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Microencapsulated and Fresh Royal Jelly: Monitoring 10-HDA Content, Antibacterial and Antifungal Activity at Different Storage Periods

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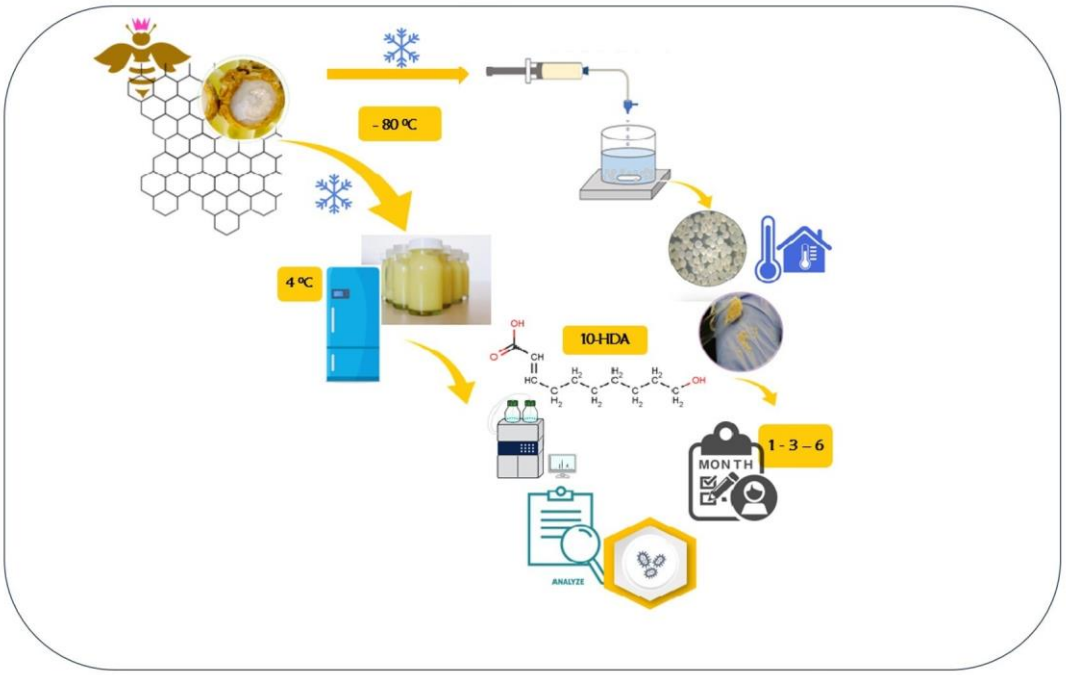
HIGHLIGHTS

- Microencapsulation maintained 10-HDA content and the antimicrobial activity of RJ for six months.
- A correlation was found between the antimicrobial activity and 10-HDA content.
- Microencapsulation of RJ is a promising technique enabling storage at room temperature.
- Innovative RJ products can be developed by advanced formula and microencapsulation.

Abstract: Royal Jelly (RJ) is a unique functional food having rich nutrient composition. Due to its extremely sensitive and perishable nature, the cold chain is inevitable to maintain the biological properties of RJ. Microencapsulation is considered as an alternative technology for commercial RJ forms, owing to the elimination of cold-chain requirements. The objective of the study is to evaluate the microencapsulation of RJ and its protective effect on the 10-HDA content as well as on the antimicrobial activity during a defined storage period. Microcapsules were formed by utilizing alginate cross-linking technique in the encapsulator (Buchi B-390, Flawil, Switzerland) under 450 mbar. The antibacterial and antifungal activity of fresh and microencapsulated royal jelly (MRJ) was examined, comparatively. The possible changes in antimicrobial activity in the 1st, 3rd, and 6th months were evaluated considering the changes in 10-HDA levels. The antimicrobial efficiency of RJ on test bacteria (*Micrococcus luteus, Staphylococcus epidermis, Salmonella Enteritidis, Escherichia coli*) and yeast (*Candida albicans, Candida parapsilosis*) was maintained throughout the storage period. On the other hand, antifungal activity on test molds (*Penicillium digitatum, Aspergillus flavus*) slightly decreased from the 3rd month. No significant difference between 10-HDA contents was observed until the end of storage (p>0.05). The results indicate that microencapsulation retains the 10-HDA content of RJ for six months and is a promising method enabling storage at room temperature.

Keywords: 10-HDA; antibacterial; antifungal; microencapsulation; royal jelly; storage

GRAPHICAL ABSTRACT



INTRODUCTION

Royal Jelly (RJ) is a secretion occurs by the biochemical reaction of nectar and pollen in the hypopharyngeal glands of worker bees and used to feed young larvae and the queen [1]. It is known as a "superfood" that plays a major role in caste differentiation through the key components that privilege the queen [2]. Morphological superiority, longevity, and fertility [1] given to the queen are associated with the special RJ diet, so it has become one of the most valuable natural products worldwide, used in traditional medicine, healthy foods, and cosmetics.

RJ, as a great source of essential nutrients with biological and health-promoting activities, mainly consists of proteins (9-18%), lipids (4-8%), carbohydrates (11-23%), amino acids, enzymes, vitamins, and minerals (0.8-3%) with a high content of water (60-70%) [3]. Proteins are the principal components of RJ and form about half of its dry matter. Bioactive ones are Major Royal Jelly Proteins (MRJP) that closely related to the therapeutic properties of RJ. In addition to the MRJPs, Royalisin, Apisimin, and Jelleines are also seen as the origin of pharmacological effect and gain multifunctional properties to RJ [4]. Lipid fraction, a characteristic and chemically interesting component in RJ consists mainly of medium-chain (8-10 carbon atoms) hydroxy and dicarboxylic free fatty acids, in contrast to the organic acids of animal or plant materials. A major fatty acid defined as trans-10-hydroxy-2-decanoic acid (10-HDA) is the predominant fatty acid among them and known as royal jelly or queen bee acid due to being a unique fatty acid that exists only in RJ [5]. 10-HDA is used as a marker to define the authenticity and quality of RJ and exhibits a broad spectrum of therapeutic and regenerative properties including antioxidant, anti-inflammatory, immunomodulatory, and antimicrobial activity [6].

The health-promoting properties of RJ are quite diverse due to the complex matrix of these bioactive compounds. Beyond being an antioxidant and antimicrobial agent, RJ possesses anti-lipidemic, antiproliferative, neuroprotective, antiaging, and estrogenic activity [7]. It also exhibits vasodilatory, hypotensive, hepatoprotective activity and modulates blood glucose with insulin-like peptides [8]. RJ is usually used in enhancing the immune system and the treatment of various diseases. Through its considerable commercial appeal, it is preferred by the pharmaceutical and cosmetic sectors besides the food industry.

RJ is widely promoted as a commercially available natural remedy, but the maintenance of biological activity in storage and transport is the main difficulty in production and marketing. As a commercial product, RJ is highly susceptible and ready to be influenced by improper production, transport, and storage conditions. The cold chain is a necessity for the preservation of the product due to its perishable nature, and this prior condition brings along the risk of cold-chain breakage [9]. Recently, the lyophilization process is applied for

the elimination of cold-chain dependence, but the lyophilized product is extremely hygroscopic and needs to be protected from humidity [10]. The lyophilized formulations offer multiple advantages like sustaining biological activity and stability [11], but also possess substantial disadvantages like expensive equipment and high costs for manufacturing [12]. Microencapsulation is thought to be a good alternative to lyophilization at that point and seen as one of the promising techniques to produce commercial products.

Microencapsulation is a method of packaging target materials in small capsules to ensure the stability and protection of sensitive substances against environmental factors [13]. It improves the bioavailability of components by controlled release and broadens the application range of products as food ingredients [14]. Microencapsulation is extensively used in many industrial areas including pharmaceuticals, textiles, and cosmetics, for many years. However, it has been recently practiced in the food industry and extremely satisfying results have been achieved [15]. Many studies have shown that microencapsulation maintains the stability of biologically active compounds during food processing and storage, although high temperature and presence of oxygen [16, 17].

Defining the effect of microencapsulation on the biological properties of RJ is very essential to learn more about the microencapsulation and probability of its application on RJ. Our hypothesis is that it is possible to produce a commercial RJ stored at room temperature with microencapsulation technology. In this study, it was intended to monitor the antimicrobial activity of microencapsulated (MRJ) and fresh RJ during the storage. The content of 10-HDA was determined and the biological activity of the product was evaluated in the light of the obtained data. Studies about microencapsulated royal jelly (MRJ) are very limited. This report will lead other studies evaluating the biological properties of microencapsulated RJ and monitoring activity during the storage period.

MATERIAL AND METHODS

The antimicrobial activity of fresh and microencapsulated royal jelly was examined, comparatively. The possible changes in antimicrobial activity in the 1st, 3rd, and 6th months were evaluated considering the changes in 10-HDA levels.

Preparation of Microencapsulated Royal Jelly

RJ was obtained from a professional beekeeper in Bursa (İnegöl region, Turkey) and samples were stored at -80 °C until it was used. The capsules were produced using a crosslinking technique by mixing RJ with sodium alginate (2% w/v) and gum arabic (0.5% w/v) using a similar method by Mohamed and coauthors [18] with some modifications. The optimal concentrations of the wall and core materials for the preservation of antimicrobial activity were adjusted with preliminary studies. Capsules were formed by dripping the pre-prepared solution into the calcium chloride (0.45 M) solution in the encapsulator (Buchi B-390, Flawil, Switzerland) under 450 mbar air pressure with a 1.00 mm nozzle size (frequency is 70 Hz). Microencapsule royal jelly was dried for 24 hours and stored at room temperature.

Particle Size and Encapsulation Efficiency of Microencapsulated Royal Jelly

The particle sizes of microcapsules were scanned by a stereo zoom microscope (Leica EZ4-E) and measured with the LibreOffice Draw (version 7.0) office program [19]. Encapsulation efficiency was determined by dissolving capsules in a proper solvent and analyzing 10-HDA content with HPLC. Microcapsules containing 10-HDA (50 mg/L) were mixed with 25 mL phosphate buffer solution (pH 7.4) and stirred at 40°C with at 1000 rpm until the capsules dissolved. The solution was made up to 50 mL with methanol and centrifuged at 10000 rpm for 5 minutes. The extracts were filtered with a 0.45 µm syringe filter and 10-HDA contents were determined by HPLC analysis. The following equation is used to calculate encapsulation efficiency.

$$\text{Encapsulation efficiency (\%)} = \text{Actual 10-HDA content} / \text{Theoretical 10-HDA content} \times 100" \quad (1)$$

HPLC determination of Trans-10-hydroxy-2-decanoic acid (10-HDA)

The 10-HDA content was determined according to the procedure reported in Kolayli and coauthors [20] with minor modifications. Hitachi High-Performance Liquid Chromatography (HPLC) with DAD detector was used under the following conditions: The column was a reversed-phase column C18-H (Thermo Scientific Hypersil ODS, 5 mm, 150 mm 4.6 mm I.D.) and set at 35°C. The mobile phase was composed of methanol/water (55:45 v/v), the pH was adjusted to 2.15 with phosphoric acid. The detector was adjusted to 215 nm. The flow rate and injection volumes were 1.0 mL/min and 20 µL, respectively. Stock solutions were

prepared as 50, 100, 150, 200, 250 µg/mL with the 10-HDA standard (Sigma Aldrich, Missouri, USA) to obtain a standard curve. RJ sample (200 mg) dissolved in 50 mL methanol/water (50:50 v/v) and MRJ samples added 0.1 M phosphate buffer (pH 7.4) solution (100 mL), stirred at 40°C until capsules dissolve. The mixtures were centrifuged at 10000 rpm for 5 min and the supernatants were filtered through a 0.45 µm syringe filter before analysis.

Preparation of Microencapsule Royal Jelly for Analysis

The medium pH was adjusted to release capsule samples in Mueller-Hinton Broth (MHA, Merck, 1.10293) for bacteria and Sabouraud Dextrose Broth (SDB, Merck, 1.08339) for yeast and mold. MHB was prepared with phosphate buffer to ensure the capsules opened at the appropriate pH in the medium; SDB was adjusted to pH 7.3 with 10% sterile (cold sterilization) sodium hydroxide solution. Stock solutions were obtained by stirring the media at 37°C for 2 h [18]. Fresh RJ was stored under the cold chain and microencapsulated RJ at room temperature during the analysis period.

Microorganisms and Culture Media

Antibacterial activity was evaluated against two Gram-positive bacteria, *Micrococcus luteus* ATCC 9341, *Staphylococcus epidermidis* ATCC 12228, and two Gram-negative bacteria, *Salmonella Enteritidis*, *Escherichia coli* ATCC 25922. *Candida albicans* ATCC 10351, *Candida parapsilosis* ATCC 22019, and two pathogenic fungi *Penicillium digitatum*, *Aspergillus flavus* were used to determine the antifungal activity. Mueller-Hinton Broth and Agar (MHA, Merck, 1.05437) were used for bacteria, Sabouraud Dextrose Broth and Agar (SDA, Merck, 1.05438) were used for yeast and mold.

Agar-Well Diffusion Assay

Agar-well diffusion assays were carried out with each of the bacterial and fungal strains. A hundred µl of inoculum from the cultures adjusted 0.5 McFarland was spread into the Petri dish with a drigalski spatula and 50 µL of the sample (500, 250, 125 mg/mL for fresh RJ and 125, 62.5, 31.25 mg/mL for MRJ) was inoculated to the well [21]. The bacteria were incubated for 24 hours at 37°C, yeasts and molds were incubated for 48-72 h at 25°C and the inhibition zone diameters (mm) were measured after incubation. Empty capsules (released on medium) were used as a negative control.

Broth Microdilution Assay

The antimicrobial activity was assessed by the minimal inhibitory concentration (MIC) and Minimum bactericidal/fungicidal concentration (MBC/MFC) under the Clinical and Laboratory Standard Institute (CLSI) protocol [22]. A twofold serial dilution of stock solutions was prepared ranging from 0.98 to 500 mg/mL to test antimicrobial activity. Bacterial/fungicidal growth was detected by optical density (OD) at 600 nm (BioTek Instruments, Inc. EPOCH SN 15062915). Ten µL inoculum was taken from the well where no growth was observed and incubated for 24 hours at 37°C for bacteria and 48 hours at 25°C for yeasts and molds. The number of CFU was counted at the end of the incubation [23].

Statistical Analysis

All independent variables were evaluated for normality via Q-Q plots and Shapiro-Wilk tests, and datasets were observed normally distributed. A post hoc analysis was performed using the multifactorial one-way analysis of variance (ANOVA) with Tukey's Multiple Comparison Test. Statistical analyses were carried out using SPSS version 22 (IBM, New York, NY), and the confidence level was set at 95% by convention.

RESULTS

Microencapsulated royal jelly was formulated based on the protection of 10-HDA. The wall and core materials were adjusted according to the preliminary analysis. The diameters of the microcapsules were 1.45 ± 0.07 mm. The royal jelly microcapsules were produced by the ionic gelation method. The concentration of wall material is a critical parameter to get high encapsulation efficiency in the method. The encapsulation efficiency of the microcapsules was determined as 79.01 ± 1.28%. The 10-HDA content of RJ and MRJ was determined as 1.74 ± 0.04 % and 2.31 ± 0.02 % before the storage, respectively. The final 10-HDA content of RJ and MRJ samples were 1.71± 0.03 % and 2.27± 0.03 % after six months of storage (Figure 1). No change in 10-HDA content was observed until the end of the storage period for RJ and MRJ (p>0.05).

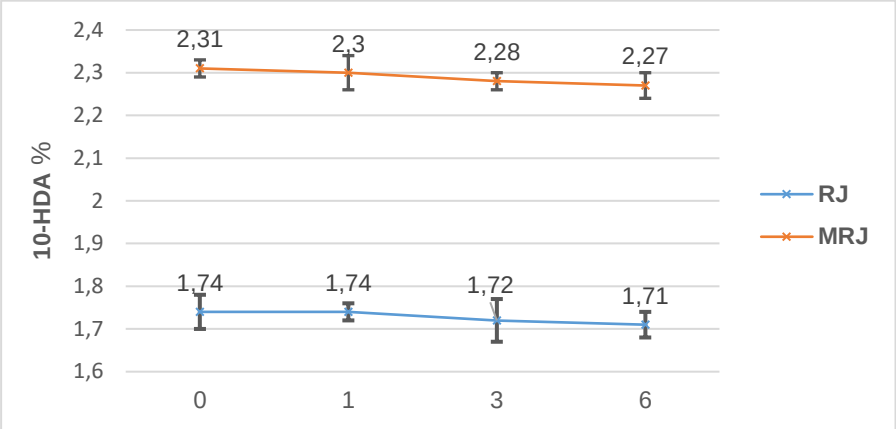


Figure 1. The 10-HDA content of RJ (at 4°C) and MRJ (at room temperature) during six months storage period.

RJ and MRJ demonstrated dose-dependent inhibition of growth for all strains of bacteria and fungi. Although MBC and MFC of RJ were higher than the MIC, the difference between MIC and MBC/MFC is not remarkable (Table 1). *S. epidermidis* was the most sensitive bacteria with the lowest MIC (15.6 mg/mL) and the highest zone diameter (25.23 mm and 16.06 mm) for both fresh and microencapsulated RJ (Table 2). Gram (+) strains were more sensitive than Gram (-) strains considering the results belonging to *M. luteus* and Gram-negative bacteria. The antifungal activity of fresh RJ on yeast (15.6 mg/mL) was more than other species of fungi. On the other hand, no major difference was found between yeast and molds according to the MICs (62.5 mg/mL) of microencapsulated RJ (Table 1). *A. Flavus* was more sensitive than *P. digitatum*, no inhibition zone was seen for the yeasts. When the inhibition zone of fresh and microencapsulated RJ was compared, there was no significant difference between the zone diameters at the concentration of 125 mg/mL for all tested strains (p>0.05) (Table 3). While the activity of MRJ on resistant ones decreased slightly, the MIC values of RJ on susceptible microorganisms did not change (Table 1). The antimicrobial activity of MRJ was protected during the storage, generally.

The antimicrobial effect of RJ was observed to proceed during the storage period against bacterial strains and yeasts whereas some alterations were determined at the 3rd month on molds in both methods (Table 1-3). The MIC of fresh royal jelly was 15.6 mg/mL for *S. epidermidis* and 31.25 mg/mL for other bacteria and these values were maintained during storage (Figure 2). Microencapsulated RJ maintained the antimicrobial activity on selected bacteria during storage at room temperature. While the inhibition zone diameters of *S. epidermidis* and *M. luteus* varied at the end of the 1st month, *E. coli* formed different inhibition zones at the end of the 3rd month (Table 2). Similar zone diameters of *S. Enteritidis* were measured for both RJ samples at the beginning and in the following months, but no inhibition zone was formed at the 6th month. The antifungal activity of royal jelly samples on *C. albicans* was preserved during six months (15.6 mg/mL) (Table 1). The efficacy of fresh royal jelly on *C. parapsilosis* (62.5 mg/mL) continued until the end of the study, while the MIC value of microencapsulated royal jelly increased to 125 mg/mL at the 3rd month. The minimum inhibitory concentration of fresh RJ increased at the 3rd month on *A. flavus* and *P. digitatum*, but the MIC of microencapsulated RJ for *P. digitatum* did not change during storage (Table 1). Zone formation was observed on *A. flavus* at all concentrations, but lower concentrations (125 and 250 mg/mL) of fresh royal jelly were insufficient to form a sporulation zone on *P. digitatum* at 3rd and 6th months (Table 3). Similarly, while the efficacy of microencapsulated royal jelly on *A. flavus* continues during the storage period, the sporulation zone did not occur at the end of the 1st month in the plate for *P. digitatum*. This result showed that the differences in the zone diameters varied with the type of RJ and tested strains.

Table 1. MIC and MBC/ MFC values [mg.mL^{-1}] for fresh and microencapsulated royal jelly at different storage periods on tested bacteria and fungi.

Forms		RJ								MRJ							
Concentration [mg·mL^{-1}]	**Storage period [months]**	***Ec***	***Se***	***Sent***	***Ml***	***Ca***	***Cp***	***Af***	***Pd***	***Ec***	***Se***	***Sent***	***Ml***	***Ca***	***Cp***	***Af***	***Pd***
MIC	0	31.25	15.6	31.25	31.25	15.6	15.6	31.25	31.25	125	15.6	125	31.25	62.5	62.5	31.25	62.5
	1	31.25	15.6	31.25	31.25	15.6	15.6	31.25	31.25	125	15.6	125	31.25	62.5	62.5	31.25	62.5
	3	31.25	15.6	31.25	31.25	15.6	15.6	62.5	62.5	125	15.6	125	31.25	62.5	125	125	62.5
	6	31.25	1.,6	31.25	31.25	15.6	15.6	62.5	62.5	125	31.25	125	31.25	62.5	125	125	62.5
MBC/MFC	0	62.5	62.5	62.5	125	31.25	250	125	125	125	125	125	125	125	nd	nd	nd
	1	62.5	62.5	62.5	125	62.5	125	125	125	125	125	125	125	125	nd	nd	nd
	3	62.5	62.5	62.5	125	62.5	250	500	125	125	125	nd	125	125	nd	nd	nd
	6	125	62.5	62.5	125	62.5	250	500	250	nd	nd	nd	125	nd	nd	nd	nd

Ec- E. coli, Se - S. epidermidis, Sent - S. Enteritidis, Ml - M. luteus, Ca - C. albicans, Cp - C. parapsilosis, Af - A. flavus, Pd - P. digitatum. nd – not determined (>125 mg.mL^{-1}).

Table 2. Mean diameter of inhibition zones [in mm ± S.D.] of bacteria*.

Forms		RJ				MRJ				
Storage period [months]	**Concentration [mg·mL^{-1}]**	***E. coli***	***S. epidermidis***	***S. Enteritidis***	***M. luteus***	***E. coli***	***S. epidermidis***	***S. Enteritidis***	***M. luteus***	**Concentration [mg·mL^{-1}]**
0	500	13.42±0.20Ac	25.23±0.03^{Aa}	13.18±0.23^{Ca}	17.71±0.32^{Ba}	8.58±0.72^{Ca}	16.06±0.57^{Aa}	8.60±0.67^{Ca}	11.68±1.52^{Ba}	125
	250	11.95±0.33Ba	21.81±0.05^{Aa}	11.70±0.40^{Ba}	13.98±0.07^{Ba}	7.51±0.24	-	-	-	62.5
	125	8.60±0.81Ca	15.24±0.22^{Aa}	8.39±0.08^{Ca}	10.99±0.60^{Ba}	8.60±0.81	-	-	-	31.25
1	500	11.53±0.36Ca	21.12±0.21^{Ab}	12.51±0.54^{Ca}	15.16±0.62^{Ba}	8.27±0.23^{Ba}	15.20±0.45^{Aa}	7.81±0.27^{BCa}	7.55±0.13^{Cb}	125
	250	10.83±0.15Ba	14.83±1.11^{Ab}	12.09±0.45^{Ba}	12.45±0.43^{Bab}	-	-	-	7.27±0.14	62.5
	125	-	11,23±0.39^{Ab}	8.37±0.58^{Ba}	8.77±0.07^{Aba}	-	-	-	-	31.25
3	500	8.41±0.23Cb	19.63±0.28^{Ac}	13.49±0.12^{Aa}	14.12±0.90^{Bab}	7.33±0.08^{Bab}	-	8.37±0.00^{Aa}	7.23±0.59^{Bb}	125
	250	8.74±0.49Dab	12.89±1.35^{Ab}	10.79±0.12^{Ba}	10.21±0.18^{Cb}	-	-	-	7.12±0.03	62.5
	125	8.07±0.41Ba	9.41±0.08^{Abc}	7.89±0.06^{Da}	7.92±0.40^{Ca}	-	-	-	-	31.25
6	500	7.76±0.98Cb	18.31±0.42^{Ac}	11.12±0.24^{Ba}	11.88±0.62^{Bb}	6.91±0.02^{Ab}	7.32±0.01^{Ab}	-	7.37±0,75^{Ab}	125
	250	6.94±0.54C	15.55±0.33^{Ab}	9.89±0.47^{Ba}	10.22±0.98^{Bb}	6.51±0.00^{B}	-	-	7.37±0.19^{B}	62.5
	125	8.60±0.81Ca	8.21±0.27^{Ac}	-	-	-	-	-	-	31.25

*The values at the same row with different capitalized letters, and those in the same column with different noncapitalized letters are significantly different at $p \leq 0.05$.

(−) No inhibition zone. Resistant ≤ 14, Intermediate 15-18, Susceptible ≥ 19 [24].

Table 3. Mean diameter of inhibition zones [in mm ± S.D.] of molds*.

Forms		RJ		MRJ		
Storage period [months]	**Concentration [mg·mL^{-1}]**	***A. flavus***	***P. digitatum***	***A. flavus***	***P. digitatum***	**Concentration [mg·mL^{-1}]**
0	500	28.93±1.01^{Aa}	16.69±0.26^{Ba}	25.19±0.45^{Aa}	14.83±0.15^{Ba}	125
	250	27.92±0.23^{Aa}	15.01±0.73^{Ba}	22.34±0.28^{a}	-	62.5
	125	27.30±0.28^{Aa}	14.92±0.44^{Ba}	-	-	31.25
1	500	28.71±0.39^{Aa}	14.35±0.48^{Bb}	21.70±0.81^{ab}	-	125
	250	25.92±1.00^{Aab}	14.38±0.64^{Ba}	20.03±0.89^{ab}	-	62.5
	125	25.67±1.61^{Aab}	14.64±1.28^{Ba}	13.08±1.04	-	31.25
3	500	27.99±0.47^{Aa}	13.40±0.33^{Bb}	22.14±3.44^{b}	-	125
	250	25.54±0.19^{b}	-	22.14±0.00^{a}	-	62.5
	125	24.62±0.37^{b}	-	-	-	31.25
6	500	24.93±0.37^{Ab}	13.05±0.35^{B}	25.41±1.25^{a}	-	125
	250	25.28±0.26^{b}	-	19.41±0.64^{b}	-	62.5
	125	22.56±0.42^{c}	-	16.85±0.69	-	31.25

*The values at the same row with different capitalized letters, and those in the same column with different noncapitalized letters are significantly different at $p \leq 0.05$. (−) No inhibition zone. Resistant ≤ 14, Intermediate 15-18, Susceptible ≥ 19 [24].

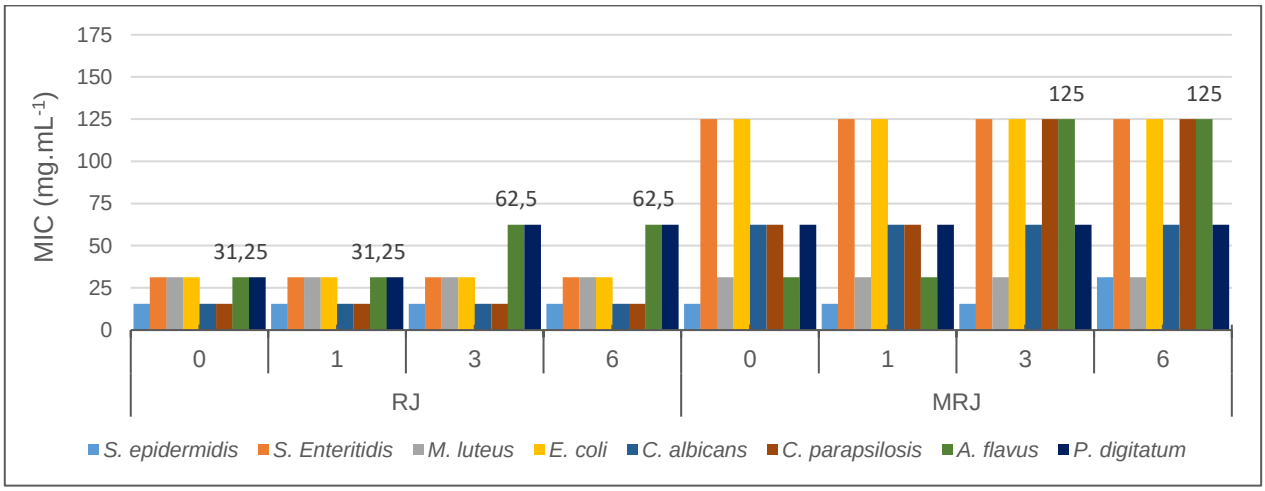


Figure 2. MIC values of fresh and microencapsulated royal jelly at different storage periods on tested bacteria and fungi.

DISCUSSION

The antimicrobial activity of fresh and microencapsulated RJ was tested on various bacteria, yeast, and molds. The results were considerable in terms of determining the effect of royal jelly on molds and monitoring antifungal activity during the storage. When the MICs and zone diameters of both RJ samples were examined, Gram-negative bacterial strains were found more resistant than Gram-positive strains. The result agrees with the hypothesis that Gram-positive bacteria are more sensitive to RJ and the role of bioactive components in the mechanism of action on different microorganisms [25]. The water-soluble royal jelly fraction was reported to have a strong inhibition activity on Gram-positive bacteria [26]. The antimicrobial effect of lipid fraction against Gram-negative bacteria has been associated with the fatty acids of RJ, particularly 10-HDA content [27]. Similar findings were observed for the antifungal activity of RJ, yeasts were found more sensitive than molds in the study. Although there are no extensive studies about the antifungal effect of RJ, the activity has been linked to the bioactive ingredients in these fractions [28]. The antifungal activity of 10-HDA against yeast is indicated by various studies [29, 30]. While the antifungal effect on yeast is attributed to 10-HDA and RJ proteins, the inhibition on molds has been referred to MRJP's and specific peptides [26,31,32].

In our study, MRJ was formulated based on the protection of 10-HDA and the relevance of antimicrobial activity with 10-HDA evaluated. Due to the preliminary studies for the effective coating materials for the microencapsulated product, high encapsulation efficiency was achieved (79.01 ± 1.28%). In a similar study aimed at optimizing conditions for microencapsulation of nisin using calcium alginate and guar gum, the encapsulation efficiency of nisin was 36.65 % [33]. For an encapsulation model of oil in ca-alginate beads, the encapsulation efficiency was found between 79% and 85% in a study by Chan [34].

The percentage of 10-HDA content throughout the storage was presented in Figure 1. No change was observed on 10-HDA content during the six-month storage period for RJ samples. Similarly, Antinelli and coauthors [35] stated that the 10-HDA level of RJ was same after 12 months when stored at -18. It has been observed that the 10-HDA level did not change when suitable conditions are provided, and the antimicrobial activity was maintained.

The effectiveness of microencapsulation on maintaining antimicrobial activity showed the ability of microencapsulation to protect the biological properties. Comparing inhibition zones of fresh and microencapsulated RJ, no significant difference was found between zone diameters at 125 mg/mL concentration (p>0.05) (Table 2-3). The MIC values of fresh and microencapsulated RJ were similar, but the activity of MRJ on resistant strains was seen slightly decreased. The difference between fresh and microencapsulated RJ is thought to be dependent on the microorganisms and probably due to the variation in components other than 10-HDA. These findings showed similarity with the literature about the lyophilization process of RJ [11, 36]. The results of the study confirm that microencapsulation can be an alternative to the lyophilization process in commercial RJ production.

The antimicrobial effect of RJ samples was observed to preserve during the storage period against bacterial strains and yeasts. On the other hand, some changes in the antifungal activity on test molds were detected at the 3rd month and the rest storage period in both methods (Table 1-3). The deterioration in the inhibitory effect of fresh royal jelly was quite low according to the broth microdilution method, and the MRJ maintained antifungal activity on P. digitatum throughout the storage period. However, the variation of the antifungal activity was slightly more in the agar well diffusion assay, and the antifungal activity maintained on *A. Flavus* until the end of the study. The inhibition effect of FRJ on *P. digitatum* finished at the 3rd month (at the concentration of 125 mg/mL), a similar situation was observed in the first month for MRJ. Comparison of the antifungal activity of fresh and microencapsulated RJ in storage was not possible due to the lack of similar studies in the literature. Nevertheless, the obtained results about antimicrobial activity in the storage period led to similar predictions. The maintenance of the inhibition depends on the species of the microorganisms and the components of MRJ in the storage.

The general opinion about the biological activity during the storage is that RJ was preserved when appropriate conditions were provided [27]. In this study, MRJ showed antimicrobial activity at room temperature and provided an advantage compared to the fresh form stored at the cold chain. Thus, the cold-chain breakage risk will be eliminated and provide the convenience of transportation and storage to the product. Moreover, this study was one of the limited studies about the biological activity of microencapsulated RJ in the storage period, it ensures an important contribution to the literature as a base for new studies.

CONCLUSION

The results of the study showed that microencapsulation maintains 10-HDA content and the antimicrobial activity of RJ throughout the storage period. Also, it is thought to be an innovative approach for royal jelly due to the elimination of cold-chain requirements. Microencapsulation of RJ is a promising technique enabling storage at room temperature. In addition, this implementation may be an alternative commercial form thanks to the low cost for production, storage, and transportation compared to other commercial products such as lyophilized, deep-freezed, and refrigerated forms. Thus, the market share of RJ and RJ-based products may increase and MRJ may become a well-liked product. In the light of the present study, innovative RJ products may be developed by different formulations and advanced microencapsulation technology.

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REFERENCES

1. Kunugi H, Ali AM. Royal jelly and its components promote healthy aging and longevity: from animal models to humans. Int. J. Mol. Sci. 2019; 20(19): 4662. DOI: 10.3390/ijms20194662
2. Slater GP, Yocum GD, Bowsher JH. Diet quantity influences caste determination in honeybees (Apis Mellifera). P. Royal Soc. B. 2020; 287(1927): 614. DOI: 10.1098/rspb.2020.0614
3. El-Guendouz S, Lousy B, Miguel M. G. Insight into the chemical composition and biological properties of mediterranean royal jelly. J. Apicult. Res. 2020; 59(5): 890-909. DOI: 10.1080/00218839.2020.1744241
4. Khazaei M, Ansarian A, Ghanbari E. New findings on biological actions and clinical applications of royal jelly. A review. J. Diet. Suppl. 2018; 15(5): 757-75. DOI: 10.1080/19390211.2017.1363843
5. Keskin M, Özkök A, Karahalil F, Kolayli, S. Arı sütü 10-Hidroksi-2-Dekanoik asit (10-HDA) miktarı ne olmalıdır?. Mediterr. Agric. Sci. 2020; 33(3), 347-50. DOI: 10.29136/mediterranean.698926
6. Strant M, Yücel B, Topal E, Puscasu A M, Margaoan R, Varadi A. Use of royal jelly as functional food on human and animal health. J. Anim. Prod. 2019; 60(2), 131-44.
7. Collazo N, Carpena M, Nuñez-Estevez B, Otero P, Simal-Gandara J, Prieto M. A. Health-promoting properties of bee royal jelly: food of the queens. Nutrients. 2021;13(2): 543. DOI: 10.3390/nu13020543
8. Pavel CI, Mărghitaş LA, Bobiş O, Dezmirean DS, Şapcaliu A, Radoi I, Mădaş MN. Biological activities of royal jelly-review. Sci. Papers Animal Sc. 2011; 44(2): 108-18.
9. Pavel CI, Mărghitaș LA, Dezmirean, DS, Bobiș O, Bărnuțiu L, Șapcaliu A. Changes in royal jelly composition during storage and possible freshness parameters. Animal Sci. Biotechno. 2011; 68(1/2): 245-8.
10. Kausar SH, More VR. Royal Jelly. Organoleptic characteristics and physicochemical properties. Lipids. 2019;6(2):20-4.
11. Nascimento AP, Moraes LAR, Ferreira NU, Moreno GDP, Uahib FGM, Barizon EA, Berretta AA. The lyophilization process maintains the chemical and biological characteristics of royal jelly. Evid-Based Compl. Alt. Medi. 2015.
12. Guiné R. The drying of foods and its effect on the physical-chemical, sensorial and nutritional properties. Int. J. Food Eng. 2018; 2(4): 93-100. DOI: 10.18178/ijfe.4.2.93-100
13. Jain A, Thakur D, Ghoshal G, Katare OP, Shivhare US. Microencapsulation by complex coacervation using whey protein isolates and gum acacia: an approach to preserve the functionality and controlled release of β-carotene. Food Bioprocess Tech. 2018; 18(8):1635-1644. DOI: 10.1007/s11947-015-1521-0
14. Gouin S. Microencapsulation: industrial appraisal of existing technologies and trends. Trends Food Sci. Tech. 2004; 15(7-8):330-47.
15. Nazzaro F, Orlando P, Fratianni F, Coppola R. Microencapsulation in food science and biotechnology. Curr. Opin. Biotech. 2012; 23(2): 182-6. DOI: 10.1016/j.copbio.2011.10.001
16. Das A, Ray S, Raychaudhuri U, Chakraborty R. Microencapsulation of probiotic bacteria, and its potential application in food technology. Int. J. Agr. Env. Biotec. 2014; 7(1):47-53. DOI: 10.5958/j.2230-732X.7.1.007
17. Dos Reis AS, Diedrich C, De Moura C, Pereira D, De Flório Almeida J, Da Silva LD, Plata-Oviedo MSV, Tavaresa RAW, Carpes ST. Physico-chemical characteristics of microencapsulated propolis co-product extract and its effect on storage stability of burger meat during storage at -15° C. LWT-Food Sci. Technol.2017; 76:306-313. DOI: 10.1016/j.lwt.2016.05.033
18. Mohamed HN, Mustafa S, Fitrianto A, Abd Manap Y. Development of alginate-gum arabic beads for targeted delivery of protein. SMU Med. J. 2016; 3(1): 486-508.
19. Oral RA. New perspectives for the encapsulation of hydrophilic compounds. J. Food Process Pres. 2017; 41(1): e12967. DOI:10.1111/jfpp.12967
20. Kolayli S, Sahin H, Can Z, Yildiz O, Malkoc M, Asadov A. A Member of complementary medicinal food: Anatolian royal jellies, their chemical compositions, and antioxidant properties. J. Evid-Based Integr. 2016; 21(4): NP43-NP48. DOI: 10.1177/2156587215618832
21. Sun X, Cameron RG, Bai J. Microencapsulation, and antimicrobial activity of carvacrol in a pectin-alginate matrix. Food Hydrocolloids. 2019; 92:69-73. DOI: 10.1016/j.foodhyd.2019.01.006
22. CLSI. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, approved standard, 9th ed., CLSI document M07-A9. Clin. Lab. Standards I., 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087, USA, 2012.
23. Arthington-Skaggs BA, Lee-Yang W, Ciblak MA, Frade JP, Brandt ME, Hajjeh RA, Harrison LH, Sofair AN, Warnock DW. Comparison of visual and spectrophotometric methods of broth microdilution mic end point determination and evaluation of a sterol quantitation method for in vitro susceptibility testing of fluconazole and itraconazole against trailing and non-trailing candida isolates. Antimicrob. Agents Ch. 2002; 46(8): 2477-81. DOI:10.1128/AAC.46.8.2477-2481.2002
24. NCCLS. Performance standards for Antimicrobial Susceptibility Testing. Third informational supplement, M100-S3; Clin. Lab. Standards I.1991.
25. Garcia MC, Finola MS, Marioli JM. Bioassay Directed Identification of Royal Jelly's Active Compounds Against the Growth of Bacteria Capable of Infecting Cutaneous Wounds. Adv. Microbiol. 2013; 3(02), 138.
26. Sauerwald N. Combined Antibacterial and Antifungal Properties of Water-Soluble Fraction of Royal Jelly. Adv. Food Sci.1998; 20, 46-52.
27. Ratanavalachai T, Wongchai V. Antibacterial activity of intact royal jelly, its lipid extract, and its defatted extract. Thammasat Int. J. Sc. Tech. 2002; 7(1).

28. Kim BY, Lee KS, Jung B, Choi YS, Kim HK, Yoon HJ, Zhong-Zheng G, Jungkwan L, Jin BR. Honeybee (Apis Cerana) major royal jelly protein 4 exhibits antimicrobial activity. J. Asia-Pac. Entomol. 2019, 22(1), 175-182. DOI: 10.1016/j.aspen.2018.12.020
29. Melliou E, Chinou I. Chemistry and bioactivity of royal jelly from greece. J. Agr. Food Chem. 2005;53(23),8987-92.
30. Isidorow W, Witkowski S, Iwaniuk P, Zambrzycka M, Swiecicka I. Royal jelly aliphatic acids contribute to antimicrobial activity of honey. J. Apic. Sci. 2018; 62(1), 111-123. DOI: 10.2478/jas-2018-0012
31. Bíliková K, Wu G, Šimúth J. Isolation of a peptide fraction from honeybee royal jelly as a potential antifoulbrood factor. Apidologie. 2001; 32(3), 275-283. DOI: 10.1051/apido:2001129
32. Fontana R, Mendes MA, De Souza BM, Konno K, César LMM, Malaspina O, Palma MS. Jelleines. A family of antimicrobial peptides from the royal jelly of honeybees (Apis Mellifera). Peptides. 2004; 25(6), 919-928. DOI: 10.1016/j.peptides.2004.03.016
33. Narsaia K, Jha SN, Wilson RA, Mandge HM, Manikantan MR. Optimizing microencapsulation of nisin with sodium alginate and guar gum. J. Food Sci. Tech. Mys. 2014; 51(12):4054-4059.DOI: 10.1007/s13197-012-0886-6
34. Chan ES. Preparation of Ca-alginate beads containing high oil content. Influence of process variables on encapsulation efficiency and bead properties. Carbohyd. Polym. 2011; 84(4): 1267-1275.
35. Antinelli JF, Zeggane S, Davico R, Rognone C, Faucon JP, Lizzani L. Evaluation of (E)-10-Hydroxydec-2-Enoic acid as a freshness parameter for royal jelly. Food Chem. 2003; 80(1): 85-89. DOI: 10.1016/S0308-8146(02)00243-1
36. Koç AN, Silici S, Kasap F, Hörmet-Öz HT, Mavus-Buldu H, Ercal BD. Antifungal activity of the honeybee products against Candida spp. and Trichosporon spp. J. Med. Food. 2011; 14(1-2), 128-134. DOI: 10.1089/jmf.2009.0296