переетерифікування – від 0,5 до 1,5 год.

При дослідженні впливу температури значення тривалості було зафіксовано на рівні 1,5 год., при дослідженні тривалості процесу значення температури було зафіксовано на рівні 115 °С.

Як свідчать отримані дані, підвищення температури плавлення олеїну пальмового більше ніж на 12 °С спостерігається при температурі переетерифікування вище 108 °С, при тривалості переетерифікування більше 1,2 год. (72 хв.).

Таким чином, на підставі вимірювання температури плавлення олеїну пальмового, встановлено раціональні умови переетерифікування у присутності каталізатору гліцерату калію, який є ефективним каталізатором переетерифікування олій та жирів.

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EFFECT OF STARCH MICROENCAPSULATION ON SURVIVAL OF *LACTOBACILLUS CASEI* AND *BIFIDOBACTERIUM BIFIDUM* IN MAYONNAISE SAUCE

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1 Introduction Probiotic bacteria are live microorganisms which, when inoculated in adequate amounts bestow health benefit on humans [5]. Probiotic bacteria such as lactobacillus and bifodobacterium strains have been reported to play therapeutic role by lowering cholesterol, preventing cancer, alleviation of constipation and reduction of lactose intolerance. Encapsulation is a new method to promote survival of probiotics during storage or adverse conditions [10].

Calcium alginate capsules entrapment of probiotics is the most used microencapsulation device for several reasons: Alginate as a natural polymer (obtained from brown alga) has been used in various probiotic encapsulations; furthermore, it is a simple and a low-cost way to immobilize cells. Alginate, a natural polymer and non-toxic to humans, can be safely used in products [1]. Probiotics are microencapsulated gently and consequently high yield are observed. The addition of prebiotic compounds, such as starch, and oligosaccahrides promotes the survival of probiotic bacteria [4, 5, 8, 15]. A limiting factor in survival of probiotic bacteria has been reported as low pH (3.6 to 4.6) of mayonnaise sauce owing to the concentration of acetic acid. It has been proven that microencapsulated probiotics survive better in acid conditions [2, 3, 9, 11, 14]. Alginate microcapsules can be prepared by emulsion and extrusion techniques. In emulsion technique, the size of capsules is much smaller than extrusion.

Probiotics have been inoculated to various food products such as sausage [10], chocolate [12, 13], ice cream [5], juices, and cream- filled cake [16]. It was reported that mayonnaise sauce might be a suitable carrier for probiotics microorganisms due to its high water activity; however, survival of free and microencapsulated *Lactobacillus casei* and *Bifidobacterium bifidum* in mayonnaise sauce containing resistant starch as a filler material has not yet been reported.

The aim of this study is inoculation of probiotics as a free and microencapsulated form with resistant starch in mayonnaise sauce and evaluation of microencapsulation on survival and sensory properties of mayonnaise sauce during refrigerated storage (4°C).

2. Materials and methods

Preparation of probiotics Pure freeze, dried cultures were inoculated in MRS broth (de Man-Rogasa-Sharpe) for 24 h under aerobic and anaerobic conditions at 37°C, respectively and biomasses were then harvested by centrifuging at 4000 rpm for 10 min at 4°C. The cultures were then washed twice by sterile saline solution (0.9 %) and used in the microencapsulation process.

Microencapsulation of bacteria All glassware and solutions used in the protocols were sterilized at 121°C for 15 min. The encapsulation way for making alginate capsules was a modified version of methods basically reported by Sheu, Marshall and Sultana et al. [15]. 2 g of resistant starch (Hi-maize 260 national starch UK) were added to 100 ml distilled water and boiled until it formed a gel, then 2 g of sodium alginate (Sigma 71238) were added until they dissolved completely. After cooling, 0.1 % probiotic cultures were transferred into the solution and stirred for 5 min. The final mixture was suspended in 200 ml vegetable oil containing 0.2 % tween 80 and mix (350 rpm for 20 min, Heydolph Stirrer, Germany) until they

appeared creamy. Alginate capsules were prepared by adding 200 ml calcium chloride 0.1 M into a mixture, the phase separation of oil/water emulsion occurred. The mixture was allowed to stand for 30 min, to separate prepared calcium alginate capsules in the bottom of the calcium chloride layer. The oil layer was drained and capsules were collected by centrifuging in 350 rcf for 15 min. The whole procedure was stored at 4°C.

Preparation of mayonnaise sauce Mayonnaise sauces were purchased from Ukrainian supermarkets. Mayonnaise sauce samples were inoculated by free and microencapsulated bacteria separately and stored for 30 days at 4°C until analysis.

Size and shape of capsules The mean diameter of capsules was measured by optical microscopy (Master sizer Malvern 2000 UK). The diameters of 100 randomly selected capsules were measured by using measurement software (Leica Qwin 550). The morphology of the capsules was observed using scanning electron microscope (SEM). In this study, the capsules were examined with scanning electron microscope (LEO 440 I, England) at an accelerating voltage of 10 kV.

Release of entrapped bacteria The capsules containing probiotic bacteria were released by phosphate buffer (pH 7.0, 0.1 M) reported by Sheu, Marshall and Sultana et al. [15]. 1 g of capsules was transferred in 9 ml buffer. The solution was vortexed on a shaker for 15 min vigorously (IKA-MS2, Minishaker, USA) until bacteria released from capsules completely. Total bacterial counts were enumerated on MRS agar (Merck, KGaA Germany). All experiments were carried out in triplicate.

Determination of pH The pH value of mayonnaise sauce samples was determined using a Digital pH-meter (744, Metrohm, Switzerland). The pH value was measured according to the standard method of Institute of Standard and Industrial Research (ISIRI number 2454, 2001). In this study, the pH-meter was calibrated using standard pH 4.0 and pH 7.0 buffer solutions.

Sensory evaluation Sensory evaluation of mayonnaise sauce was conducted after 30 days of refrigerated storage. A panel consisting of 20 panelists evaluated the mayonnaise sauce samples using a sensory rating of 1 to 5 for the color and appearance, 1 to 5 for body and texture, and 1 to 10 for flavor and taste [6].

Statistical analysis All statistical analyses were carried out by SPSS 20 software. Analysis of variance by the general linear model (GLM) procedure and mean differences ($P < 0.05$) between treatments were analyzed by Duncan's multiple range test.

3. Results and discussion

Size and morphology of microcapsules The morphology of capsules was measured by SEM and optical microscopy. All capsules were spherical in shape, and starch particle were present on the surface of the capsules. This finding is in agreement with Sultana et al. [15], which reports that the shape of the microcapsules was spherical, and resistant starch granules filled the cavities of alginate matrix and assisted to support the structure of microcapsules. The mean diameter of microcapsules was 160 μm. Furthermore, the results showed that this diameter was desirable for mayonnaise sauce and delivered soft texture to product. Several reports

have shown that larger capsules (more than 1 mm) give sandy texture to product. Similar shapes of microcapsules were also shown by many researchers [8, 9, 15, 16].

pH changes during mayonnaise sauce storage The pH value of mayonnaise sauce was not affected ($P > 0.05$) during storage at 4°C for a period of 30 days (see Table 1). The pH of control samples reached 4.02 at the end of 30 days storage and the final pH of mayonnaise sauce samples with free probiotics decreased same as control. This may be due to low survival of free probiotics in lower pH (4.6), thus there were no significant differences between free and control samples [2, 3, 9, 11, 14]. The pH of samples containing encapsulated cells of *L. casei* and *B. bifidum* attained 4.02 and 4.01, respectively. Many studies have shown that microencapsulation of probiotic bacteria could be slow absorption of nutrients and decelerating release of metabolites across the alginate shell of capsules [5, 15].

Survival of free and encapsulated bacteria in mayonnaise sauce In this study, we used free or encapsulated *L. casei* and *B. bifidum* with resistant starch for the first time in mayonnaise sauce.

Table 1. pH changes of mayonnaise sauce during storage.

Storage (days)	Control[a]	Mayonnaise sauce with free probiotics		Mayonnaise sauce with encapsulated probiotics	
		A[b]	B[b]	A[b]	B[b]
0	4.10	4.10	4.10	4.10	4.10
10	4.07	4.06	4.06	4.07	4.08
20	4.04	4.04	4.03	4.05	4.06
30	4.02	4.02	4.02	4.02	4.01

[a]Control mayonnaise sauce without probiotic; [b]A — *Lactobacillus casei*, B — *Bifidobacterium bifidum*.

The mayonnaise sauce was evaluated through storage of 30 days at 4°C for monitoring the survival carefully. On the one hand, the free *L. casei*, approximately decreased 6 log cycles in number of cells, but on the other hand the *B. bifidum* cells were sensitive to lower pH (4.6) and did not survive after 10 days in the free state. These decreases of *L. casei* and *B. bifidum* in the free state might be credited to the bactericide activity of acetic acid in mayonnaise sauce. The survival of encapsulated *L. casei* and *B. bifidum* with resistant starch declined slightly after 30 days to 1.3×10^7 and 9×10^5 CFU/g, respectively. The results showed that there were significant differences ($P < 0.05$) between the free and encapsulated probiotics in mayonnaise sauce at the end of 30 days refrigerated storage. We have shown the positive effect of alginate capsules with resistant starch during storage time.

Table 2. Sensory properties of mayonnaise sauce.

Samples	Color and appearance (1-5)	Body and texture (1-5)	Flavor and taste (1-10)	Total acceptability (1-20)
A	4.50^{a}	4.52^{a}	9.50^{a}	18.52^{a}
B	4.55^{b}	4.53^{c}	9.50^{ab}	18.58^{c}
C	4.57^{c}	4.53^{c}	9.50^{ab}	18.60^{d}
D	4.59^{d}	4.54^{d}	9.51^{b}	18.64^{e}
E	4.50^{a}	4.53^{b}	9.50^{ab}	18.53^{b}

$^{a–d}$Means in the same column followed by different letters were significantly different ($P < 0.05$). A: Mayonnaise sauce with free *L. casei*, B: Mayonnaise sauce with free *B. bifidum*, C: Mayonnaise sauce with encapsulated *L. casei*, D: Mayonnaise sauce with encapsulated *B. bifidum*, E: Mayonnaise sauce without probiotic (control).

Many studies have shown that incorporation of resistant starch into the alginate mix could increase the survival of bacteria due to curbing the diffusion of calcium ions outside of capsules [7, 15]. Sultana et al. reported that encapsulation with resistant starch could help the protection of bacterial cells in adverse condition [15]. Furthermore, microencapsulation with resistant starch may improve viable cells of bacteria in acidic foods. This finding is in agreement with those of Sultana et al. [15], Homayouni et al. [5] and Mirzaei et al. [8]. The hi- maize starch could give a higher survival ability of probiotics (105 to 106 / g) in acidic environment of mayonnaise sauce.

Sensory evaluation of mayonnaise sauce The sensory scores of 30 days mayonnaise sauces samples are given in Table 2. The results showed that there were no significant differences ($P > 0.05$) in the flavor and taste of the mayonnaise sauce samples. It was expected that addition of resistant starch to alginate capsule could slightly flavor the mayonnaise sauces [18]. However, the panelist could not identify the differences in flavor between mayonnaise sauces with encapsulated probiotics from controls and samples containing free cells. The points allocated for color and appearance, and body and texture showed that the addition of free and encapsulated probiotics had significant effect ($P < 0.05$) on sensory properties of probiotic mayonnaise sauce. Some scientists reported that the low color scores of mayonnaise samples containing free cells or control phase of probiotics might be due to oxidation rancidity of oil during storage. Furthermore, other scientists reported that formation of secondary oxidation products could affect the color and appearance of mayonnaise during storage time. The production of exopolysaccharide by bifidobacteria and lactobacilli may improve the body and texture of mayonnaise samples. This finding is in agreement with those of Kailasapathy [7].

Conclusion.

This study indicates that microencapsulation with resistant starch enhanced the survival of *L. casei* and *B. bifidum* compared to free cells in mayonnaise sauce

during 30 days storage, and provided a good protection of bacteria cells from the bactericide activity of acetic acid in mayonnaise sauce [20]. It may also be noted *L. casei* and *B. bifidum* have a different response to the acidic environment [19]. No significant differences in capsule shapes were detectable by a SEM. Furthermore, microencapsulation with resistant starch may improve sensory evaluation of the mayonnaise sauce. Further work should focus on microencapsulation processes such as spray-drying and centrifugal extrusion [17].

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Розробка складу супозиторієв на основі каротинвмісного препарату “Аєкол”

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Враховуючи гостру потребу практичної охорони здоров'я в ефективних різноманітних лікарських формах для лікування гнійно-запальних процесів