EFFECT OF MINT ESSENTIAL OIL ADDITION ON LACTIC ACID FERMENTATION IN STIRRED TANK BIOREACTOR

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Abstract

The increasing consumer's demand for healthy beverages lead to the production of lactic acid fermented wort-based beverages. They can be classified as functional because of wort vitamins, antioxidants and fibres content and probiotic properties of used lactic acid bacteria strain. However, lactic acid wort-based beverages are not well accepted by consumers because of their poor sensory characteristics. The aim of this study was to investigate the effect of the addition of 0.025 and 0.05 % (v/v) mint (Mentha piperita) essential oil on probiotic, antioxidant, and sensorial properties on lactic acid wort-based beverages. Lactic acid fermentation was carried out at 25°C in a stirred bioreactor without aeration. The addition of mint essential oil inhibited lactic acid fermentation but all the beverages produced can be classified as functional. Although the beverage with 0.05% mint essential oil showed the highest total phenolic compounds and antioxidant activity, measured by cupric reducing antioxidant power (CUPRAC), ferric reducing antioxidant power (FRAP) and ABTS radical scavenging assay, it received the lowest score by tasting panel because its strong taste and aroma. The results obtained will be used for modeling of lactic acids fermentation with addition of mint essential oil for the production of beverage with high biological value and acceptable sensory profile.

Keywords: *lactic acid fermentation, wort, mint essential oil, phenolic compounds, antioxidant activity*

1. INTRODUCTION

The increasing consumer's demand for healthy beverages lead to the production of new types functional beverages. Functional beverages should provide benefits for human health, basic nutrition, and reduce the risk of diseases. Therefore, they are often enriched with different bioactive substances such as: vitamins, antioxidants, plant extract, probiotics, etc. Beverages can be classified as functional only if their ethanol content is up to 1.2% v/v (1,2). Lactic acid wort-based fermented beverages are an alternative functional beverage for vegetarians and lactose-intolerant people. These beverages are non-alcoholic, with low pH value (3.5-4.5), produced by wort fermentation of lactic acid bacteria (LAB) strains (3). Wort is produced after a mixture of milled malt and precisely determined amount of water is mashed and this mash is lautered subsequently. Wort consists of many beneficial substances such as: fibres (β-glucan and arabinoxylan), antioxidants (phenolic compounds and melanoidins), and vitamins (folate, riboflavin, pantothenic acid, pyridoxine and niacin) (4). Lactic acid bacteria are Gram positive bacteria, which have beneficial effects on humans, including biotherapeutic action, prevention efficacy, and food preservation (5). *Lacticaseibacillus rhamnosus* (former *Lactobacillus casei* ssp. *rhamnosus*) LBRC 11 is a LAB strain, isolated from home-made cheese. It showed antimicrobial activity against pathogenic and saprophytic microorganisms, antibiotic resistance and resistance to different concentrations of bile salts and different pH values, possibility for conduction of industrial processes (fermentation, freeze-drying) with accumulation and maintaining of a high concentration of viable cells,

thus being very suitable for the development and production of functional beverages (6). It was an excellent choice for lactic acid fermentation of wort (7,8). Lactic acid wort-based fermented beverages can be considered as probiotic if LAB are maintained alive in it until the time of consumption and present in significant numbers, at levels of at least 10^7 viable cells/mL (1).

The main problem with lactic acid wort-based fermented beverages is that they are not accepted by consumer due to objectionable off-flavor and taste (3). Therefore, different herbs or essential oils can be used for their flavouring. Mints (*Mentha piperita*) comprise a group of species of the genus *Mentha* which belong to the family *Lamiaceae.* Mint is widely used in the food, medical, cosmetic, health, and pharmaceutical industries. The aerial parts of the herb on distillation yields essential oil containing a large number of volatile aroma chemicals, flavonoids, organic acids, quinones. The essential oils of some *Mentha* species, including *M. piperita*, *M. spicata*, *M. arvensis* and *M. longifolia* have antimicrobial, antioxidant, radical-scavenging and cytotoxic activities. Mint essential oils are generally used externally for antipruritic, astringent, rubefacient, antiseptic purposes, and for treating neuralgia, myalgia, headaches, and migraines (9-11). Some authors have made lactic acid wort-based beverages with peppermint addition as dried herb (7) or as essential oil (8). The results obtained were promising for the application of mint essential oil in this beverage production. However, in both papers fermentation was carried out statically in bottles and there is no data for effect of stirring on the lactic acid fermentation of wort in bioreactor.

The aim of this study is to produce lactic acid wort-based fermented beverage with mint essential oil in stirred bioreactor. The dynamics of the concentration of viable LAB, phenolic compounds and antioxidant activity was determined in order to estimate the biological value of the beverages produced. The sensorial characteristics of the beverages were also described.

2. MATERIALS AND METHODS

2.1.Materials

2.1.1. Microorganisms

Lacticaseibacillus rhamnosus (former *Lactobacillus casei* ssp. *rhamnosus*) LBRC 11 with proven probiotic properties was isolated from home-made cheese.

2.1.2. Media

MRS Broth Composition (g/L): peptone from casein - 10; yeast extract - 4; meat extract - 8; glucose - 20; K_2HPO_4 - 2; sodium acetate - 5; diammonium citrate - 2; $MgSO_4$ - 0.2; MnSO4 - 0.04; Tween 80- 1 mL/L; pH = 6.5. Sterilization – 15 minutes at 118ºC. The medium was used for cultivation of *Lb. rhamnosus* LBRC 11 at 37±1°C for 24 hours.

LAPTg10 Agar Composition (g/L): peptone - 15; yeast extract - 10; tryptone - 10; glucose – 10; Tween 80 – 1 mL/L; agar – 15; pH = 6.6-6.8. Sterilization - 20 minutes at 121ºC. The medium was used for the determination of the number of viable lactobacilli cells.

2.1.3. Essential oil

Mint essential oil was purchased from Bulgarian rose Plc, Karlovo, Bulgaria.

2.1.4 Reagents

Folin-Ciocalteu (FC) reagent, gallic acid, caffeic acid, quercetin, DPPH (2,2-diphenyl 1-picrylhydrazyl), TPTZ (2,4,6-tripyridyl-s-triazine), ABTS (2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt), neocuproine, $FeCl_3x6H_2O$, and Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) were purchased by Sigma Aldrich. Hydrochloric acid was purchased by Merck, Germany. All the other reagents were of analytical grade.

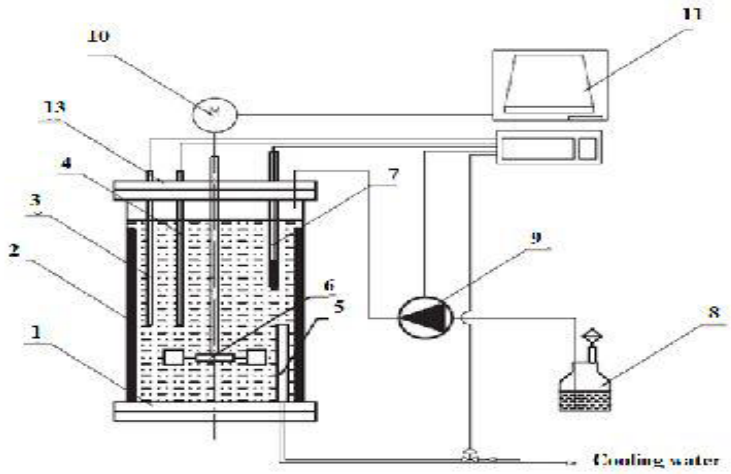
2.2. Methods

2.2.1. Wort production

Wort was produced with 60% Pilsen malt, 20% Vienna malt and 20% Caramel Munich II malt (Bestmaltz, Germany), as described in Trendafilova et al., 2021 (8). The wort extract was 11 % (w/w) and the pH was 5.30.

2.2.2. Wort fermentation

Fermentations were carried out in stirred bioreactor shown in Fig. 1. The apparatus has a geometric volume of 2 L and a working volume of 1.5 L and is equipped with a Sartorius A2 control device, which includes all the measuring instruments for the fermentation process: temperature, pH, dissolved oxygen, etc. Three fermentations were carried out: without mint essential oil (reference), with addition of 0.025% (v/v) mint essential oil, and with the addition of 0.05% (v/v) mint essential oil. Wort was inoculated with 2% (v/v) *Lacticaseibacillus rhamnosus* LBRC 11 and fermentations were carried out at constant temperature of 25±1°C and a stirring speed of 150 rpm.



1 - vessel with a geometric volume of 2 dm3; 2 - baffles; 3 - temperature electrode (thermometer); 4 - cooling/heating device (water jacket); 5 - an additional cooling/heating device; 6 - turbine stirrer; 7 - pH/Eh electrode; 8 - fermentation medium/inoculum/pH adjustment medium; 9 -peristaltic pump; 10 - stirrer drive; 11 – Sartorius A2 reference device;

Fig. 1. Stirred tank bioreactor

2.2.3. Analytical procedures

Fermentation parameters.

The extract was measured according to EBC standard methods (12) by the means of Anton Paar DMA 35 density meter (Anton Paar, Graz, Austria). pH was measured by bioreactor itself at every 5 minutes.

Determination of the number of viable LAB cells

Appropriate tenfold dilutions of the samples were prepared using sterile saline solution. 1 mL of the last three dilutions was used for pour plating in Petri dishes with LAPTg10–agar. The inoculated and solidified Petri dishes were incubated for 48-72 hours at 37 ±1°C until the appearance of countable single LAB colonies.

Determination of phenolic compound content and antioxidant activity

The wort and lactic acid wort-based beverages were diluted in a proper ratio with methanol, rested for 30 minutes and filtered using Whattman No. 1 filter paper. The filtrate was used to analyse the phenolic compounds and the antioxidant activity of beverages.

Total phenolic compounds (TPC) with Folin-Ciocalteu (FC) reagent, content of phenolic compounds (TPC, phenolic compounds, and flavonoids) by the modified Glories method, antioxidant activity (AOA) against the DPPH radical, AOA by the FRAP method, AOA by the ABTS method, and AOA by the CUPRAC method were measured according to Shopska et al., 2021 (13).

Sensory analysis

A sensory evaluation of the beverages was carried out by a trained, 6-member tasting panel. The samples were evaluated by their taste and aroma using descriptive analysis and ranking method (methods 13.10 and 13.11) (12).

Statistical analysis

The results of all the analyses were expressed as the mean values±standard deviation of three replicates using Microsoft Excel 2013.

3. RESULTS AND DISCUSSION

In our preliminary studies, the antimicrobial activity of mint essential oil against Lacticaseibacilluscasei rhamnosus LBRC11 was investigated using the disc-diffusion method. It was found that it did not show antimicrobial activity against the tested microorganism in concentrations up to 1% (v/v) (unpublished data). Because of the oil strong taste and aroma, it was used in concentrations up to 0.05% (v/v).

Fermentation dynamics

The results for fermentation dynamics, measured 1 by pH drop and viable cells concentration are shown in Fig. 2 and 3, respectively. The results for pH drop (Fig. 2) showed that fermentation lasted 24 hours for all the beverages produced. The extract consumption was very low, because the wort produced by the used mashing regime contained approximately 30-35% fermentable sugars, the main one of which is maltose (14). The extract decreased with 0.1% (w/w) for the beverage with 0.05% mint essential oil addition and with 0.2 % (w/w) in the other beverages. Although the difference was not significant, we can assume that the increase in mint essential oil concentration inhibited lactic acid fermentation. This was also confirmed by the data on the concentration of viable cells in the beverages (Fig. 3).

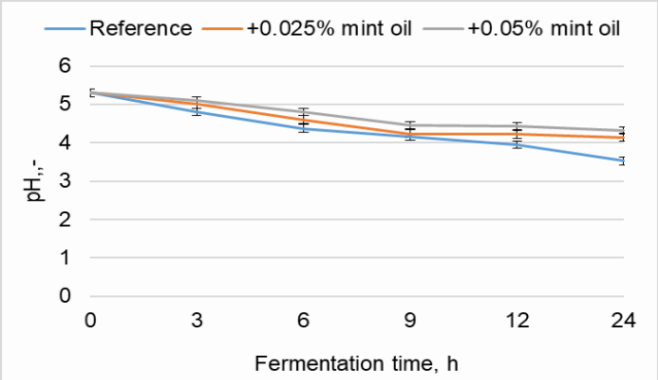


Fig. 2. pH changes during fermentation

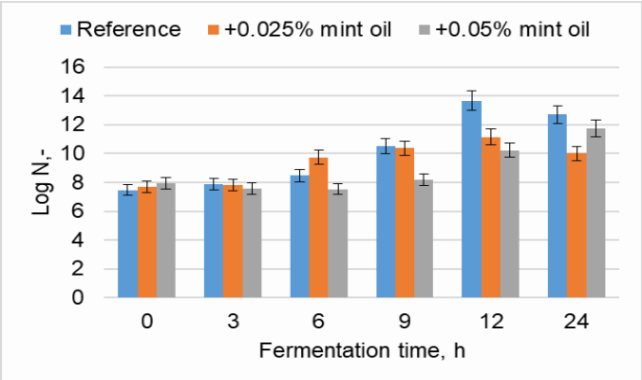


Fig. 3. Changes in viable cells concentration during fermentation. Log *N* on *Y* axis is log10*N*, where *N* is the number of viable *L. rhamnosus* cells, CFU/mL

Until the 12th hour an increase in the number of viable LAB cells was observed for all the beverages produced. At 24th hour the amount of viable cells decreased in the reference and the beverage with 0.025% mint essential oil but increased in the beverage with 0.05% mint essential oil. However, at the end of fermentation the viable cells concentration was highest in the reference. All the beverages obtained could be classified as functional because the viable LAB concentration in them was over 10^{10} CFU/mL. The pH changes during fermentation (Fig. 2) also confirmed the hypothesis for mint essential oil inhibition of lactic acid fermentation even in these low concentrations. In all the beverages produced pH decreased gradually from the beginning of the fermentation. At the end of the fermentation the pH of the reference was 1.7 units lower than the initial pH. The pH drop for the beverages with 0.25% and 0.05% mint essential oils were 1.2 and 1, respectively. Stirring was the main reason for shorter fermentation (24 hours in stirred bioreactor vs 48 hours in bottles without stirring (8)). Moreover, stirring keep the LAB cells in suspension, which led to higher concentration of LAB in the beverages produced in stirred bioreactor (over 10^{10} for all beverages produced by dynamic fermentation vs over 10^{10} only for refernce, produced by static fermentation (8)).

Biological value

All the beverages produced were analysed for determination of phenolic compounds content and AOA because mint essential oil was a source of different biologically active compounds and it was interesting to see if they would interact with wort constituents. The results are shown in Table 1.

	Reference		**+0.025% mint essential oil**		**+0.05% mint essential oil**	
	0h	**24 h**	**0h**	**24 h**	**0h**	**24 h**
TPC by FC method, mg/L	593	580	619	217	673	884
TPC by modified Glories method, mg/L	835	825	727	678	796	915
Phenolic acids, mg/L	187	194	153	135	178	117
Flavonoids, mg/L	110	110	74	58	97	48
AOA by DPPH method, µmol TE/L	1072	1756	1294	1712	2001	1634
AOA by FRAP method, µmol TE/L	1148	1373	998	1390	1538	2005
AOA by ABTS method, µmol TE/L	2304	2038	2601	2713	2709	2801
AOA by CUPRAC method, µmol TE/L	13692	3136	14247	3108	7923	13692

Table 1. Changes in phenolic compounds concentration and antioxidant activity of beverages produced without or with mint essential oil addition.

The results for TPC measured by FC method showed a sharp drop in the TPC concentration in the beverage with 0.025% mint essential oil at the end of fermentation. On the contrary, the TPC concentration in the beverage with 0.05% mint oil increased approximately 1.3 times. Only in the reference sample, the amount of phenolic compounds was relatively constant. The tendencies for TPC determined by the modified Glories method were similar to these for TPC determined by FC method. However, the modified Glories method is based on the characteristic absorption of the benzene cycles of the majority of phenols at 280 nm and is less influenced by the oxidative status of the analyzed molecules (15). It has to be highlighted that the results for the TPC, measured by both methods in the beverage with 0.05% mint essential oil was almost equal, which indicated that the beverage contained mainly unoxidized phenolic compounds. In the reference, about 200 mg/L were oxidized phenolic compounds, while in the beverage with 0.025% mint essential oil, mainly oxidized phenolic compounds predominated.

The concentrations of phenolic acids and flavonoids in the reference sample remained relatively constant during the fermentation and they were the highest among the final beverages. At the end of fermentation, the beverage with 0.05% essential oil had the lowest concentration of the both phenolic compounds. The results observed differed from those of the static fermentation, where the lowest content of phenolic acids and flavonoids was in the reference (8). It can be assumed that stirring affected the phenolic acids and flavonoids content by facilitating their interaction with some of the mint essential oil constituents.

From a modern point of view, several methods should be used to evaluate AOA in vitro to cover all aspects of antioxidant capacity (21->16). The AOA of the investigated samples was determined by 4 different methods - activity against DPPH and ABTS radicals; measurement of total antioxidant activity against Cu^{2+} (CUPRAC) and against Fe^{3+} (FRAP). At the beginning at fermentation, the addition of 0.05% mint essential oil led to the highest AOA, measured by DPPH, FRAP and ABTS methods and the lowest AOA, measured by CUPRAC method (Table 1). At the end of fermentation, the AOA of the beverage with 0.05% mint essential oil was highest against ABTS radical, Cu^{2+}, and Fe^{3+}. The highest AOA against DPPH radical showed the reference sample.

It can be summarized that, in general, the drink with 0.05% mint essential oil showed the highest AOA. It can be ascribed to the highest concentration of TPC, measured in this beverage. Moreover, the results confirmed the observation that AOA of polyphenols could be assessed most accurately by scavenging free radicals or delaying the generation of free radicals using different *in vitro* methods, including the DPPH antioxidant assay and the ABTS radical cation decolourization assay, and the FRAP assay (15).

Sensory analysis

At the end of the fermentation process, a sensory analysis of the beverages obtained was made (Fig. 4). The beverage with addition of 0.05% mint oil showed a strong mint aroma and taste that succeed in dulling all other sensory characteristics. Therefore, it received the lowest rank. In the beverage with 0.025% mint oil, the mint aroma and taste again dominated, but a slight fermentation tone can also be detected. The combination of these two parameters made the drink very interesting and quite reasonably it was rated higher than the beverage with 0.05% mint essential oil. The highest score was given to reference, which was viscous, with a pleasant sour taste, which has a refreshing character.

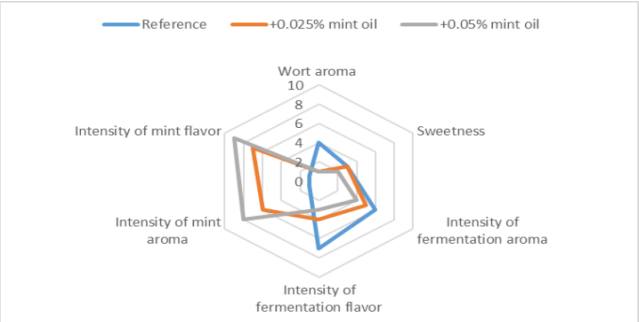


Fig. 4. Sensory analysis of beverages produced

4. CONCLUSIONS

Lactic acid fermentation of wort with addition of mint essential oil in concentrations of 0.025% (v/v) and 0.05% (v/v) in stirred tank bioreactor was made. A fermentation without mint essential oil was used as a reference. Stirring led to reduction of fermentation time with 24 hours and increase in LAB concentration compared to static fermentation. All the beverages produced can be classified as functional because the viable LAB concentration was above 10^{10} CFU/mL. Although the beverage with 0.05% mint essential oil showed the highest TPC and antioxidant activity, measured by ABTS, FRAP and CUPRAC methods, it received the lowest score by tasting panel because its strong taste and aroma. The results obtained will be used for modeling of lactic acids fermentation with addition of mint essential oil for the production of wort-based beverages in stirred bioreactor for the production of beverage with high biological value and acceptable sensory profile.

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