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# The beneficial effect of peppermint (*Mentha X Piperita* L.) and lemongrass (*Melissa officinalis* L.) dosage on total antioxidant and polyphenol content during alcoholic fermentation

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# ABSTRACT

Our research aimed to create an herbal fermented alcoholic beverage with high antioxidant and polyphenol content. In this study, continuous sampling was performed throughout the fermentation period, and the changes in total antioxidant (TA) and total polyphenol (TP) contents were determined. After processing the raw material, the prepared herbs were added in 0.5 and 1.0 v/v% concentrations to the samples. The TP content of the control sample was between 1.17 and 1.57 mg/g, and the TA content was 2.12 and 2.54 mg/g during the fermentation process. The lemongrass dosage increased 77.86 % the antioxidant and 70.98 % the polyphenol content by the end of the fermentation process. In the best case, the peppermint dosage increased 72.80 % of the antioxidant content and 72.05 % of the polyphenol content. Overall, fermentation combined with herbs dosage could increase the bioavailability of products made from its polyphenol and antioxidant contents and can be used to develop novel functional foods.

# Introduction

Most foods contain essential ingredients for the human body. Advances in food production and medicine have led to the addition of valuable additives to food products. In this way, these beneficial components can be introduced into the human body in an increased manner by the food consumed. Nowadays, lot of research is based on the production and examination of food products supplemented with various herbs (Marimuthu, 2019; Thongkhao et al., 2020) and its essential oils in dairy products (Mishra et al., 2020), chocolate (Belščak-Cvitanović et al., 2012) or pork meat products (Araújo et al., 2021).

The demand for functional food or drinks with healing applications has increased mainly due to easy access. It has been an emerging trend in the last decade, which provides the tools for improving quality of life (Shahidi & Ambigaipalan, 2015). The consumption of infusions of leaves, flowers, fruits, and seeds of some vegetable species is widely practiced in terms of beverages and produces essential effects on human health. It allows for an improvement in the oxidative balance due to the

phytochemical composition of these species (Valduga et al., 2019).

Oxidation, free radical formation, and absorbing reactions occur in human health and every living organism and biological system. The functionality of consuming foods rich in polyphenols has been confirmed: they increase the antioxidant capacity of blood plasma, reduce the risk of chronic human disease by preventing harmful oxidative chain reactions of cell constituents, and reduce DNA damage to lymphocytes. (Pandey & Rizvi, 2009). Natural phenols play an important role in protection against several pathological diseases such as atherosclerosis, cancers (Ames et al., 1995), and cerebral dysfunction (Cardona et al., 2013). Food antioxidants, added to foodstuffs, have the same role as the antioxidants of the human body, to protect the food and conserve its texture organoleptic and consumption safety properties (Carocho et al., 2018).

Apples are among the most common fruits due to their price, taste, and ability to obtain a wide range of processed products (juices, purees, wines, and ciders). Apples and their products include considerable amounts of phenolic compounds (Khanizadeh et al., 2008), which play

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an important role in human health. They have a preventive impact against various diseases such as cancer, cardiovascular diseases (Rodriguez-Mateos et al., 2013), neuropathies, and diabetes (Shahidi, 2012). Fruits contain polyphenols from various groups, but their concentration is not high and strongly depends on which part of the plant they are taken, for example, from fruit variety or climatic conditions (De Paepe et al., 2015; Simmonds & Howes, 2016) or cultivar (Saidania et al., 2017). In addition, the antioxidant and polyphenol content decreases when processing apples (Alberti et al., 2016).

Fruits and vegetables are essential food sources that are highly recommended because of their low caloric intake combined with substantial amounts of micronutrients and dietary fibre (Genser, 2008). Furthermore, fruit juices, as well as alcoholic drinks (especially red wine) are increasingly consumed because of the pharmacologic (e.g., antioxidant) effects of specific constituents (Gruenwald, 2008). However, some fruits and vegetables being eaten whole or in concentrate form may cause clinically relevant drug-food inter-actions.

The polyphenolic content of apple juice depends on apple varieties (Laaksonen et al., 2017) or processing methods. These technologies are for example, the utilization of pectolytic enzymes to release phenolic compounds from polysaccharide structures with different treatments. (Ye, Yue, & Yuan, 2014).

Fermentation is an ancient technique used to enhance the shelf-life and nutritional and organoleptic qualities of food (Frias et al., 2005). Fruits and vegetables contain many antioxidant compounds, including phenolic compounds, carotenoids, anthocyanin's, and tocopherols (Naczk & Shahidi, 2006). Polyphenols play important roles in the quality of alcoholic products as they are related to the colour, bitterness, and astringency, whose balance defines the overall organoleptic properties of the beverage (Lea & Drilleau, 2003).

In recent years, apple wine consumption has increased and has become a trendy drink. Apple wine combines the advantages of wine and fruit juice, and it has a low alcohol content between 1.2 and 8.5 % v/v as well as a mellow taste (Laaksonen et al., 2017). Apple wine production starts with pressed apple juice. During the fruit crushing, pressing only a fraction of phenolic compounds is extracted (Van der Sluis, Dekker, & van Boekel, 2005). This is mainly due to the discarding the peel and seeds rich in different phenolic compounds (Francini & Sebastiani, 2013). A significant proportion of phenolic compounds are concentrated in the fruit's peel. Due to the poor extraction efficiency, the TA and TP content of the pressed juices shows lower values than the remaining pulp (Nogueira et al., 2008).

The various fruits and many herbs have antioxidant and polyphenol content. Herbs are used in many domains, including medicine, nutrition, flavoring, beverages, colour materials, insect repellents, flavor, cosmetics (Djeridane et al., 2006). Many species have been recognized to have medicinal properties and a beneficial impact on health. These effects are, for example, antioxidant activity, digestive stimulation action, anti-inflammatory, antimicrobial (Shakeri et al., 2016, Sandasi et al., 2017) hypolipidemic, antimutagenic effects, and anticarcinogenic potential (Luo et al., 2004). The herbs have been used as antioxidants as whole or ground forms, extracts, encapsulated kinds of stuff, or as emulsions. However, besides its antioxidant effect, spices and herbs are all-natural, an attractive quality for consumers. Thus, they may be used to control lipid oxidation in foods (Embuscado, 2015).

Therefore, in this study, the mash was made from apples with added herbs, and then they were subjected to ethanol fermentation to obtain wines or ciders.

#### Materials and methods

# Raw materials and fermentation process

#### Fruit, herbs and additives

Malus domestica Borkh. 'Idared' fruits (15 kg) were derived from a Hungarian retail unit in a state of complete ripeness. Plant parts inappropriate for consumption and unripe fruits were discarded right after the arrival of the material, and we started the preparation immediately. As an additional additive to our experiments, we used two different herbs. These are lemongrass (*Melissa officinalis* L.) and peppermint (*Mentha* × *piperita* L.), which were freshly collected from the herb garden of the Department of Food Science (Széchenyi István University, Hungary) on the day of the experiment.

For the enzyme treatment, we used 2 g / 100 kg pectinase enzyme (Safizym Clean, Fermentis, France; *endo*-polygalacturonase (>2,450 PG/g), such as pectin-methylesterase (>490 PE/g), and pectin lyase (>70 PL/g).

During the fermentation process, *Saccharomyces bayanus* Saccardo yeast strain (Safspirit Fruit - Fermentis, France) was used as a starter culture for fruit fermentation with a recommended dosage of 20 g /100 kg. Before use, they were rehydrated in 10 fold volume of apple juice at 25 °C for 30 min. As for the yeast nutrient (20 g / 100 kg), we used SpringFerm<sup>TM</sup> (Fermentis, France), which includes inactivated yeast (rich in growth factors), zinc sulphate, and manganese sulphate.

# Conditions of fermentation

After washing, stoning, and chopping was made five types of samples from the 15 kg of apple material in the preparation process. The experiments were carried out using five fermentation-related parameters (liquid volume, pH, fermentation temperature, and fermentation time). Samples (3 L/sample) were put in a 5 L Erlenmeyer flask, sealed with an airlock tube that released the CO<sub>2</sub> by-product. Fermentation lasted for 17 days at 18 °C of temperature. The initial pH of all samples was set to 3.2 with 20 % phosphoric acid (Merck, Germany). Freshly harvested herbs were added after washing in chopped form without drying to the mash (wet material).

Five types of fermentation was carried out: (1) Control sample (C) pH adjustment, pectin digestion, yeast inoculation, and additional yeast nutrient. (2) Sample with 0.5 % (10 g) peppermint dosage (M-0.5) pH adjustment, pectin digestion, and yeast inoculation. (3) Sample with 1.0 % (20 g) peppermint dosage (M-1.0) pH adjustment, pectin digestion, and yeast inoculation. (4) Sample with 0.5 % (10 g) lemongrass dosage (L-0.5) pH adjustment, pectin digestion, and yeast inoculation. (5) Sample with 1.0 % (20 g) peppermint dosage (L-1.0) pH adjustment, pectin digestion, and yeast inoculation.

Sampling was taken eight times: on day 1 (sweet mash), then on days 3, 5, 8, 10, 12, 15, and 17. Samples (40 mL) were stored in an ultralow freezer at -55 °C (Bio-Medlab B-HL 100, China). Three parallels were carried out to measure the ethanol, TA, and TP content.

The mash was pressed at the end of the fermentation process, so we got the fermented apple wine and remaining pomace. The TA and TP content of the apple wine and pomace were examined.

# Analytical methods

#### Sample preparation of antioxidant and polyphenol determination

During sample preparation of antioxidant and polyphenol determination, 10 g of each sample (fermented mash and pomace) into a 250 mL Erlenmeyer flask and diluted with 15 mL of high-purity water (Zeneer-Power 1, Human Corporations, Korea), 35 mL methanol and 0.05 mL hydrogen chloride (Merck) was measured, then stored for 1 h on a rotary shaker (Elphan 358S, Bohemia) for extraction at room temperature (24  $^{\circ}$ C). After storing, the samples were centrifuged at 3500 g for 20 min. Measurements were then continued with the centrifuged supernatant. In the case of wine, centrifugation without extraction was the sample preparation method.

# Determination of antioxidant

The TA content of a sample was determined using the ferric reducing ability of plasma FRAP assay by Benzie and Strain (1996). The FRAP reagent should be made immediately before using 2.5 mL of the 10 mM TPTZ stock solution, 25 mL of acetate buffer (300 mM, pH 3.6), and 2.5 mL of 20 mM FeCl<sub>3</sub> solution (1:10:1). To make a 10 mM 2,4,6-tri(2-pyr-idyl)-1,3,5-triazine (TPTZ) solution 0.31 g to 100 mL HCl (40 mM) was added. To make acetace (300 mM, pH 3.6) buffer 0.16 g of sodium acetate in 100 mL of 0.28 M acetic acid was dissolved, the pH should be 3.6 (adjusted using 1 M HCl). To ferric chloride stock solution preparing, 20 mM FeCl<sub>3</sub> solution was added to 0.135 g of the compound to 25 mL of distilled water.

The assay was based on the reducing power of a compound (antioxidant). A potential antioxidant will reduce the ferric ion (Fe<sup>3+</sup>) to the ferrous ion (Fe<sup>2+</sup>); the latter forms a blue complex (Fe<sup>2+</sup>/TPTZ), which increases the absorption at 593 nm. The FRAP reagent (3 mL) extracted sample (100 µl) and distilled water (100 µl) were added to each well and mixed thoroughly. The absorbance was taken with a spectrophotometer (Pharo 100, Merck) at 593 nm after 10 min. The standard curve was prepared using different ascorbic acid concentrations (40–500 mg/L). All solutions were used on the day of preparation. All determinations were performed in triplicate (n = 3).

# Determination of polyphenols

TP content was measured using Folin–Ciocalteu colorimetric method described previously by Gao et al. (2000). Plant extracts (100  $\mu$ l) were mixed with 2.5 mL of Folin–Ciocalteu reagent (10 %) and 1.5 mL of H<sub>2</sub>O and incubated at room temperature for 3 min. After adding 1 mL of 20 % sodium carbonate to the mixture, TP was determined after 90 min of incubation at room temperature. The absorbance of the resulting blue colour was measured at 765 nm with a spectrophotometer (Pharo 100, Merck). Quantification was done concerning the standard curve of gallic acid (25–500 mg/L). The results were expressed as gallic acid equivalents (GAE), milligrams per 100 g of dry weight (DW). All determinations were performed in triplicate (n = 3).

#### Sample preparation and determination of alcoholic content

During sample preparation of alcoholic content, 1 g of each mash sample into 250 mL Erlenmeyer flask and diluted it with 20 mL of highpurity water (ZeneerPower 1, Human Corporations, Korea) was measured, then stored for 1 h on a rotary shaker (Elphan 358S, Bohemia) for extraction at room temperature (24 °C). Extracted samples were centrifuged for 30 min at 6000 RCF (Labnet Hermle Z206A, USA) in a 15 mL tube, then 1.5 mL of each supernatant was centrifuged for an additional 20 min 14 500 RCF (Biosan Micro spin 12, Latvia) in 2 mL Eppendorf tube. Subsequently, the samples were simply filtered through a membrane filter (polyvinylidene difluoride [PVDF nylon] 0.22 µm, Filter Bio) into screw topped HPLC vials of 2 mL with an inner septum (Berrytec, Germany). Ethanol was measured with an HPLC-UV system. Before analyses, the measuring system was calibrated with solutions of 0.05–10 mg/mL concentration made from the measurand (Ethanol 96 % a.r. - Merck, Germany). Measuring solutions were diluted to the graduation mark with the eluent (high purity water). The separation of ethanol was also carried out using an ion-exchange HPLC system (LC 900, Jasco, Japan); components were identified with a refractive index detector (RI 71, Merck, Germany). The column (Supelcogel H, Sigma Aldrich, USA) was kept at room temperature, and the flow rate of the eluent was 0.5 mL/min.

# Statistical analyses

Data were expressed as the mean (n = 3)  $\pm$  relative standard deviation (RSD). One-way analyses of variance (ANOVA) were used to compare the significant difference for the data. The predicted values were considered significant at p  $\leq$  0.05. The statistical analyses were performed using Microsoft Office Excel 2016® software.

#### **Results and discussion**

#### Results of the TA content of the samples during fermentation

In the case of the control sample, it can be seen (Table 1) that the antioxidant content increased by 19.8 % compared to the initial value at the end of the fermentation process. This value was 52.8 % in the case of 0.5 v/v% peppermint and 52.8 % in the case of 0.5 v/v% lemongrass dispensed sample. The antioxidant content of the sample containing 1 v/ v% peppermint increased by 60.7 %, while the 1 v/v% addition of lemongrass resulted in a 58.7 % increase. On the first five days of fermentation, the antioxidant levels of the samples developed similarly. For all samples, it can be observed that the antioxidant content increases continuously until the 10th day of fermentation. The antioxidant content shows a decreasing trend from here, 10.3 % for the control. This decomposition was 0.9 % for 0.5 % peppermint dosage and 2.9 % for 0.5 % lemongrass dosage. Antioxidant degradation was 8.3 % (peppermint) and 8.9 % (lemongrass) in case of observed at 1 % herbal dosing. The number of antioxidants was the highest in lemongrass dosage samples compared to the control and samples containing peppermint. Kawa-Rygielska et al. (2019) has observed significant differences in the amount of antioxidant content between mead without additives used as a control sample (MW) and mead with the addition of chokeberry syrup or red grape seeds mead (MGS) samples. A significant increase in the total polyphenols was observed in the MGS samples after fermentation and aging when their content was twofold higher than in the MW. Peppermint and also lemongrass contain significant antioxidant compounds (menthol; menthone; 1-8-cineole; neomenthol; carvone; limonene), resulting in increased levels of these compounds during fermentation.

#### Results of the TP content of the samples during fermentation

The content of polyphenols in fruits and processed fruit products depends on the variety of fruits and their pre-treatment and processing methods (Kucharska et al., 2017; Oszmiański & Kucharska, 2018). In the case of control sample, the number of polyphenols increased by 34.1 % from the initial value by the end of the fermentation (Table 2). This upward trend was 48.7 % (0.5 % dosing) and 66.4 % (1 % dosing) in the case of peppermint dosage samples. The increase of polyphenol amount was 59.3 % for the sample containing 0.5 v/v% lemongrass and 61.8 %

#### Table 1

Results of the TA content of samples during fermentation process (C - Control sample; M-0.5 - Sample with added 0.5 % (v/v) peppermint; L-0.5 - Sample with added; 0.5 % (v/v) lemongrass; M-1.0 - Sample with added 1.0 % (v/v) peppermint; L-1.0 - Sample with added 1.0 % (v/v) lemongrass).

Day of	TA content mg ascorbic acid/ g					
fermentation	С	M-0.5	L-0.5	M-1.0	L-1.0	
1	$\begin{array}{c} \textbf{2.12} \pm \\ \textbf{0.03}^{\text{a}} \end{array}$	$\begin{array}{c} \textbf{2.21} \pm \\ \textbf{0.06}^{a} \end{array}$	$\begin{array}{c} \textbf{2.23} \pm \\ \textbf{0.06}^{ab} \end{array}$	$\begin{array}{c} \textbf{2.29} \pm \\ \textbf{0.07}^{ab} \end{array}$	$\begin{array}{c} \textbf{2.35} \pm \\ \textbf{0.09^{bc}} \end{array}$	
3	$\begin{array}{c} \textbf{2.25} \ \pm \\ \textbf{0.06}^{a} \end{array}$	$\begin{array}{c} \textbf{2.67} \pm \\ \textbf{0.06}^{b} \end{array}$	$\begin{array}{c} 2.71 \ \pm \\ 0.08^{b} \end{array}$	$\begin{array}{c} \textbf{2.91} \pm \\ \textbf{0.06}^{c} \end{array}$	$\begin{array}{c} \textbf{2.98} \pm \\ \textbf{0.08}^{c} \end{array}$	
5	$\begin{array}{c} 2.32 \ \pm \\ 0.05^{a} \end{array}$	$\begin{array}{c} 3.01 \ \pm \\ 0.05^{b} \end{array}$	$\begin{array}{c} 3.15 \pm \\ 0.12^{b} \end{array}$	$\begin{array}{c} {\rm 3.29} \pm \\ {\rm 0.11}^{\rm bc} \end{array}$	$\begin{array}{c} \textbf{3.47} \pm \\ \textbf{0.12}^{c} \end{array}$	
8	$\begin{array}{c} 2.63 \pm \\ 0.12^a \end{array}$	$\begin{array}{c} 3.32 \pm \\ 0.05^{b} \end{array}$	$\begin{array}{c} 3.45 \pm \\ 0.10^{b} \end{array}$	$\begin{array}{c} \textbf{3.75} \pm \\ \textbf{0.05}^{c} \end{array}$	$\begin{array}{c} 3.96 \pm \\ 0.10^d \end{array}$	
10	$\begin{array}{c} \textbf{2.89} \pm \\ \textbf{0.09}^{a} \end{array}$	$\begin{array}{c}\textbf{3.48} \pm \\ \textbf{0.07}^{b} \end{array}$	$\begin{array}{c} 3.66 \pm \\ 0.07^b \end{array}$	$\begin{array}{c} \textbf{3.94} \pm \\ \textbf{0.16}^{c} \end{array}$	$\begin{array}{c} \textbf{4.18} \pm \\ \textbf{0.07^c} \end{array}$	
12	$\begin{array}{c} \textbf{2.83} \pm \\ \textbf{0.07}^{\rm a} \end{array}$	$\begin{array}{c} \textbf{3.41} \pm \\ \textbf{0.09}^{b} \end{array}$	$\begin{array}{c} 3.51 \ \pm \\ 0.15^{\rm b} \end{array}$	$4.01 \pm 0.09^{\rm c}$	$\begin{array}{c} 4.09 \pm \\ 0.15^{c} \end{array}$	
15	$\begin{array}{c} \textbf{2.58} \ \pm \\ \textbf{0.05}^{\text{a}} \end{array}$	$\begin{array}{c} 3.39 \ \pm \\ 0.19^{b} \end{array}$	$\begin{array}{c} \textbf{3.49} \pm \\ \textbf{0.08}^{b} \end{array}$	$\begin{array}{c} \textbf{3.88} \pm \\ \textbf{0.11}^{c} \end{array}$	$\begin{array}{c} \textbf{3.86} \pm \\ \textbf{0.08}^{c} \end{array}$	
17	$\begin{array}{c} \textbf{2.54} \pm \\ \textbf{0.12}^{\text{a}} \end{array}$	$\begin{array}{c} \textbf{3.38} \pm \\ \textbf{0.12}^{b} \end{array}$	$\begin{array}{c} 3.41 \ \pm \\ 0.13^b \end{array}$	$\begin{array}{c} \textbf{3.68} \pm \\ \textbf{0.17}^{c} \end{array}$	$\begin{array}{c} 3.73 \pm \\ 0.13^c \end{array}$	

Values are means  $\pm$  SE (n = 3). Within each line, values with different letters are significantly different (p < 0.05).

#### Table 2

Results of the TP content of samples during fermentation process (C - Control sample; M-0.5 - Sample with added 0.5 % (v/v) peppermint; L-0.5 - Sample with added; 0.5 % (v/v) lemongrass; M-1.0 - Sample with added 1.0 % (v/v) peppermint; L-1.0 - Sample with added 1.0 % (v/v) lemongrass).

Day of	TP content mg GAE/ g				
fermentation	С	M-0.5	L-0.5	M-1.0	L-1.0
1	$1.17~\pm$	$1.19~\pm$	1.23 $\pm$	$1.25 \pm$	1.31 $\pm$
	0.04 <sup>a</sup>	0.03 <sup>a</sup>	0.07 <sup>a</sup>	0.07 <sup>ab</sup>	$0.06^{b}$
3	1.35 $\pm$	1.39 $\pm$	$1.42 \pm$	1.43 $\pm$	$1.52 \pm$
	$0.07^{a}$	$0.03^{a}$	0.04 <sup>a</sup>	$0.06^{ab}$	$0.09^{b}$
5	1.54 $\pm$	1.63 $\pm$	1.75 $\pm$	$1.84~\pm$	$1.97 \pm$
	0.03 <sup>a</sup>	$0.08^{a}$	$0.11^{ab}$	0.09 <sup>ab</sup>	$0.08^{\mathrm{b}}$
8	1.67 $\pm$	1.84 $\pm$	$1.93~\pm$	$\textbf{2.07}~\pm$	$\textbf{2.18} \pm$
	0.09 <sup>a</sup>	$0.07^{b}$	$0.07^{b}$	0.05 <sup>c</sup>	0.10 <sup>c</sup>
10	1.78 $\pm$	1.96 $\pm$	$2.09~\pm$	$\textbf{2.16} \pm$	$\textbf{2.24} \pm$
	$0.11^{a}$	$0.07^{\rm b}$	$0.13^{b}$	$0.16^{bc}$	0.13 <sup>c</sup>
12	1.72 $\pm$	1.88 $\pm$	$2.05~\pm$	$\textbf{2.12} \pm$	$\textbf{2.22} \pm$
	$0.07^{a}$	$0.11^{b}$	$0.10^{c}$	0.09 <sup>c</sup>	0.09 <sup>cd</sup>
15	1.63 $\pm$	1.79 $\pm$	$1.97~\pm$	$2.08~\pm$	$2.17~\pm$
	0.05 <sup>a</sup>	$0.07^{b}$	0.08 <sup>c</sup>	0.08 <sup>c</sup>	0.08 <sup>cd</sup>
17	1.57 $\pm$	1.77 $\pm$	$1.96~\pm$	$\textbf{2.08}~\pm$	$\textbf{2.12} \pm$
	0.12 <sup>a</sup>	0.12 <sup>b</sup>	0.09 <sup>c</sup>	0.11 <sup>d</sup>	0.11 <sup>d</sup>

Values are means  $\pm$  SE (n = 3). Within each line, values with different letters are significantly different (p < 0.05).

and 1 v/v% herb dosage by the end of fermentation. Similar to the antioxidant results, the amount of polyphenols increases up to the 10th day of fermentation, however, by the end of fermentation, their amount had decreased.

Yeasts, for example, *Saccharomyces* strains may considerably affect the polyphenol content of the fermented product, because the metabolites generated during fermentation such as pyruvic acid and acetaldehyde, can react further with the phenolic compounds (Romano et al., 2008). The most common phenolic compounds in apples are catechins ((+)-catechin; procyanidin B2; (-)-epicatechin; phloretin glycosides (phloretin-2'-xyloglucoside; phloridzin), quercetin glycosides (hyperoside; isoquercitrin; glycoside + Rutin), and hydroxycinnamic acids (chlorogenic acid).

# Results of the TA and TP content of wine and pomace

Fermented samples were squeezed at the end of the process and were also examined the antioxidant and polyphenol content of wine and

#### Table 3

TA and TP content of fermented apple wine and remaining pomace of the end of the fermentation process (17th day) with and without herbs dosage.

1 ,	57	0
Sample	TA content mg ascorbic acid/ g	TP content mg GAE/ g
Control - pomace	$2.14\pm0.06^a$	$1.21\pm0.02^{\rm a}$
Control - wine	$1.49\pm0.07^{\rm b}$	$0.67\pm0.01^{\rm b}$
Peppermint dosage 0.5 % - pomace	$2.69\pm0.13^{c}$	$1.37\pm0.01^{\text{c}}$
Peppermint dosage 0.5 % - wine	$2.05\pm0.02^d$	$\textbf{0.89}\pm\textbf{0.01}^{d}$
Peppermint dosage 1.0 % - pomace	$2.94\pm0.05^e$	$1.55\pm0.07^e$
Peppermint dosage 1.0 % - wine	$2.38\pm0.12^{\rm f}$	$1.02\pm0.01^{\rm f}$
Lemongrass dosage 0.5 % - pomace	$2.81\pm0.03^{e}$	$1.67\pm0.03^{\text{e}}$
Lemongrass dosage 0.5 % - wine	$2.48\pm0.06^{\rm f}$	$1.21\pm0.01^{\text{g}}$
Lemongrass dosage 1.0 % - pomace	$3.26\pm0.05^g$	$1.83\pm0.05^{d}$
Lemongrass dosage 1.0 % -	$2.71\pm0.06^e$	$1.34\pm0.01^{c}$

Values are means  $\pm$  SE (n = 3). Within each column, values with different letters are significantly different (p < 0.05).

pomace (Table 3). The results show that for all samples, the amount of antioxidants and polyphenols in the remaining pomace was higher than in the fermented pressed wine. In the case of the control sample, a difference of 43.4 % was observed. This difference in case of peppermint dosage samples was 31.2 % (0.5 v/v%) and 42.0 % (1 v/v%), while samples containing lemongrass it was 13.3 % (0.5 v/v%) and 20.3 % (1v /v%) resulted in a difference. This can be explained by the fact that a significant part of the phenolic compounds is concentrated in the fruit skin. This phenomenon is supported by another reference that justifies the quantitative differences between pressed wine and pomace due to the poor extraction efficiency of polyphenolic compounds (Nogueira et al., 2008). The results also show that the antioxidant and polyphenol content of the samples supplemented with lemongrass was higher.

# Results of the ethanol content

During the experiment, the amount of ethyl alcohol content was examined in the case of fermented mash samples (Table 4). Of the samples, the control sample had the lowest alcohol content by the end of fermentation, followed by the samples containing 0.5 % and then the highest amount of 1 % herb. Compared to the control sample, the alcohol content resulted in 4.6–13.3 % (peppermint 0.5 and 1 v/v%) and 14.0–17.2 % (lemongrass 0.5 and 1 v/v%) higher amounts in the case of herbal samples. In some cases, it has been shown the use of additives positively influences the dynamics of fermentation (Kawa-Rygielska et al., 2019).

Previous research has shown an increase in the antioxidant activity of herbs during fermentation (Bose & Kim, 2013). The ability of fermentation to improve antioxidant activity is primarily contributed by an increase in the amount of phenolic compounds and flavonoids during fermentation, which is the result of a microbial hydrolysis reaction (Hur et al., 2014).

# Conclusion

In all cases, the control sample had significantly lower (p < 0.05) amounts of antioxidant and polyphenol compounds during fermentation. This property can be said for the end of the fermentation and the end product. Our experimental work and the end product are a novelty among alcoholic products. Although there have been examples of similar measurements, only a few research work on supplementing apple mash with such have been carried out herbs. The measurements performed during the experiments support the positive effect of herbal dosage on the amount of polyphenols and antioxidants during alcoholic fermentation. Based on the results, the processes that take place during alcoholic fermentation have amplified the extraction of these beneficial agents into the product. The effects of active substances on human health have already been demonstrated, but no such cider or cider product has been produced.

#### CRediT authorship contribution statement

Rita Székelyhidi: Conceptualization, Supervision, Writing – review & editing. Erika Lakatos: Funding acquisition. Beatrix Sik: Data

#### Table 4

The ethanol content of the pressed samples (wine) of the end of the fermentation process (17th day).

Sample	Ethanol content v/v %
Control	$2.85\pm0.05^a$
Lemongrass dosage 0.5 %	$2.98\pm0.04^{\rm a}$
Lemongrass dosage 1.0 %	$3.23\pm0.08^{\rm b}$
Peppermint dosage 0.5 %	$3.25\pm0.10^{\rm b}$
Peppermint dosage 1.0 %	$3.34\pm0.09^{b}$

Values are means  $\pm$  SE (n = 3). Within each column, values with different letters are significantly different (p < 0.05).

curation, Writing – review & editing. Ágnes Nagy: Formal analysis, Methodology. Laura Varga: Investigation. Zoltán Molnár: Writing – review & editing. Viktória Kapcsándi: Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

#### **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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# R. Székelyhidi et al.

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