groups” (Alimentarius, 1994). It is practised in areas where the problems of malnutrition are prevalent. According to FAO/WHO guidelines (FAO/WHO, 1995), essential nutrients can be added for many reasons, starting from replacing losses during manufacturing to providing a balanced intake of micronutrients in a specific case (dietetic foods) (Arora *et al.*, 2011; Gomaa *et al.*, 2022).

In the food industry, consumers have become increasingly aware of the relationship between diet and health. Thus, the demand for a balanced diet and functional food products that provide certain health benefits is growing progressively. The addition of probiotic strains, prebiotic substances, or e in the form of synbiotics

is one of the latest widely used approaches that provide nutritional benefits to consumers while preserving food.

Probiotics are “live microorganisms which when administered in adequate amounts confer a health benefit on the host” (FAO/WHO, 2001). Lactic acid bacteria (LAB) have a long history of safe use and are part of the gut microflora of humans and animals. In addition, LAB produce several antimicrobial substances such as organic acids, hydrogen peroxide, bacteriocins, and bacteriocin-like compounds that are already used by the food industry (O’Byrne *et al.*, 2015; Allam *et al.*, 2018; Teneva-Angelova *et al.*, 2018). A prebiotic is “a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon”. Prebiotics are found as natural components in some fruits and vegetables such as onion, artichoke, chicory, leek, garlic, banana, rye, barley, and salsify (Mussatto and Mancilha, 2007). Isomaltulose is a potential prebiotic that is present naturally in honey,

sugarcane juice, and products derived from them (treacle or food-grade molasses) (Lina *et al.*, 2002). Moreover, xylooligosaccharides (XOS) are also an emerging prebiotic found in bamboo shoots, fruits, vegetables, milk, and honey (Vazquez *et al.*, 2000). Galactooligosaccharides are naturally present in human and bovine milk (Alander *et al.*, 2001). Seeds of legumes, lentils, peas, beans, chickpeas, and mustard are rich in raffinose oligosaccharides (Johansen *et al.*, 1996; Sánchez-Mata *et al.*, 1998). Prebiotics alter the composition of the microflora of the colon and lead to the dominance of some potentially health-promoting bacteria, especially, but not exclusively, lactobacilli and bifidobacteria (Gibson and Roberfroid, 1995).

The mixture of probiotics and prebiotics is referred to as “synbiotics,” which positively affect the host by enhancing the survival and implantation of probiotics in GIT (Collins and Gibson, 1999; Gibson *et al.*, 1999). The term synbiotics is used to demonstrate a synergistic effect and should be used to describe that prebiotics can specifically support the development of probiotics (Roberfroid *et al.*, 2010).

The significance of synbiotics as dietary supplements for the prevention of non-communicable and communicable diseases has only recently been recognized. It is a novel area of research in the field of functional foods and nutraceuticals that is being explored and is becoming increasingly important because of its many hidden health benefits.

Fortification should not alter the organoleptic properties (taste, smell, colour, consistency) and shelf life (conditions for storage and transportation) of the product (Gomaa, 2015). There is often a delicate balance between bioavailability and other properties of fortified food. Milk and milk products are a convenient and useful vehicle for fortification with micronutrients. The risks associated with fortification are minimal except if good manufacturing practices are not followed. This study aims to design new functional dairy products without any negative effects on organoleptic properties. Using plant parts and plant extracts in fortification will increase the nutritional value of the final product in addition to its prebiotic effect.

## MATERIALS AND METHODS

### 2.1. Strains origin and culturing conditions

Lactic acid bacteria (LAB) cultures used in this study (*Lactobacillus delbrueckii* subsp. *bulgaricus* STY9, *Streptococcus thermophilus* STY1, and *Lactobacillus rhamnosus* MGRE) were obtained from Food Science Department, Faculty of Agriculture, Saba Basha, Alexandria University, Culture Collection (FABA).

All strains were maintained for long preservation on MRS slant agar (De Man *et al.*, 1960) (Biolife, Italy) for further study. Bacterial growth from the slant culture was reactivated in 10 ml broth medium at 37 °C for 24 h at anaerobic conditions.

### 2.2. Media and growth conditions

Selective enumeration of *Lactobacillus delbrueckii* subsp. *bulgaricus* STY9 and *Lactobacillus rhamnosus* MGRE was performed on pH-modified (4.58) MRS (deMann, Rogasa, and Sharpe) agar (Oxoid Ltd., Hampshire, England); M-17 agar (Oxoid Australia Ltd) was used for selective enumeration of *Streptococcus thermophilus* STY1 (Dave & Shah, 1996). All bacteria were incubated in anaerobic jars (AnaeroGen™, Oxoid Ltd., Hampshire, England), except for *S. thermophilus*, which was incubated under aerobic conditions. *L. delbrueckii* subsp. *Bulgaricus*, *L. rhamnosus* MGRE, and *S. thermophilus* were incubated for 24 h at 37, 37, and 45 °C, respectively.

### 2.3. Inoculums preparation

The inoculum for bacterial cultures was prepared using a direct colony suspension method. Micro-organisms were cultured in MRS or M-17 agar for 24 hours. Colonies were then transferred directly from the isolated colonies to the appropriate broth medium and vortexed for 2 min using a vortex mixer (Falc, Italy).

### 2.4. Growth of probiotic strains under concentrations of selected plants.

The inoculum of the probiotic bacterial strain *L. rhamnosus* MGRE was prepared by suspending the bacteria in sterile 0.85% NaCl (0.5 McFarland standard equivalent concentration of 10<sup>8</sup> CFU/mL) and serially diluting (1:100; corresponding to ~1 × 10<sup>6</sup> CFU/ml during the colony count assay). The MRS broth (5 mL) was then inoculated with 100 µL of the bacterial suspension, which was supplemented with three different plants (*Lepidium sativum*, date palm pollen, and date kernel powder) and three plant extracts (Hibiscus, Hyphaene, and Hibiscus+Hyphaene 1:1), each at five concentrations (1.0, 1.5, 2.0, 2.5, and 3.0% w/w), in addition to the control under aseptic conditions, and incubated at 37 °C. The optical density at 600 nm (OD<sub>600</sub>) of each culture was determined after 0, 18, 24 and 48 h, respectively. Subsequently, the delta optical density (ΔOD) of bacteria in each selected plant was calculated by subtracting the initial OD from the reading and expressed as log ΔOD<sub>600</sub>, as described by Reuben *et al.* (2019).

### 2.5. Milk

Fresh full-fat buffalo milk was purchased from the local market in Alexandria Governorate. The milk samples were collected and transported

in an ice box under cooling within 60 min to the laboratory where the experiments were carried out.

### 2.5.1. Milk analysis

The fat, SNF, protein, lactose, density, freezing point (calculated), and ash of milk samples were determined using the Funke Gerber 3510 Laktostar milk content analyser (Funke Gerber, Berlin, Germany) according to the manufacturer's instructions. The measurement depends on a thermo-optical procedure combination. The milk sample (12 to 20 ml) was pumped into two different measuring cells. It is analyzed through these two measuring units: the blue box (opto-unit) and the red box (thermal unit). This indicates that the milk samples are analyzed using a completely different measuring method. The blue box is a turbidity measurement that analyzes the undissolved (visible) substances, fat and protein. It is also involved in impedance or conductivity measurement. The red box contains two thermoanalytical measuring cells; the measurement is performed at two different temperatures (40°C/65°C). The fat content and fat-free dry matter were measured by thermal effects at different measurement temperatures (Yıldız, 2008).

### 2.5.2. Milk pH determination

The pH of the full-fat buffalo milk used in yoghurt production was measured with a pH meter (Jenway 3505, England).

### 2.6. Yoghurt manufacture

Yoghurt was prepared by lactic acid fermentation of buffalo milk heat treated at 80°C for 10 minutes. Probiotic starter culture (*L. delbrueckii subsp. Bulgaricus*, *S. thermophilus* and *L. rhamnosus* MGRE) was added at a ratio of 1:1:1 to reach 10<sup>8</sup> cfu/mL in the final mixture at 42 °C and mixed well. The inoculated milk was poured into cups, 100 mL each, and incubated at 42 °C for about 3 h to reach a pH of 4.5-4.6, followed by cooling at 4 °C. Different eighteen treatments were prepared using three different plants (*Lepidium sativum*, date palm pollen, and date kernel powder) and three plant extracts (Hibiscus, Hyphaene, and Hibiscus+Hyphaene 1:1) each at five concentrations (1, 1.5, 2, 2.5, and 3% w/w), in addition to the control.

#### 2.6.1. pH and viscosity determination

The pH values of the yoghurt samples were measured with a pH meter (Jenway 3505, England).

The viscosity of the yoghurt treatments was measured at 15 °C using a viscometer (D.P. SELECTA, S.A. ST-2020R, Korea) at a speed of 60 to 200 rpm with spindle R5. The viscosity

expressed in mPa.s. and the temperature is automatically corrected.

### 2.6.2. Diacetyl and acetaldehyde contents in Yoghurt

For the determination of diacetyl, the method of Owades and Jakovac modified by Pack et al. (1964) was used. The sample (1 g) was used instead of the 20 g described in the original procedure to accommodate the modern equipment. Acetaldehyde was determined on a separate aliquot of the culture at the same time as diacetyl using a modified 3-methyl-2-benzothiazolone hydrazone procedure (Lindsay and Day, 1965).

### 2.7. Sensory evaluation

Nine experienced panellists aged 20–61 years, participated in the evaluation of the sensory attributes of yoghurt samples one day after production. The tasting panel consisted of students and staff members from the Faculty of Agriculture, Saba Basha, Alexandria University. The yoghurt samples (100 mL cups) were presented on white plates in random order to the panellists in random order. The samples were organoleptically evaluated after 1 day of manufacturing. All sensory attributes assessed by the panellists were rated on a 5-point scale, with the worst characteristic scored as 1 and the best as 5. The procedures for evaluating the sensory properties of yoghurts were divided into flavour (smell and taste), appearance, and texture.

First, the smell was assessed by removing the yoghurt cup lid and rating the intensity of the volatile odorants. Second, appearance was evaluated by visual observation and textural properties by breaking the yoghurt and agitating the product. Finally, the taste of yoghurts was assessed by swallowing 10 g (a teaspoon portion) of the sample. Overall acceptance was rated at the end of the sensory evaluation of each sample (Soukoulis et al., 2007).

### 2.8. Statistical analysis

The statistical analysis was performed by the IBM SPSS program 25, Armonk, New York, United States. The data were analyzed by one-way ANOVA tests at a confidence level of 95% ( $p < 0.05$ ); the obtained data were expressed in the mean of three replicates  $\pm$  standard deviation (SD).

## 3. RESULTS AND DISCUSSIONS

### 3.1. Milk chemical composition and physical properties

Table 1 shows the chemical composition and physical properties of whole buffalo milk: the milk consisted of 6.36% fat. In addition, the milk comprises 11.28% SNF, including 3.73% protein, 5.41% lactose, and 1.70%. The milk had a density of 1.0355 g/cm<sup>3</sup> and a freezing point of - 0.530 °C; the milk had a pH of 6.7 at 4.5 °C.

**Table 1: Chemical composition and physical properties of full-fat buffalo milk used in Yoghurt making.**

Component	Average value
Fat (%)	6.39 ± 0.01
SNF (%)	11.28 ± 0.01
Protein (%)	3.73 ± 0.02
Lactose (%)	5.41 ± 0.01
Density g/cm <sup>3</sup>	1.0355 ± 0.00
Freezing point (°C)	-0.530 ± 0.00
Ash (%)	1.70 ± 0.00
pH	<b>6.70 ± 0.00</b>

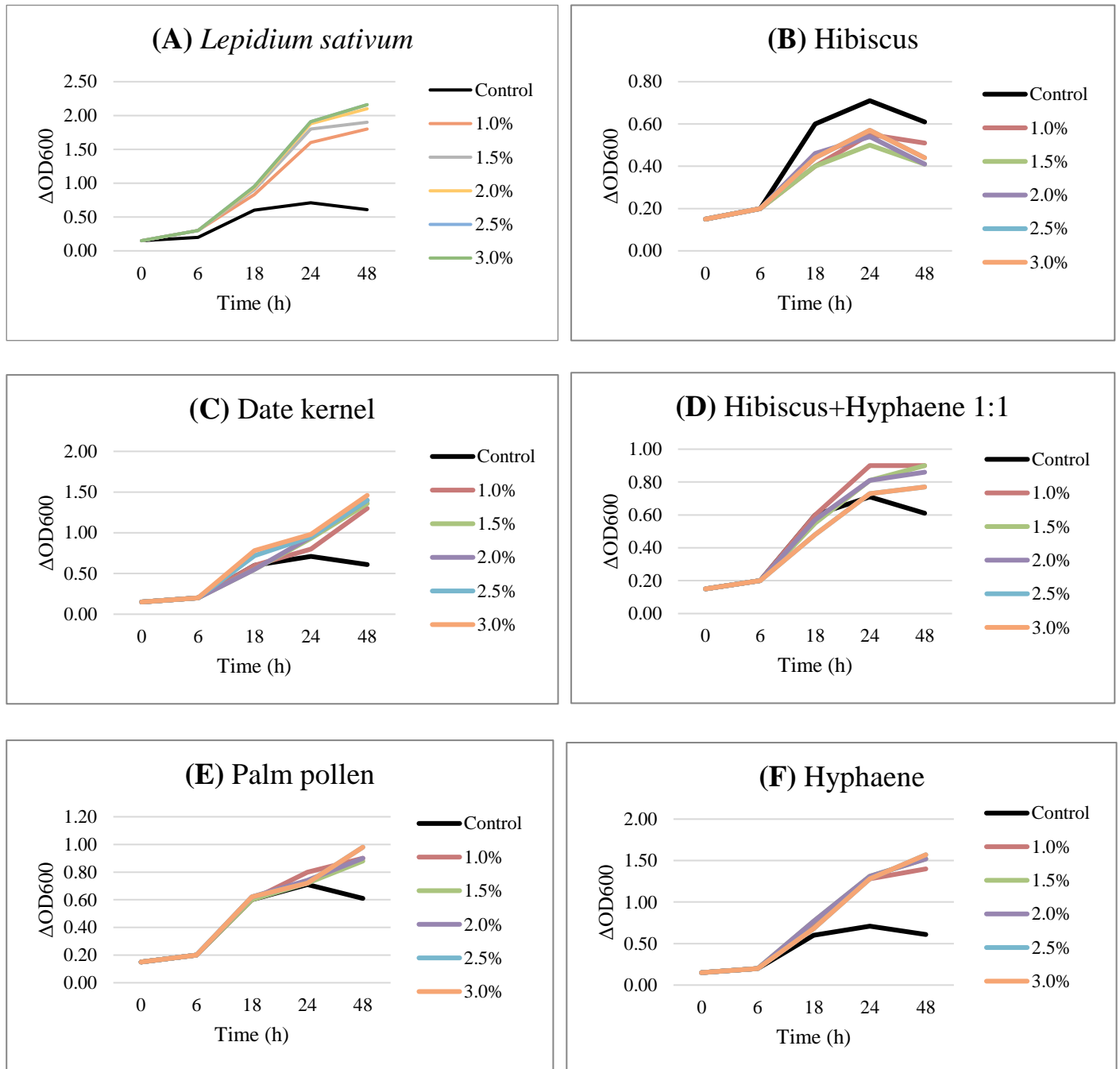
Data are presented as mean ± SD

### 3.2. Relationship of concentration of selected plant and growth curves probiotic strains

Bacterial growth ( $OD_{600}$ ) during 0 - 48 h fermentation was significantly affected by the additive and its concentration (1–3% w/w) (Figure 1). The addition of *Lepidium sativum* increased  $OD_{600}$  throughout the fermentation period (Figure 1A). The increase was directly related to the concentration of the additive ( $p < 0.05$ ):  $\Delta OD_{600}$  increased from 0.10 after 0 h of fermentation to 0.23-0.35, 0.89-1.20, and 1.19-1.55 after 6, 18, 24, and 48 h respectively. A similar pattern was observed when hyphaene extract and date kernel powder 1-3% were added. At all concentrations of hyphaene extract and date kernel powder, an increase in  $\Delta OD_{600}$  of 0.79 - 0.96 (Figure 1F) and 0.069 - 0.85 (Figure 1C), respectively, was observed after 48 h, which was higher than the control. However, using palm pollen or Hibiscus+Hyphaene extract in a 1:1 ratio resulted

in  $\Delta OD_{600}$  values close to the control until 18 h after fermentation ( $p > 0.05$ ), Figures 1E and 1D, respectively. From 24 h of fermentation onward, a slight increase in  $OD_{600}$  values was observed compared to the control. On the other hand, hibiscus extract caused a decrease in  $\Delta OD_{600}$  readings for all concentrations between 0.10 and 0.17 units compared with the control ( $p < 0.05$ ) (Figure 1B).

Similarly, Goderska (2019) reported that prebiotic properties are strain-dependent, so they can only be used judiciously if their effect on a specific probiotic strain is known. The effect depends on the dose, the type of prebiotic, and the species of probiotic microorganism; each strain needs to be tested individually. This combination can be employed as a synbiotic by choosing the right prebiotic to best stimulate the growth of a specific probiotic bacterium.



**Figure 1:** Symbiotic relationship between *Lactobacillus rhamnosus* MGRE probiotic strain and three different plants (*Lepidium sativum*, Date palm pollen, and Date kernel powder) and three plants extracts (Hibiscus, Hyphaene, and Hibiscus+Hyphaene 1:1) each in five levels (1.0, 1.5, 2.0, 2.5, and 3.0% w/w), in addition to the control. The figure shows the growth curve at 0, 18, 24 and 48 h reported as  $\Delta OD_{600}$ .

### 3..Yoghurt fermentation time

The fermentation time of the control and enriched yoghurts varied between 2h and 23min to 3h and 21min (Table 2): The lowest value was for the control, while the longest period was for the samples with 3% *Lepidium sativum* and palm pollen. The data showed that the incubation times of yoghurt production changed gradually with the addition of all plants or extracts. Each 1% increase in plant concentration significantly changed the

fermentation time by 5-15 min (10 min on average). However, the incubation times of the date kernel-containing trials at different concentrations (1, 1.5, 2, 2.5, and 3%) were similar to those of the control yoghurt ( $p > 0.05$ ). The prolongation of the coagulation time could be due to the antioxidant and antimicrobial effects of the additives.

### 3.4 Chemical Composition and physical properties of the Yoghurts

#### 3.4.1.pH values

The pH of plain-yoghurt samples containing 0 (control), 1.0, 1.5, 2.0, 2.5, and 3% of all trials ranged from 4.34 to 4.45. All Yoghurt samples showed similar or close pH values to the control, with a difference of  $\pm 0.11$ , as shown in Table (2).

The acidification of milk is the most important step in yoghurt production. It controls most of the chemical and rheological characteristics of the final product (Dalglish and Law, 1989; Lucey, 2004). The applied treatments did not cause a great change in the final pH compared to the control. Therefore, it can be concluded that the fortification did not affect the final pH of the yoghurt (Table 2).

#### 3.4.2.Viscosity

The viscosity of the yoghurts was measured after manufacturing and overnight cooling; it ranged from 1319 and 2816 mPa.S (Table 2). All samples

had a higher viscosity than the control (1395 mPa.S), except for the treatment containing 2% *Lepidium sativum*, which recorded the lowest viscosity value (1319 mPa.S). The highest viscosity (2816 mPa.S) was observed in the presence of 1.5 % Hyphaene. The viscosity value of the samples containing date kernel was slightly higher than the control but lower than the other trials, ranging from 1691 to 1793 mPa.S. The resulting yoghurts had lower viscosity values than those reported by Hanif et al. (2012) (2340 - 2800 mPa), which could be because adding fruits and other additives affects the viscosity of the yoghurts, as mentioned by Labropoulos et al. (1984). **Table 2: Incubation periods, pH value, and viscosity of plain yoghurt and yoghurt enriched with five concentrations (1, 1.5, 2, 2.5, and 3%) of *Lepidium sativum*, Hibiscus, Hyphaene, Hibiscus+Hyphaene 1:1, and Date palm.**

**Table 2: Incubation periods, pH value, and viscosity of plain yoghurt and yoghurt enriched with five concentrations (1, 1.5, 2, 2.5, and 3%) of *Lepidium sativum*, Hibiscus, Hyphaene, Hibiscus+Hyphaene 1:1, and Date palm.**

Trial	Yoghurt treatment	The incubation period	pH	Viscosity (mPa.S.)
Control	Plain	2:23	4.43 $\pm$ 0.01	1395 $\pm$ 3.06
	Lepidium sativum	2:35	4.41 $\pm$ 0.06	1998 $\pm$ 2.52
1%	Hibiscus	2:31	4.45 $\pm$ 0.03	2471 $\pm$ 2.08
	Hyphaene	2:32	4.44 $\pm$ 0.00	2566 $\pm$ 1.53
	Hibiscus+Hyphaene 1:1	2:33	4.42 $\pm$ 0.02	2416 $\pm$ 3.21
	Palm pollen	2:33	4.42 $\pm$ 0.02	2542 $\pm$ 2.08
	Date kernel	2:23	4.34 $\pm$ 0.03	1716 $\pm$ 2.08
	Lepidium sativum	2:48	4.44 $\pm$ 0.01	2198 $\pm$ 3.61
1.5%	Hibiscus	2:45	4.4 $\pm$ 0.01	2720 $\pm$ 2.89
	Hyphaene	2:47	4.39 $\pm$ 0.01	2816 $\pm$ 3.00
	Hibiscus+Hyphaene 1:1	2:45	4.41 $\pm$ 0.00	2654 $\pm$ 3.61
	Palm pollen	2:47	4.42 $\pm$ 0.01	2756 $\pm$ 2.08
	Date kernel	2:24	4.35 $\pm$ 0.02	1771 $\pm$ 3.06
	Lepidium sativum	3:00	4.38 $\pm$ 0.00	1319 $\pm$ 3.46
2%	Hibiscus	3:00	4.41 $\pm$ 0.01	1621 $\pm$ 1.53
	Hyphaene	3:02	4.4 $\pm$ 0.00	1701 $\pm$ 1.15
	Hibiscus+Hyphaene 1:1	3:00	4.42 $\pm$ 0.01	1599 $\pm$ 2.52
	Palm pollen	3:01	4.2 $\pm$ 0.18	1705 $\pm$ 2.08
	Date kernel	2:26	4.44 $\pm$ 0.05	1691 $\pm$ 2.08
	Lepidium sativum	3:07	4.38 $\pm$ 0.01	1833 $\pm$ 2.08
2.5%	Hibiscus	3:05	4.43 $\pm$ 0.01	2264 $\pm$ 2.52
	Hyphaene	3:06	4.41 $\pm$ 0.01	2318 $\pm$ 2.52
	Hibiscus+Hyphaene 1:1	3:05	4.45 $\pm$ 0.01	2215 $\pm$ 3.06
	Palm pollen	3:06	4.44 $\pm$ 0.00	2308 $\pm$ 3.06
	Date kernel	2:28	4.42 $\pm$ 0.01	1793 $\pm$ 2.00
	Lepidium sativum	3:21	4.38 $\pm$ 0.03	1827 $\pm$ 2.31
3%	Hibiscus	3:15	4.44 $\pm$ 0.00	2256 $\pm$ 3.06
	Hyphaene	3:16	4.42 $\pm$ 0.01	2332 $\pm$ 3.21
	Hibiscus+Hyphaene 1:1	3:15	4.45 $\pm$ 0.07	2209 $\pm$ 0.58
	Palm pollen	3:21	4.44 $\pm$ 0.03	2309 $\pm$ 2.08
	Date kernel	2:28	4.42 $\pm$ 0.00	1793 $\pm$ 3.21

Data are presented as mean  $\pm$  SD

### 3.4.2. Diacetyl and acetaldehyde contents

A gradual increase in acetaldehyde and diacetyl concentrations was observed in the fortified treatments containing *Lepidium sativum*, Hibiscus, Hyphaene, Hibiscus+Hyphaene 1:1, and date palm compared with the control (Table 3). In the case of plain yoghurt, the acetaldehyde concentration was the lowest (9.04 ppm). On the other hand, the yoghurt with 2.5% Hyphaene had the highest acetaldehyde content (13.28 ppm). The same trend was observed for diacetyl concentrations: the control recorded the lowest value (10.16 ppm), while yoghurts with 2 and 3% Hyphaene reached a diacyl concentration of 17.07 ppm.

Concerning the fortified yoghurt, all treatments achieved higher acetaldehyde and diacetyl concentrations than the plain yoghurt up to 46%. Samples containing Hyphaene had higher contents than those of the other treatments. The data also showed that all fortified yoghurt samples had a slight increase in acetaldehyde and diacetyl in the presence of a high concentration of the plants and extracts. Such a trend reflects a positive effect on the starter culture, marking the probability of a prebiotic effect. This point needs further investigation in order to assess the prebiotic properties of all additives.

**Table 3: Acetaldehyde and diacyl concentrations of plain yoghurt and yoghurt enriched with five concentrations (1, 1.5, 2, 2.5, and 3%) of *Lepidium sativum*, Hibiscus, Hyphaene, Hibiscus+Hyphaene 1:1, and Date palm.**

Trial	Yoghurt Treatment	Acetaldehyde (ppm)	Diacetyl (ppm)
<b>Control</b>	Plain	9.04	10.16
	Lepidium sativum	9.37	10.60
<b>1%</b>	Hibiscus	12.28	16.18
	Hyphaene	12.95	16.96
	Hibiscus+Hyphaene 1:1	11.72	15.62
	Palm pollen	12.28	16.18
	Date kernel	12.50	16.29
	Lepidium sativum	9.60	10.38
<b>1.5%</b>	Hibiscus	12.28	15.96
	Hyphaene	12.83	16.63
	Hibiscus+Hyphaene 1:1	12.05	15.40
	Palm pollen	12.50	16.07
	Date kernel	13.06	17.07
	Lepidium sativum	9.71	10.71
<b>2%</b>	Hibiscus	12.61	16.52
	Hyphaene	13.17	17.07
	Hibiscus+Hyphaene 1:1	12.28	15.51
	Palm pollen	12.95	16.07
	Date kernel	11.38	15.74
	Lepidium sativum	9.82	11.16
<b>2.5%</b>	Hibiscus	12.50	16.74
	Hyphaene	13.28	17.07
	Hibiscus+Hyphaene 1:1	12.28	15.85
	Palm pollen	12.95	16.52
	Date kernel	12.16	16.29
	Lepidium sativum	10.16	11.49
<b>3%</b>	Hibiscus	12.50	16.63
	Hyphaene	13.06	17.07
	Hibiscus+Hyphaene 1:1	12.50	15.85
	Palm pollen	13.15	16.52
	Date kernel	12.16	16.29

Data are presented as mean  $\pm$  SD

### 3.5. Sensory evaluation

Sensory analysis of the yoghurt trials was carried out by nine experienced panellists. The panellists evaluated each treatment based on four main criteria: smell, taste, appearance, body and texture, and overall grade.

Table (4) demonstrates the scores of sensory attributes of plain and fortified yoghurt with three different plants (*Lepidium sativum*, date palm pollen, and date kernel powder) and three plant extracts (Hibiscus, Hyphaene, and Hibiscus+Hyphaene 1:1) at five concentrations (1, 1.5, 2, 2.5 and 3%). Evaluation of the smell scores of the samples showed that the samples containing *Lepidium sativum* gained the highest, which were close to the control with an overall score of 4.5. Samples containing 1-3% Hibiscus, or Hibiscus+Hyphaene 1:1 achieved higher than samples that contained only 1-3% Hyphaene, with average overall scores of 4.0, 4.0, and 3.5, respectively. Panellists rated the taste of the three treatments as creamy and acidic, in addition to the control. The control treatment received the highest score of 5.0; the treatment containing *Lepidium sativum* was very close to the control, with a score of 4.5. The treatments with Hibiscus or Hibiscus +Hyphaene 1:1 scored 3.5 points, and the other treatments scored the lowest (3 points).

Most treatments produced a well-formed plain yoghurt that resembled the control in appearance. The appearance of the yoghurt samples containing *Lepidium sativum* and Hyphaene was similar to the control treatment (soft and thick), scoring 4.0 and 3.5, respectively. A slightly different attribute was reported in the case of Hibiscus or Hibiscus +Hyphaene 1:1 as thick and coloured in violet-red, with a score of 3.0. On the other hand, treatments containing date palm pollen or date kernel were heterogeneous, with scores of 3.5.

Comparison of the “body and texture” attribute of the samples showed that samples containing

*Lepidium sativum*, Hibiscus, or Hibiscus +Hyphaene 1:1 were thick with a score of 3.5 in all treatments. When Hyphaene was added at concentrations 1-3% body and texture were light with a rating of 3.0. All treatments containing date palm pollen or date kernel had a floury or sandy texture, respectively as negative attributes in contrast to the control, with the lowest rating (2.5).

The overall acceptance score for all samples ranged from 70 to 97%. The highest value was obtained for the control, while the yoghurt samples containing *Lepidium sativum*, Hibiscus, or Hibiscus +Hyphaene 1:1 had excellent overall scores of 90, 85, and 85%, respectively. Treatments containing 1-3% Hyphaene scored high overall score (80%). The lowest overall score (70%) was recorded for treatments containing date palm pollen or date kernel. It should be mentioned that the scores of all samples were higher than the unacceptable limit (60%).

No obvious differences in sensory attributes were found between the control and the samples containing *Lepidium sativum*. Slight deviations were noted in the case of Hibiscus, or Hibiscus +Hyphaene 1:1, except for colour. Samples containing Hyphaene were overall very good but slightly lighter in texture. Floury or sandy texture (negative attributes) was noted as the least favourable trial for samples containing date palm pollen or date kernel, averaging 2.5 points.

### 4. CONCLUSION

There are an expanding number of synbiotics on the market. Prebiotics influence the survivability of probiotic bacteria in products during storage and promote their development. It appears encouraging that *Lepidium sativum*, Hyphaene, and date kernel could be used as prebiotics. Yoghurt prepared using *Lepidium sativum* and Hyphaene showed the best organoleptic properties with synbiotic potential.



**Table 4: Sensory evaluation of plain and fortified Yoghurt with *Lepidium sativum*, Hibiscus, Hyphaene, Hibiscus+Hyphaene 1:1, Date palm pollen, and Date kernel in five concentrations 1, 1.5, 2, 2.5 and 3%.**

Treatments	Smell		Taste		Appearance		Body and texture		Overall score 100%	
	Score <sup>a</sup>	Description	Score <sup>a</sup>	Description	Score <sup>a</sup>	Description	Score <sup>a</sup>	Description		
<b>Control</b>		5.0	Yoghurt, milky	4.5	Creamy, Acidic	5.0	Soft, Thick	5.0	Homogenous	97
<b>Lepidium sativum</b>	1.0%	4.5	Yoghurt, cheesy	4.0	Creamy, Acidic	4.5	Soft, Thick	3.5	Light, Thick	90
	1.5%	4.5	Yoghurt, cheesy	4.0	Creamy, Acidic	4.5	Soft, Thick	3.5	Light, Thick	90
	2.0%	4.5	Yoghurt, cheesy	4.0	Creamy, Acidic	4.5	Thick	3.5	Thick	90
	2.5%	4.5	Yoghurt, cheesy	4.0	Creamy, Acidic	4.5	Thick	3.5	Thick	90
	3.0%	4.5	Yoghurt, cheesy	4.0	Creamy, Acidic	4.5	Thick	3.0	Thick	88
<b>Hibiscus</b>	1.0%	4.0	Yoghurt	3.5	Creamy, Acidic	3.0	Thick, Colored	3.5	Thick	85
	1.5%	4.0	Yoghurt	3.5	Creamy, Acidic	3.0	Thick, Colored	3.5	Thick	85
	2.0%	4.0	Yoghurt	3.5	Creamy, Acidic	3.0	Thick, Colored	3.5	Thick	85
	2.5%	4.0	Yoghurt	3.5	Creamy, Acidic	3.0	Thick, Colored	3.5	Thick	85
	3.0%	4.0	Yoghurt	3.5	Creamy, Acidic	3.0	Thick, Colored	3.5	Thick	85
<b>Hyphaene</b>	1.0%	3.5	Yoghurt	3.0	Creamy, Sweety	3.5	Soft, Thick	3.0	Light	80
	1.5%	3.5	Yoghurt	3.0	Creamy, Sweety	3.5	Soft, Thick	3.0	Light	80
	2.0%	3.5	Yoghurt	3.0	Creamy, Sweety	3.5	Soft, Thick	3.0	Light	80
	2.5%	3.5	Yoghurt	3.0	Creamy, Sweety	3.5	Soft, Thick	3.0	Light	80
	3.0%	3.5	Yoghurt	3.0	Creamy, Sweety	3.5	Soft, Thick	3.0	Light	80
<b>Hibiscus +Hyphaene 1:1</b>	1.0%	4.0	Yoghurt	3.5	Creamy, Acidic	3.0	Thick, Colored	3.5	Thick	85
	1.5%	4.0	Yoghurt	3.5	Creamy, Acidic	3.0	Thick, Colored	3.5	Thick	85
	2.0%	4.0	Yoghurt	3.5	Creamy, Acidic	3.0	Thick, Colored	3.5	Thick	85
	2.5%	4.0	Yoghurt	3.5	Creamy, Acidic	3.0	Thick, Colored	3.5	Thick	85
	3.0%	4.0	Yoghurt	3.5	Creamy, Acidic	3.0	Thick, Colored	3.5	Thick	85
<b>Date palm pollen</b>	1.0%	3.0	Yoghurt	3.0	Earthy	3.5	Heterogeneous	2.5	Floury	70
	1.5%	3.0	Yoghurt	3.0	Earthy	3.5	Heterogeneous	2.5	Floury	70
	2.0%	2.5	Yoghurt	3.0	Earthy	3.5	Heterogeneous	2.5	Floury	70
	2.5%	2.5	Yoghurt	3.0	Earthy	3.5	Heterogeneous	2.5	Floury	70
	3.0%	2.5	Yoghurt	3.0	Earthy	3.5	Heterogeneous	2.5	Floury	70
<b>Date kernel</b>	1.0%	2.5	Yoghurt	3.0	Acidic	3.5	Heterogeneous	2.5	Sandy	70
	1.5%	2.5	Yoghurt	3.0	Acidic	3.5	Heterogeneous	2.5	Sandy	70
	2.0%	2.5	Yoghurt	3.0	Acidic	3.5	Heterogeneous	2.5	Sandy	70
	2.5%	2.5	Yoghurt	3.0	Acidic	3.5	Heterogeneous	2.5	Sandy	70
	3.0%	2.5	Yoghurt	3.0	Acidic	3.5	Heterogeneous	2.5	Sandy	70

Score <sup>a</sup>: 1=bad, 2= fair, 3= good, 4=very good, 5=excellent

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## المخلص العربي

الخصائص الحسية والفيزيائية الكيميائية للزبادي الوظيفي المحتوي على بروبيوتيك  
*Lactobacillus rhamnosus* MGRE المدعوم بستة نباتات ومستخلصات.

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البريبايوتك هي أغذية وظيفية ذات خصائص تعزز الصحة وتستخدم في العديد من الجوانب الصحية والتغذية. جنبًا إلى جنب مع البروبيوتيك المناسب، فإنها تنتج منتجات synbiotic ذات القيمة الغذائية العالية والفوائد الصحية. في مصر، تم الكتابة عن أن العديد من النباتات العضوية التقليدية لها خصائص حيوية. في هذه الدراسة، تمت دراسة التأثيرات المحتملة لثلاثة نباتات مختلفة (بذور الشيا وحبوب لقاخ النخيل ومسحوق نواة البلح) وثلاثة مستخلصات نباتية (الكركديه و الدوم وخليط الكركديه مع الدوم بنسبة 1:1) عند خمسة تراكيز لكل منها (1.0 و 1.5 و 2.0 و 2.5 و 3.0٪ وزن / وزن). تم استخدام النباتات المختارة لتقييم خصائص البريبايوتك مع بكتيريا بروبيوتيك *L. rhamnosus* MGRE كما تم انتاج ثمانية عشر نوعًا من الزبادي الحيوي المدعوم لتقييم الخصائص الحسية والفيزيائية الكيميائية. بعض هذه النباتات المختارة لها تأثير بريبيوتيك. كان لبذور الشيا تأثير كبير على نمو البكتيريا أثناء التخمر. زاد  $\Delta OD_{600}$  معنويًا من 0.10 إلى 1.19-1.55 (بطريقة تعتمد على التركيز)، بينما أثر الكركديه سلبيًا على نمو البكتيريا مقارنة بالعينة الضابطة. تراوح الأس الهيدروجيني لتجارب الزبادي من 4.34 إلى 4.45، والذي كان قريبًا من مستوى العينة الضابطة ، بفارق  $\pm 0.11$ . تراوحت لزوجة التجارب من 1319 إلى 2816 mPa.S ، أعلى من تلك بالعينة الضابطة (1395 mPa.S)، باستثناء المعاملة التي تحتوي على 2٪ بذور الشيا (1319 mPa.S) أظهر الزبادي المحضر باستخدام الدوم وبذور الشيا أفضل الخصائص الحسية. يبدو من الواعد استخدام بذور شيا والدوم ومسحوق نواة البلح كمواد بريبيوتك.

## الكلمات المفتاحية:

بذور الشيا، البريبايوتك، synbiotics، الألبان الحيوية، خصائص حسية، اللزوجة