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ORIGINAL RESEARCH PAPER

FERMENTATION OF *TAGETES PATULA* L. INFUSION BY NATIVE MICROORGANISMS

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Abstract: The fermentation of *Tagetes patula L.* flowers infusion by native microorganisms has been studied. The composition of marigold flower microorganisms, which includes yeasts, bacteria, and microscopic molds was determined. It was established that yeasts from the genera *Saccharomyces*, *Brettanomyces*, and *Rhodotorula*, along with lactic acid bacteria from the genera *Lactobacillus* and *Leuconostoc* participate in the spontaneous fermentation of the infusion. The influence of sugar and citric acid concentrations on the fermentation duration, as well as physicochemical, microbiological, and organoleptic indicators of the fermented infusion, was demonstrated. It was established that acidification with citric acid effectively promotes fermentation of the infusion primarily at a sugar concentration of 7.0 %. The *p*H value significantly impacts the dynamics of spontaneous fermentation and the properties of the fermented marigold infusion. It was found that increasing the initial citric acid concentration and lactic acid adversely affects yeast, particularly *Saccharomycetes*, during the fermentation of the marigold infusion.

Keywords: *acid lactic bacterium, beverage, Brettanomyces, fermented infusion, Lactobacillus, Leuconostoc, marigolds, yeast, Rhodotorula, Saccharomyces*

INTRODUCTION

Marigolds from the genus *Tagetes* are grown as ornamental plants in various countries. Among the different species in Ukraine, the most common is *Tagetes patula* L. Various anatomical parts of these plants are utilized to treat gastrointestinal, emotional, and nervous disorders and enhance vision, among other applications [1]. The fungicidal, bactericidal, and insecticidal properties of *Tagetes* are widely employed in agriculture [2, 3]. Additionally, marigolds serve as a source of orange and yellow carotenoids, food preservatives, and natural colorants [4, 5]. They represent a promising raw material for developing functional beverages in the food industry. Recommendations have been established for producing both unfermented and fermented health drinks with probiotic, antioxidant, and hepatoprotective properties, using cheese whey, berry fillers, and an infusion of *Tagetes patula* L. flowers as sources of biologically active substances [6].

As consumers increasingly focus on health and seek organic and functional foods, manufacturers create new products and beverages that cater to these demands [7, 8]. Consequently, fermented drinks made with various microbial cultures have garnered significant interest.

For thousands of years people have employed fermentation processes to extend shelf life and enhance the taste and safety of various foods and beverages. Initially, fermentation occurred through spontaneous microorganisms entering substrates from the environment. Nowadays, pure cultures of microorganisms and/or their associates are used for fermentation, ensuring a quality product [9, 10]. Specifically, a fermented beverage is produced by processing fresh elderflower blossoms from the wild flora of Romania using the yeast *Saccharomyces cerevisiae* [11].

However pure cultures may cause a loss of the complex and subtle flavor tones inherent in spontaneous fermentation. Therefore, so-called “non-traditional” cultures (yeasts, lactic acid bacteria, and other bacteria) are increasingly employed to produce fermented beverages, enhancing the organoleptic properties of classic and new innovative drinks.

The non-traditional yeasts species in beer production, particularly in sour ales, cause a unique flavor profile [12]. Consumer interest in sour ales obtained through spontaneous fermentation is growing [13]. One such example is Gueuze, a Belgian beer produced through spontaneous fermentation over two years [14]. The production of Belgian sour ale involves both alcoholic and lactic acid fermentation. In the early stages, bacteria from the *Enterobacteriaceae* family participate in the fermentation, contributing to an aroma typical of lambic. As fermentation progresses, *Enterobacteria* disappears, while alcoholic fermentation, driven by various *Saccharomyces* species, takes over. Despite differences in raw materials and fermentation initiation, the primary microbial changes during subsequent fermentation stages are similar for both Belgian sour ale and lambic. Mixed fermentation occurs, involving yeast of the genus *Brettanomyces* and lactic acid bacteria such as *Pediococcus*.

In kvass production, the fermentation stage shapes the distinctive characteristics of beverage, which arise from microbial development. Lactic acid bacteria convert sugars into lactic acid, and the combined lactic-alcoholic fermentation involves two physiological groups of microorganisms (bacteria and yeast) that coexist symbiotically. This microbial consortium provides a unique taste and aroma of kvass [15].

Cider is produced through the spontaneous fermentation of diluted juice from specific apple varieties. Native yeasts and bacteria, naturally present on the surface of fruit, trigger

fermentation, resulting in a beverage with an elegant and pure fruit aroma. Temperature, sugar concentration, and fermentation duration significantly impact the alcohol content of the final product. In mediums with low sugar content, microbial metabolic processes occur more rapidly, whereas in concentrated mediums, microorganisms experience osmotic pressure, leading to a slowdown in enzymatic processes and alcohol accumulation [16].

The fermentation beverage from *Tagetes* is a low-alcohol, naturally fermented drink with a pleasant, refreshing taste and aroma, enriched with valuable chemical and biologically active substances. *Tagetes erecta* and *Tagetes patula* flowers contain leucine (0.95 %), glutamic acid (1.1 %), aspartic acid (0.83 %), carotenoids (0.005 %), tocopherols (0.6 %), ascorbic acid (1.78 %), water-soluble polysaccharides (16.26 %), pectic substances (11.87 %), and hemicelluloses A and B (0.91 % and 0.55 %) [17]. During the infusion of marigolds in water, extractive substances dissolve, supporting microbial activity. After fermentation, these compounds further enhance the nutritional value of the beverage.

MATERIALS AND METHODS

Products and substances used in the study

Marigolds of the species *Tagetes patula* L., drinking water (DSTU 7525:2014. Drinking water. Requirements and quality control methods), sugar (DSTU 4623:2023 Sugar. Technical conditions), and citric acid (DSTU GOST 908:2006 Citric acid monohydrate, food grade. Technical conditions (GOST 908-2004, IDT) were used as raw materials. Marigolds were collected in the Lviv region during the growing season (August-September) in 2024.

Nutrient media

A nutrient media was prepared for the isolation of yeasts from the native microorganisms of marigolds, with the composition (in $g \cdot L^{-1}$):

1) glucose - 20.0; yeast extract - 2.0; agar - 18.0; oxytetracycline - 0.05; initial *p*H value 6.4 - 6.6; sterilization conditions pressure at 0.1 MPa, duration of 20 min.;

2) wort-agar with a dry matter concentration of 8 - 12 % and *p*H 6.6; the initial *p*H value was adjusted to 3.5 using sulfuric acid; sterilization conditions pressure at 0.05 MPa, duration of 30 min.; for the isolation of lactic acid bacteria (in $g \cdot L^{-1}$) - glucose - 20.0; yeast extract - 5.0; meat extract - 8.0; peptone - 10.0; Tween-80 - 1.0 mL; K_2HPO_4 - 2.0; $CH_3COONa \cdot 3H_2O$ - 5.0; $(NH_4)_2C_6H_6O_7$ - 2.0; $MgSO_4 \cdot 7H_2O$ - 0.2; $MnSO_4 \cdot 2H_2O$ - 0.05 g; *p*H 6.0 - 6.5; sterilization conditions pressure at 0.05 MPa, duration of 20 min.; sucrose - 100.0 g; meat-peptone broth - 1000 mL; agar - 15.0; for the isolation of microscopic molds - sucrose - 30.0 g; $MgSO_4 \cdot 7H_2O$ - 0.5 g; KH_2PO_4 - 1.0; KCl - 0.5 g; $NaNO_3$ - 2.0 g; $FeSO_4$ - 0.01 g; agar – 20 g; the initial *p*H was adjusted to 4.0 - 5.5 using a 10 % citric acid solution; sterilization conditions pressure at 0.1 MPa, duration of 15 min.

Meat-peptone agar was employed as a universal nutrient medium for detecting most microorganisms (initial *p*H 7.0 - 7.2; sterilization conditions pressure at 0.1 MPa, duration of 20 min.).

Experimental Method

Fresh flowers of marigolds (20 g) were kept in 800 g of drinking water for 3 days at the temperature of 25 ± 1 °C. The infusion was filtered through a sieve with a hole diameter of 1 mm. Sugar and citric acid were added to the filtrate and poured into flasks. The flasks were closed with a water lock filled with concentrated sulfuric acid, diluted four times with distilled water, and left to ferment. The amount of citric acid added was 0.13 and 0.28 % of the infusion weight. The amount of sugar added was 4.8, 7.0, and 9.1 % of the combined weight of the infusion, sugar, and citric acid. The medium underwent fermentation for 8 - 40 days at a temperature of 24.5 ± 1.0 °C by native microorganisms from marigolds. Fermentation intensity was monitored by weight loss due to CO_2 release, which involved weighing the flasks at specified intervals.

Analysis Methodology

Physical and Chemical Analysis Methodology

In the infusion of marigolds, the dry matter content was measured using a Kruss AR-4 refractometer, the *p*H value with a Mettler Toledo SevenCompact *p*H meter S220, and titratable acidity (in mL of 1 M NaOH solution consumed for neutralizing acidic substances in 20 mL of fermented infusion as Delbrueck degrees, ^{o}D) trough titration with a 0.1 M NaOH solution.

The fermented infusion was evaluated organoleptically (taste, aroma, color, transparency), along with measures of *p*H, alcohol and dry matter concentration (post-distillation measured by a Mettler Toledo 30330857 Densito electronic densitometer with U-shaped oscillating tubes at a temperature of 20 ± 0.5 °C), reducing substances (following the Willstetter-Schudl method), acidic substances (by titration with a 0.1 M NaOH solution), and the optical density of the fermented infusion filtrate in a 10 mm wide cuvette at a light wavelength of 380 - 750 nm using an LLG uniSPEC 2 spectrophotometer. The degree of fermentation shown in the graphs was calculated by monitoring changes in the infusion weight over time, based on the assumption that the sucrose in the fermented infusion was converted solely into alcohol.

Microbiological analysis methodology

Microorganisms from the flower infusion were isolated using traditional microbiological methods [18, 19], applying morphological, physiological, and cultural features [20]. Yeasts and lactic acid bacteria were isolated on selective agar media [11 – 24]. Microorganisms from unfermented and fermented marigolds infusion were primarily identified using a monocular microscope XS-5510 LED (MICROmed) with subsequent computer processing of images (photos) according to [25, 26]. Cell morphology was established through microscopy of preparations prepared from individual colonies, which were stained with a basic fuchsin solution. Yeast concentration was assessed by direct cell counting using a Goryaev chamber.

RESULTS AND DISCUSSION

Infusing flowers *Tagetes patula* L. in a covered chemical glass with air access for three days resulted in the development of a pale pink color and the formation of gas bubbles on

the surface of the infusion, indicating the initiation of spontaneous fermentation by native microorganisms (Figure 1). The dry matter content extracted from the flowers was negligible, the *p*H value of the infusion was 6.30, and its titratable acidity was 0.02 cm^3 of 1 M NaOH per 20 mL (°D).

The spontaneous fermentation of marigold infusion under aerobic conditions occurs due to the sequential action of various microorganisms responsible for fermentation (primarily yeasts and lactic acid bacteria) on the water-soluble carbohydrates from flowers. According to microscopy and preliminary identification results, yeasts and lactic acid bacteria were the most abundant microorganisms in the marigold infusion samples (Figures 2 and 3).

Figure 1. *Infusion after three days of marigold immersion in water at a temperature of 24.5 ± 1.0 °C*

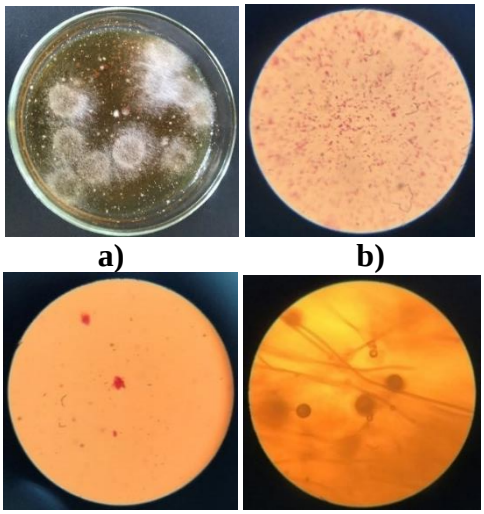


Figure 2. *Microorganisms isolated from the infusion of marigolds on meat-peptone agar (a), preparations of isolated colonies of lactic acid bacteria (b), yeasts (c), and molds (d)*

The fermentation intensity of unacidified marigold infusion under anaerobic conditions, with sugar added at concentrations of 4.8, 7.0, and 9.1 %, was assessed based on weight loss (Figure 4). The initial *p*H of the marigold infusion was 6.30, which resulted in a slow fermentation process. After the fourth day, fermentation was inhibited, as the optimal *p*H for yeasts and lactic acid bacteria is between 4.0 and 4.5. It is likely that other bacterial microorganisms utilized part of sugar, and their metabolic by-products negatively affected the yeasts and lactic acid bacteria.

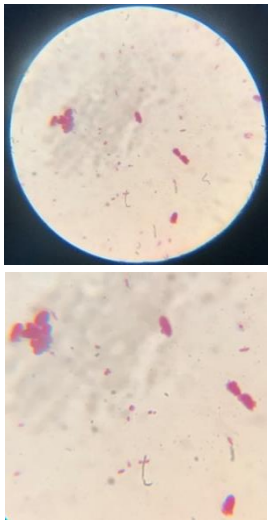


Figure 3. *Fuchsin staining preparations of yeasts and lactic acid bacteria from marigold infusion*

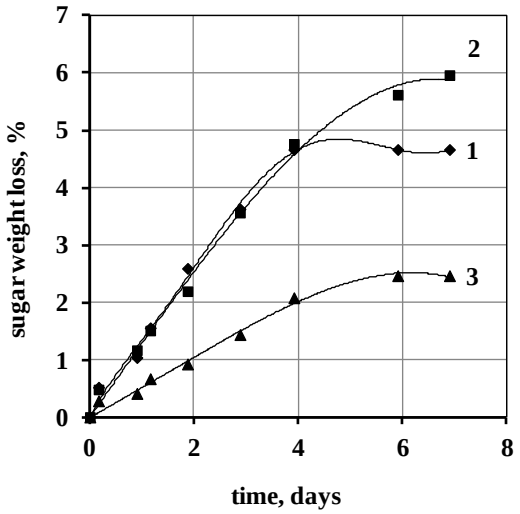


Figure 4. *Fermentation intensity of unacidified marigold infusion temperature: 24.5 ± 1.0 °C;*
initial sugar content (%): 1-4.8; 2-7.0; 3-9.1

The sucrose content, as the primary nutrient for microorganisms, influences the dominant type of fermentation at the initial stage. Higher carbohydrate concentrations increase the likelihood of alcoholic fermentation driven by yeasts. Additionally, yeast strains vary in their ability to ferment carbohydrates, particularly sucrose. Notably, by the fourth day under anaerobic conditions, only the infusion with 4.8 % sucrose exhibited minor gas bubble formation, characteristic of alcoholic fermentation, along with turbidity in all flasks (Figure 5). This suggests that, in addition to slow alcoholic fermentation, lactic acid fermentation also occurred, resulting in the formation of lactic and other organic acids. This is supported by the greater weight loss in the sample with 7.0 % sugar, the increased turbidity of the infusion after seven days of fermentation, and its higher viscosity.

Figure 5. *Samples of unacidified marigold infusion at the beginning (a) and after (b) fermentation - Initial sugar content (%): 1-4.8; 2-7.0; 3-9.1*

Lactobacillus and *Leuconostoc* thrive in a wide temperature range. Active growth of these bacteria is observed within the temperature range of 20 - 35 °C and *p*H 3.5 - 6.5 [27]. The fermentation temperature of 24.5 °C for the marigold infusion and a *p*H of 6.2 - 6.3 promote the development of these bacteria in microaerobic conditions. The high sugar (sucrose) concentration in the liquid medium at the initial stage inhibits lactic acid bacteria among other native microorganisms since yeasts tolerate higher sugar concentrations better than bacteria. Therefore, it can be assumed that there was slight yeast dominance during the first four days of alcoholic fermentation. At a sugar concentration of 9.1 %, less intense fermentation was observed due to the yeast's adaptation to sucrose and its high concentrations. The organoleptic analysis of the infusion, fermented over seven days, confirms the predominance of other types beyond alcoholic fermentation. The viscosity of the fermented infusion was increased significantly, and an odor that is uncharacteristic of alcoholic fermentation appeared. Microscopy of samples of the marigold infusion fermented over seven days revealed yeasts with damaged cells in a state of autolysis (Figure 6). During fermentation, acidity decreased, and, apparently, metabolic products from lactic acid and other bacteria accumulated, negatively affecting the yeasts. As a result of these changes, those yeast species sensitive to increased acidity perished and underwent autolysis. Consequently, they provided lactic acid and other bacteria with valuable nutritional components that fostered their development in the fermented infusion. The observations under a microscope indicate a significant number of lactic acid bacteria, as well as sporadic cells of sporogenous bacteria of the genus *Bacillus* in the fermented infusion (Figure 6). These bacteria can produce substances that increase fermented infusion viscosity, observed with an initial sugar content of 7.0 %. Heterofermentative lactic acid fermentation is often initiated by bacteria of the genus *Leuconostoc*, which produce CO_2 from glucose and dextran from sucrose forming mucous capsules in the fermentation medium [28]. The absence of this phenomenon in the infusion with a sugar concentration of 9.1 % accounts for the degree of fermentation of only 2.5 %.

Figure 6. *Stained microorganisms from unacidified marigold infusion after 7 days of fermentation*

Therefore, the *p*H value significantly impacts the fermentation of marigold infusion. Lowering the infusion acidity creates more favorable conditions for the activity of natural marigold microorganisms within a sugar concentration ranging from 4.8 to 9.1 %. Adding 0.28 % citric acid to the marigold infusion reduced the medium *p*H value from 6.20 to 3.50 units. This change immediately affects the brightness of the infusion color (Figure 7). The infusion color depends on the *p*H since marigold flowers and their extracts contain orange or yellow carotenoids [29]. As a result of acidifying with citric acid, the infusion acquires a pink hue, which remains nearly unchanged after fermentation

(Figure 7). Although the color intensity of the acidified infusion at the optimal wavelength of 500 nm for determining the pink color of the marigold infusion was only slightly higher than that of the unacidified sample (0.283 versus 0.247), its brightness and transparency appeared noticeably greater.

The *p*H of the fermented medium significantly influences microbial activity. Metabolic products from foreign bacteria, including pathogenic strains, can contaminate beverages, degrade their consistency and sensory qualities, and affect safety. Most pathogenic bacteria do not thrive at low *p*H values. Therefore, acidifying the marigold infusion to a *p*H of 3.5 prevents the growth of undesirable foreign microorganisms. Lactic acid bacteria from marigold flowers produce lactic acid lowering the medium *p*H. The anaerobic conditions during fermentation also inhibit the thriving of unwanted obligate aerobes.

Figure 7. *Flasks with unacidified (a) and acidified (b) marigold infusion before fermentation and with fermented unacidified (c) and acidified (d) marigold infusion (4.8 % sugars content and 0.28 % citric acid)*

The fermentation intensity in an acidified infusion, based on its weight loss and the change in fermentation degree calculated from weight loss over time, is illustrated in Figure 8.

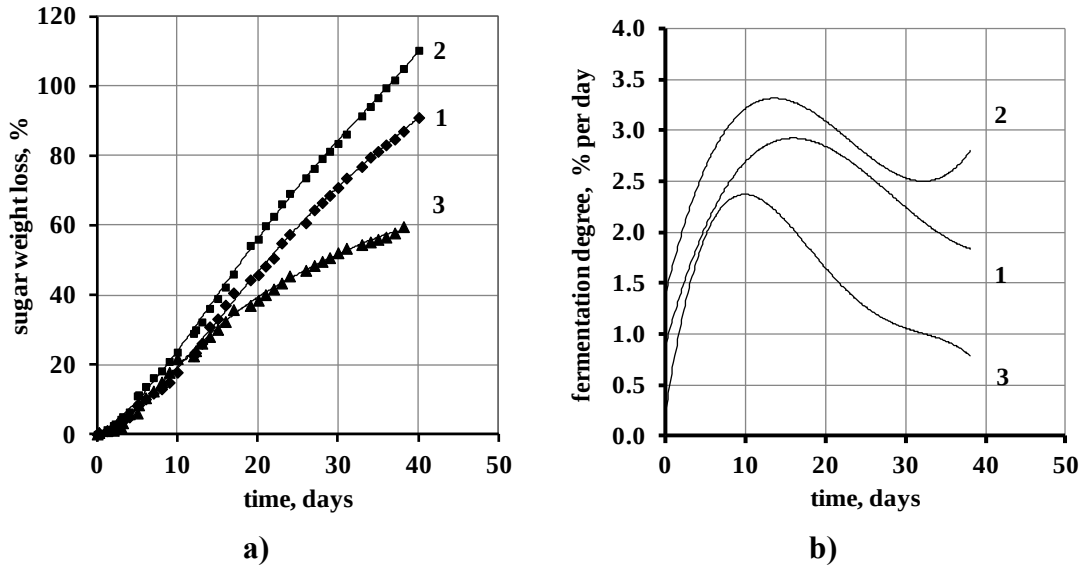


Figure 8. *Fermentation intensity of acidified marigold infusion (a) and the change in the fermentation degree calculated from weight loss over time (b)*

The complete fermentation process lasted over 40 days. The infusion with an initial sugar concentration of 7.0 % underwent the most intense fermentation, while the medium with an initial sucrose concentration of 9.1 % fermented the slowest. The maximum fermentation degree of the marigold infusion, calculated from its weight loss, is achieved between the 10th and 17th day, at approximately 3.3, 3.0, and 2.3 % per day for initial sugar concentrations of 7.0, 4.8, and 9.1 %, respectively. The significant weight loss of the infusion with an initial sugar concentration of 7.0 % on the 40th day of fermentation suggests that microorganisms assimilate some fermentation products, indicating the potential for processes beyond just alcoholic fermentation.

The analysis results of the fermented infusions are presented in Table 1 and Figures 9 and 10. Some results correlate with the initial sugar concentration in the marigold infusion. Specifically, higher initial sugar concentrations correlate with higher density in the fermented infusion. The same applies to the density of the bottom residue obtained after distilling the fermented infusion. The content of residual dry matter in the fermented infusion increases from 1.667 to 6.374 % as the initial sugar concentration rises from 4.8 to 9.1 %. However, the density of the fermented infusion distillate shows insignificant variation with increased initial sugar concentration, with alcohol concentrations of 1.94 wt. % at sugar concentrations of 4.8 and 7.0 %, and 2.45 wt. % at a sugar concentration of 9.1 %. These results support the assumption that additional types of fermentation occur alongside alcoholic fermentation.

Table 1. *Indicators of marigold infusion fermentation by native microorganisms at different initial sugar concentrations*

Sugar amount [wt.%]	**Concentration [wt.%]**		***p*H**	**Acidity [°D]**	**Reducing substances concentration [g/100 mL]**	**Microorganism concentration**		**Sucrose consumption for formation [%]**		
	alcohol	**dry matter**				**[million cells per mL]**	**[g·L^{-1}]**	**alcohol**	**biomass**	**total**
4.8	1.94	1.667	3.08	0.9	0.46	12.45	0.83	54.1	21.6	75.7
7.0	1.94	2.991	3.03	2.4	0.08	11.85	0.79	36.8	13.8	50.6
9.1	2.45	6.374	3.08	1.1	2.01	10.75	0.72	39.7	9.9	49.4

The initial citric acid concentration is 0.28 %, before acidification, the pH is 6.20, and after acidification pH is 3.50

The *p*H value decreases from 3.50 to 3.03 - 3.08 during fermentation. Concurrently, the titratable acidity of the fermented infusion at an initial sugar concentration of 7.0 % reaches 2.4 °D, while at initial concentrations of 4.8 and 9.1 %, it measures 0.9 and 1.1 °D, respectively.

Obviously, at the initial sucrose content of 7.0 %, the most optimal conditions are established for simultaneous lactic acid fermentation and alcoholic fermentation. This assumption correlates with the amount of sucrose consumed for alcohol formation at a sugar concentration of 7.0 %, which is the lowest at only 36.8 % (Table 1). Only 13.8 % of sucrose is utilized for biomass accumulation under these conditions. The fermentation degree of the marigold infusion, calculated by weight loss in terms of solely alcohol formation, exceeded 100 %, providing further evidence of lactic acid and other types of fermentation. The sucrose consumption for alcohol production was 39.7 % at a higher initial sugar concentration in the marigold infusion (9.1 %), with sucrose consumption for microorganism biomass accumulation being only 9.9 %. In this case, the total sucrose consumption for alcohol formation and microorganism biomass

accumulation was 49.4 % and differs by only 10.2 % from the weight loss (in %) calculated solely for the sugar consumption for alcohol production.

At an initial sugar concentration of 4.8 %, the sucrose consumption for alcohol formation in the fermented infusion reached 54.1 %, and the accumulation of microorganism biomass was 21.6 %. The proportion of alcoholic fermentation was significantly higher at a sugar concentration of 4.8 %, and side reactions were insignificant. The same effect occurred at the initial sucrose concentration of 9.1 %.

Therefore, the most intensive lactic acid fermentation occurs at an initial sugar content of 7.0 %.

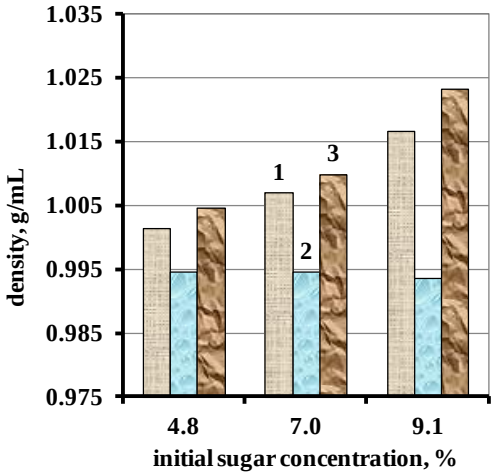


***Figure 9**. Density of fermented infusion (1), distillate (2) and vat residue (3)*

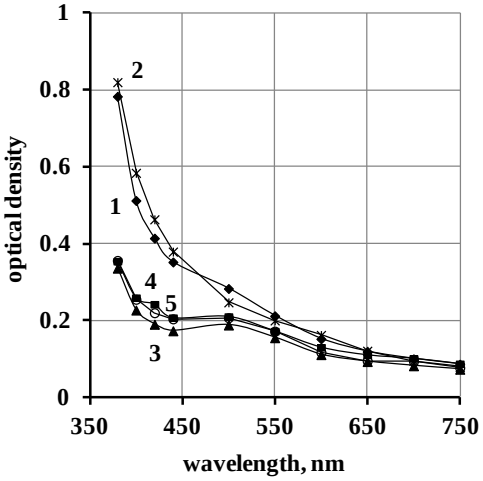


***Figure 10**. Optical density of acidified (1) and unacidified (2) marigold infusion and fermented infusion (3-5) samples (initial sugar content (%): 3-4.8; 4-7.0; 5-9.1)*

With an increase in the initial sugar concentration, the microorganism concentration in the fermented infusion and the biomass yield decreased from 12.45 to 10.75 million·mL^{-1} and from 0.83 to 0.72 g·L^{-1}, respectively. These results correlate with the sucrose consumption for the accumulation of microorganism biomass. A high initial sugar concentration promotes the fermentation of sucrose by native microorganisms from flowers. At low initial sugar concentrations, sucrose consumption for the biomass accumulation increases. This can be explained by the fact that at the beginning of fermentation, dissolved oxygen is present in the subsequent process; therefore, under aerobic conditions with a low sugar concentration, cells generally prefer respiration over fermentation (known as the “Pasteur effect”).

The initial sucrose concentration also affects the redox properties of the fermented medium. The lowest concentration of reducing substances is 0.08 g (in terms of glucose) per 100 mL and occurs at an initial sugar concentration of 7.0 %. Simultaneously, 0.46 and 2.01 g per 100 mL of reducing substances accumulate at initial sugar concentrations of 4.8 and 9.1 %, respectively.

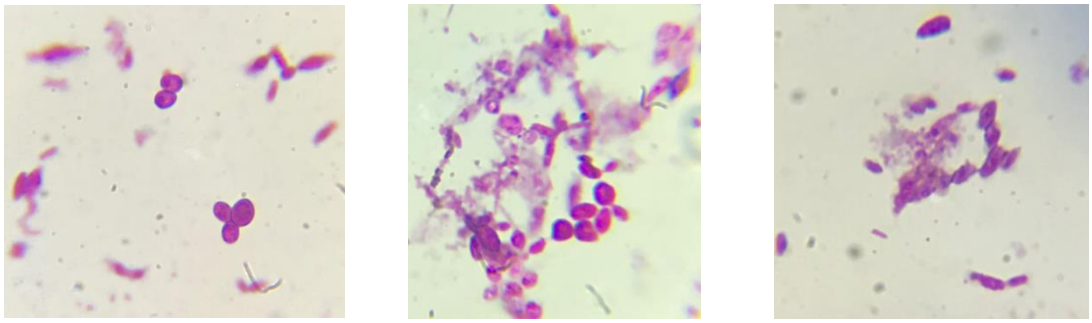
The relationship between the optical density of the fermented infusion filtrate and the wavelength is nearly the same for varying initial sugar concentrations in the marigold infusion (Figure 10). The difference in optical density of each fermented infusion is only

slightly affected by the initial sugar concentration. However, at wavelengths from 380 to 600 nm, it is significantly lower than the optical density of the acidified infusion.

According to weight loss in the fermented infusion, the rate of CO_2 release at different initial sugar concentrations in the marigold infusion varies slightly at the beginning of fermentation and then significantly decreases at an initial sucrose content of 9.1 %. This may indicate a decrease in native microorganism activity, as low initial acidity and acids produced by lactic acid bacteria inhibit alcoholic fermentation. The further active fermentation of the infusion with the initial sucrose concentration of 7.0 % over 35 - 40 days indicates favorable conditions for the thriving heterofermentative bacteria of the genus *Lactobacillus*. These conditions include a decrease in sucrose concentration, a decrease in the *p*H of the medium, and an increase in alcohol produced by yeasts.

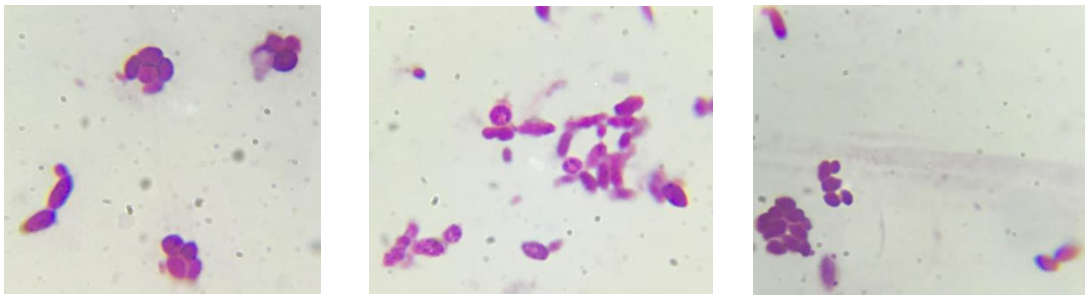
Observations under a microscope of samples of fermented infusions reveal yeasts from different genera (Figure 11).

Samples were plated on selective agar media to isolate and identify the yeasts observed through microscopy in fermented infusions. Detected yeast genera included *Brettanomyces*, *Rhodotorula*, and *Saccharomyces*. Notably, increasing the initial sucrose concentration caused a decrease in yeast cell size (Figure 12). This indicates a potential effect of osmotic stress on yeast morphology.



a) **b)** **c)**

Figure 11. *Stained yeasts of fermented marigold infusions with initial sugar content a): 4.8 %; b) 7.0 %; c 9.1 %*
(initial citric acid content: 0.28 %; temperature: 24.5 ± 0.1 °C)



a) **b)** **c)**

Figure 12. *Stained isolated yeasts on agar media from fermented infusion with initial sugar content: a) 4.8%; b) 7.0 %; c) 9.1 %*

Dead yeast cells with damaged membranes and lysed cells were observed, around which lactic acid bacteria were present in the fermented infusions (Figure 13). Low *p*H values and volatile acids negatively impact microorganisms, particularly the genus

Saccharomyces. Consequently, these cells perish, leading to gradual autolysis. The decomposition products are released into the medium, enriching it with growth sources for lactic acid bacteria of the genus *Lactobacillus*.

Active CO_2 production after 10 days in a flask with low initial sucrose concentration (4.8 % and 7.0 %) is attributed to the activity of heterofermentative lactic acid bacteria from the genera *Lactobacillus* and *Leuconostoc*. These bacteria convert sucrose into lactic and other acids, releasing carbon dioxide, which may suggest ongoing fermentation. The proliferation of these bacteria is characterized by a reduction in sugar content and an increase in titratable and volatile acids due to lactic and acetic acid's production. Heterofermentative lactic acid bacteria thrive best at temperatures between 15 and 30 °C [30]. Their presence was confirmed through microscopy of fermented infusion samples and their isolation on selective agar media (Figure 14).

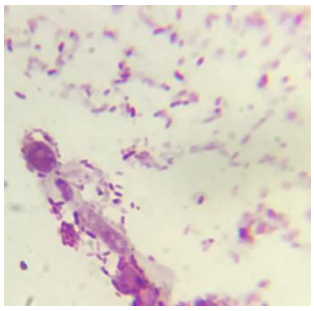
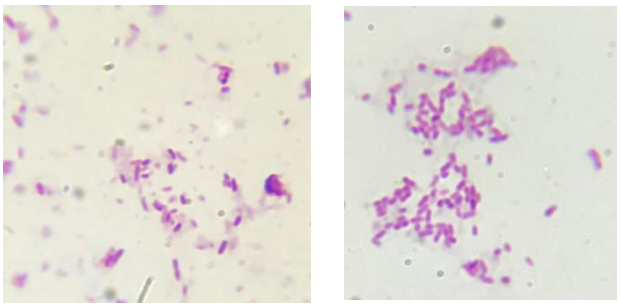


Figure 13. *Stained lysed yeasts of fermented marigold infusion with an initial sugar concentration of 7.0 %*



a) **b)**

Figure 14. *Stained lactic acid bacteria in fermented infusion (a) and isolated on agar medium (b) (the initial sugar concentration 7.0 %)*

A high concentration of titratable acids (2.4 °D) indicates that in a fermented infusion with an initial sugar concentration of 7.0 %, alcoholic fermentation occurs first with native yeasts, followed by heterofermentative lactic acid fermentation. This results in CO_2 release and the accumulation of lactic and acetic acids, among others. Consequently, the *p*H of the fermented infusion decreased from 3.5 to 3.03. Therefore, *Lactobacillus* is regarded as a secondary microorganism in fermented products [31].

All fermented infusion samples exhibited a distinct pleasant floral aroma, most pronounced at an initial sugar concentration of 7.0 %. The highest sucrose fermentation occurred at the lowest initial sugar concentration, resulting in a dry taste due to reduced residual sugar. Additionally, this sample displayed acidity due to lactic acid bacteria activity, which proliferates more rapidly in media with lower carbohydrate concentrations, as well as a glycerin aftertaste. In the other two samples, acidity was barely noticeable. Although at an initial sugar concentration of 7.0 % lactic acid fermentation occurred with a significant accumulation of weakly dissociated organic acids, the residual unfermented sugar contributed to a sweet taste in the fermented infusion.

The effect of added citric acid (0.13 and 0.28 %) on the fermentation of marigold infusion was studied at an initial sugar concentration of 7.0 %. The fermentation intensity (%) and weight loss (% per day) of the fermented infusion over time are shown in Figure 15. The fermentation intensity of both infusions was similar for the first 10 days; however, the

weight loss of the fermented medium with a higher citric acid concentration continued to decrease by 2.5 - 3 % per day, while the rate of CO_2 release in the medium with a lower citric acid concentration declined sharply.

The initial *p*H of the infusions was 6.32 and 6.20, and after adding 0.13 % and 0.28 % citric acid, the *p*H values decreased to 3.40 and 3.50, respectively. By the 40th day of fermentation, the *p*H values were nearly identical (3.00 and 3.03). However, the titratable acidity of the infusion with the lower initial citric acid concentration was significantly lower (0.9 vs. 2.4 °D).

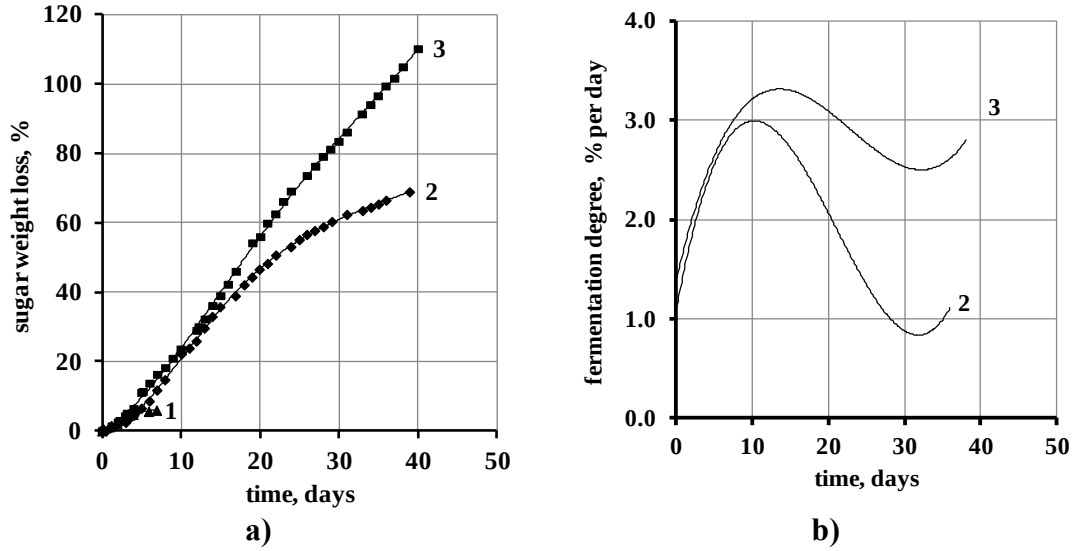


Figure 15. *Fermentation intensity of marigold infusion with added sugar (a) and change in the fermentation degree calculated by weight loss (b) over time at different citric acid concentrations (%): 1-0, 2-0.13, 3-0.28. The temperature is 24.5 ± 1.0 °C*

Consequently, with a lower citric acid concentration, a greater percentage of sucrose was utilized for alcoholic fermentation (40.6 % vs. 36.8 % for 0.28 % citric acid), leading to a higher alcohol concentration (2.45 wt. % vs. 1.94 wt. %). Simultaneously, the microorganism concentration in the fermented infusion with lower citric acid was 1.26 times higher (14.91 vs. 11.85 million cells per mL), and the microorganism biomass yield was 1.00 $g \cdot L^{-1}$ compared to 0.79 $g \cdot L^{-1}$. Sucrose consumption for biomass formation was also higher (25.0 % vs. 13.8 %). Overall, 14.3 % more sucrose (65.0 %) was utilized for alcohol production and biomass formation at an initial citric acid concentration of 0.13 %. Considering that the residual dry matter content in both experiments was similar (3.169 % and 2.991 %), it can be inferred that fermentation at higher initial titratable acidity favors pathways leading to increased production of fermentation by-products (Table 2).

The concentration of reducing substances in the fermented infusion with a citric acid concentration of 0.13 % is 1.0 g/100 mL compared to 0.08 g/100 mL when adding 0.28 % citric acid.

The initial citric acid concentration affects the intensity and brightness of the fermented infusion's color (Figure 16). Notably, with a higher citric acid concentration, the optical density of the infusion is greater in visible light, than that of the fermented infusion with the citric acid content of 0.13 % (Figure 17).

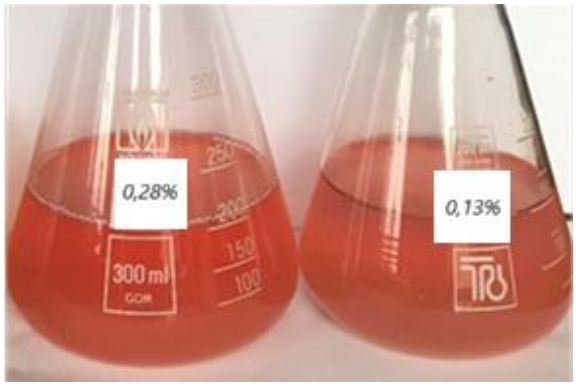


Figure 16. *Flasks with fermented marigold infusions. The sugar concentration is 7.0 %. The citric acid concentration is 0.13 % and 0.28 %*

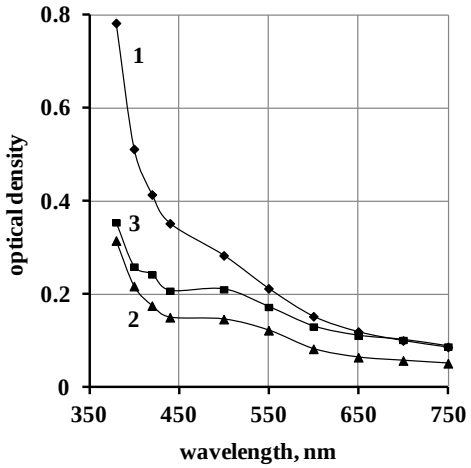


Figure 17*. Optical density of acidified infusion (1) and fermented infusion samples at initial citric concentration of 0.13 % (2) and 0.28 % (3)*

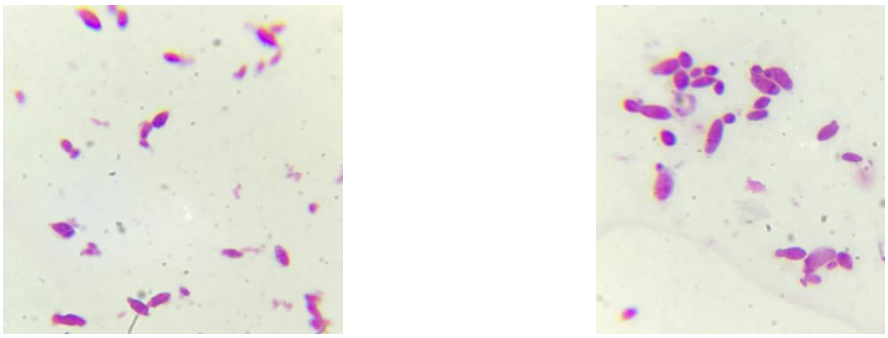
Both samples of fermented marigold infusions exhibited a distinct pleasant floral aroma. A slight sourness was noted in the fermented infusion with a lower citric acid concentration. Apparently, the fermentation by-products in the infusion with a higher initial citric acid concentration mask the acidity.

In the fermented infusion with a lower citric acid concentration, yeast cells were primarily oval, elongated, and small. Yeasts with buds were also observed, though few lysed cells were present (Figure 18 a). Sowing samples of this fermented infusion onto agar media and subsequent microscopy of individual colonies confirmed these conclusions (Figure 18 b).

Table 2. *Indicators of marigold infusion fermentation by native microorganism at different initial citric acid concentrations*

Citric acid amount [wt.%]	Concentration, [wt.%]		*p*H	Acidity [°D]	Reducing substances concentration [g/100 mL]	Microorganism concentration,		Sucrose consumption for formation [%]		
	alcohol	dry matter				[million cells per mL]	[g·L^{-1}]	alcohol	biomass	total
0.13	2.45	3.169	3.00	0.9	1.0	14.91	0.99	47.2	17.8	65.0
0.28	1.94	2.991	3.03	2.4	0.08	11.85	0.79	36.8	13.8	50.6

The initial sugar concentration is 7.0 %



a) **b)**

Figure 18. *Stained yeasts of fermented infusion (a) and yeasts isolated from the agarized media (b). The citric acid concentration is 0.13 %*

Instead, in a fermented infusion with higher citric acid concentration, the cells were more varied in shape and larger. Round-licked cells were available.

CONCLUSIONS

It was determined that yeasts, bacteria, and microscopic molds are components of the microflora of *Tagetes patula* L. flowers. Yeasts from the genera *Saccharomyces*, *Brettanomyces*, and *Rhodotorula*, along with lactic acid bacteria from the genera *Lactobacillus* and *Leuconostoc*, contribute to the fermentation of marigold flower infusion. It was established that in the absence of citric acid, fermentation of the infusion is inhibited by the metabolic products of lactic acid and other bacteria. It was observed that fermentation occurs most intensively with the acidification of marigold infusion using 0.13 % citric acid at a sugar concentration of 7.0 %. It has been found that increasing the initial citric acid concentration and lactic acid bacteria negatively impacts yeasts during the infusion fermentation. The results of the experiments are significant in terms of expanding the range and clarifying the mode of fermented beverages production.

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