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ANTIOXIDANT PROFILE OF MULLED WINE

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ABSTRACT
The aim of the study was to compare chemical and nutritional profile of wine and heat-treated wine, called mulled wine. The experiment was focused on simulation of ordinary produce mulled wine by the majority of consumers. Cabernet Moravia (bottled in Velkobílovická vína s.r.o., Czech Republic) was used for the experimental production of mulled wine. Following spices were added to wine during cooking: cloves (Vitana, Czech Republic) and cinnamon (KOTÁNY, Austria). The samples of wine were heat treated in stainless steel pot for 5 minutes. The relative density, acidity, alcohol content, phenol content and antioxidant capacity were monitored in experimentally produced wine and mulled wine. The gained results showed that samples of mulled wine with added cloves had statistically significant (p <0.05) higher phenol content and higher antioxidant properties in comparison with wine before heat treatment and spices addition. The results clearly showed that mulled wine can be considered as the product with better health beneficial nutritional profile than wine from which it is produced; in addition, mulled wine sample had significantly (p <0.05) lower alcoholic content (8.27 ±0.04 vol.%).

Keywords: Cabernet Moravia; mulled wine; spice; clove; cinnamon; antioxidant

INTRODUCTION
Consumers interest in the binominal diet (diet related to health) has been constantly increasing due to clear evidences how specific dietary patterns can reduce a chronic diseases development **(Zorraquin-Peña et al., 2019)**. The most often diet connected with health benefits is the Mediterranean diet and the most common food commodity in this type of diet is wine **(Chiva-Blanch et al., 2013; Artero et al., 2015)**. Wine health benefits are mostly connected with high polyphenols content and their positive influence on human health, beside organoleptic properties **(Snopek et al., 2018; Zorraquin-Peña et al., 2019)**.

Nowdays, wine is broadly consumed and used in culinary preparation. Wine is very often thermally threated when it is added to certain meals. It is also consumed as a warm beverage with addition of spices. This popular drink is called mulled wine and its physical-chemical properties are changed due to processing **(Mudnić et al., 2011)**. It is well known that high phenol content means food with higher antioxidant activity due to phenols ability to donate hydrogen. The role of phenols in so complex system such as food is hard to predict due to the presence of other antioxidants, polyphenols, oxidative enzymes, metals, etc. This statement is crucial for food that is fortified with different polyphenols and it means that each food fortifcation should enclude a specific experimental study **(Pinelo et al., 2004)**. It was also found that thermally treated wine still posses high antibacterial properties **(Boban et al., 2010)**. Thermal treatment of wine even at lower temperatures, such as 45 ºC during 20 days, also can make changed in wine chemical and sensorial properties. It was found that this kind of treatment significantly changed floral character of the wines and increased aromas described as oak, honey and smoky **(Leino et al., 1993)**.

Cabernet Moravia is a wine variety that was made by crossing of Zweigeltrebe and Cabernet Franc. It is grown mostly in Moravian region in the Czech Republic. This wine variety contains a high content of anthocyanins, pigments and polyphenols content **(Balík and Kumšta, 2008; Bajčan et al., 2016)**.

The most often used spices for the preparation of mulled wine are cloves (*Eugenia caryophyllata*) and cinnamon (*Cinnamomum cassia*). Cloves are already known for their medical usage and antimicrobial activities. Cloves are also known to serve as preservative during shelf life of different food products **(Santin et al., 2011; Sukorini, Sangchote and Khewkhom, 2013)**. Cinnamon (*Cinnamomum cassia*) has been the one of the most commonly used spice since 2800 BCE. Cinnamon used as a spice has many beneficial effects encompassing antioxidant, antiinflamatory, antidiabetic, antibacterial and anticancer **(Ben-Arfa et al., 2019)**.

Scientific hypothesis

Mulled wine is better solution for consumers than heat not treated wine without spices addition.

The aim of the study was to evaluate heat treated wine (mulled wine) with and without spices addition and compare their antioxidant profile and chemical-physical parameters.

MATERIAL AND METHODOLOGY

The mulled wine was prepared from Moravian Country wine, dry red wine Cabernet Moravia (bottled in Velkobílovická vína s.r.o., Czech Republic). The mulled wine was made with the addition of spices: clove (*Eugenia caryophyllata*) (Vitana, Czech Republic, batch: L1104182) and cinnamon (*Cinnamomum cassia*) (KOTÁNY, Austria, batch: L327392111511). The addition of spices was done according to the Table 1.

The samples 2 to 5 were boiled in stainless steel pot during 5 minutes. In the samples 3 to 5 the spices **(**clove/cinnamon**)** were added before boiling (Table 1). The sample 1 was control sample including only wine (Cabernet Moravia).

The relative density was measured with the use of capillary tube pycnometers (capacity: 10 mL). The samples' weights were measured in analytical balance with 0.0001 g precision **(Cepeda and Villarán, 1999)**. The blank sample was distilled water and it was measured according to the following equation:

$*\rho v = (mpv - mpp)/Vp$

*mpv: weight of pycnometer filled with wine sample; mpp: weight of empty pycnometer; Vp: volume of the pycnometer

The pH of each sample was measured by the pH meter GRYF 259 (GRYF HB, Czech Republic) with electrode PCL 124 (GRYF HB, Czech Republic).

The determination of titratable acidity was measured by the titration of sample by 0.1 M NaOH with the bromthymol blue as an indicator up to the color change to green. The concentration of titratable acid was calculated as the content of tartaric acid ($g.L^{-1}$) by formula: $TA = a \cdot 0.75$; where a is the volume of 0.1 M NaOH. This method is recommended by the Compendium of International Methods of Wine and Must Analysis **(OIV, 2009)**.

The determination of alcohol was measured by Ebulliometer 160450T (Laboratories DUJARDIN-SALLERON, France).

The total polyphenols content (PCA) was measured with the Folin-Ciocalteau solution diluted by water (1:10) and Na_2CO_3 (75 $g.L^{-1}$) by **Talcott, Howard and Brenes (2000)** with slightly modification. The sample of wine was diluted 100 times and then 1 mL was used for analysis. 5 mL of Folin-Ciocalteau solution and 4 mL Na_2CO_3 and then the sample was incubated in dark for 30 minutes. The absorbance was measured at 765 nm and the gallic acid was used as the standard **(Talcott, Howard and Brenes, 2000)**.

The determination of FRAP – ferric reducing/antioxidant power was measured at the absorbance of 593 nm. FRAP reagent was prepared by mixing 10 volumes of 300 $mmol.L^{-1}$ acetate buffer with 1 volume of 10 $mmol.L^{-1}$ TPTZ (2,4,6-tripyridyl-s-triazine) in 40 $mmol.L^{-1}$ hydrocloric acid and with 1 volume of 20 $mmol.L^{-1}$ ferric chloride. The dilution of sample in reaction mixture was 1:34. Absorbance readings were done after 8 minutes of incubation.

The determination of polyphenols by high pressure liquid chromatography (HPLC). A 2 g of baked cookies sample was weighed into 200 mL volumetric flask and 50 mL methanol/water (50/50, v/v) was added. The samples were left for 5 days in the dark. Extracts were then filtered by a syringe filter (Agilent Captiva Premium Syringe Filter Regenerated Cellulose, 0.45 µm, 25 mm, p/n 5190 5111) and filtrates were used directly for injection.

The HPLC mobile phase was following: A) water + 1% phosphoric acid; B) acetonitrile; with the following gradient: 10% B, from 0 to 20 min; 20% B, from 20 to 25 min; 30% B, from 25 to 35 min; 40% B, from 35 to 40 min; post time was 10 minutes. The column was Agilent ZORBAX Eclipse Plus, 4.6 × 250 mm, 5 µm. Detection was done with the use of diode array detector (DAD) at 324nm. The method was slightly modification of the method developed by **Naegele (2013)**.

Statistic analysis

Statistical significance at $p < 0.05$ was evaluated by one-way ANOVA analysis of variance, and parametric Tukey post hoc test (in the case when Levene's test showed equal variances $p > 0.05$) and nonparametric Games–Howel post hoc test (in the case when Levene's test showed unequal variances $p < 0.05$) for finding differences within individualgroups. Principal component analysis (PCA) was done (with Promax rotation) for finding overall differences among wine and experimentally produced mulled wine samples. SPSS 20 statistical software **(**IBM Corporation, Armonk, NY**)** was used.

RESULTS AND DISCUSSION

Alcohol percentage, titrated acids content and pH value of wine and developed mulled wine samples are shown in Table 2.

Titrated acids were the highest in the sample of mulled wine that was prepared with cloves addition, which can be explained by low pH of clove oil (2.81) **(Santin et al., 2011)**. Alcohol content in evaluated samples was significantly (p <0.05) reduced in mulled wine samples (samples number: 2, 3, 4, 5) due to heat treatment. In the study of **Boban et al. (2010)** during 45 minutes heat treatment of wine at 75 ºC and 125 ºC alcohol content in mulled wine was also lowered by 20% and more than 90%, respectively. The content of alcohol was almost at the same level in the both mulled wines (45 minutes treatment at 125 ºC) and dealcoholized wines (under vacuo in rotary evaporator) **(Boban et al., 2010)**. Alcohol content in wine can be up to 15% **(**vol/vol**)** in wines produced in warmer climate regions due to higher sugar content **(Contreras et al., 2014)**. At the present time there is a trend of producing wines with reduced alcohol content due to public health recommendations to lower alcohol consumption **(Grant, 2010; MacAvoy, 2010)**. The results obtained by analysis of total phenol content represented as gallic acid ($mg.L^{-1}$) and antioxidant capacity (FRAP) are shown in Table 3.

The total phenol contents in were the highest (p <0.05) in mulled wine samples with cloves addition (samples 3 and 5). The increment can be explained by cloves addition which is known as good source of phenolic compounds **(Gulcin et al., 2004)**, but also wine heat treatment leads to increase of phenolic compounds due to loss of volume **(Boban et al., 2010)**. Though, Authors **(Boban et al., 2010)** found that heat treatment and dealcoholizing of wine result in the loss of some individual phenolic compounds, but total phenol content increases. On the other side, wine and especially mulled wine represent the model of mixed polyphenolic compounds that are well protected during heating. It was also indicated that certain polyphenolic compounds serve as polyphenols' protector during heat-induced decomposition **(Yamaguchi et al., 2003)**. Although, heat-induced polyphenol interactions are very hard to predict and their paths toward degradation or increment **(Pinelo et al., 2004)**. The complexity of mulled wine phenolic content changing is also supported by the observation that even small physical-chemical properties changes of hydroalcoholic polyphenolic solutions significantly affect their solubility, perception behavior and also their interactions with other compounds, such as proteins **(Serafini, Maiani and Ferro-Luzzi, 1997; Zanchi et al., 2008)**. The differences in polyphenolic profile among wine and experimentally produced mulled wine sample can be also seen in Figure 2 that is clearly showing the increase of polyphenolic compounds, especially in samples with added cloves. Antioxidant capacity represented as ferric reducing antioxidant power (FRAP) was the highest (p <0.05) among mulled wine samples with cloves addition (samples 3 and 5). These results are corresponding with higher polyphenol contents in these samples. The finding is supported by the observation of previous studies that found higher antioxidant activity in heat treated food product in comparison to raw materials. This swift to higher antioxidant capacity is explained by two possibilities: i) the production of stronger antioxidants during heating and ii) oxidative enzymes are inactivated by thermal processing **(Boban et al., 2010)**. **Mudnić at al. (2011)** found the increase of phenol content in heat treated wine (mulled wine). The authors also stated that thermal degradation of phenolic compounds is still poorly understood and that it is hard to predict these process.

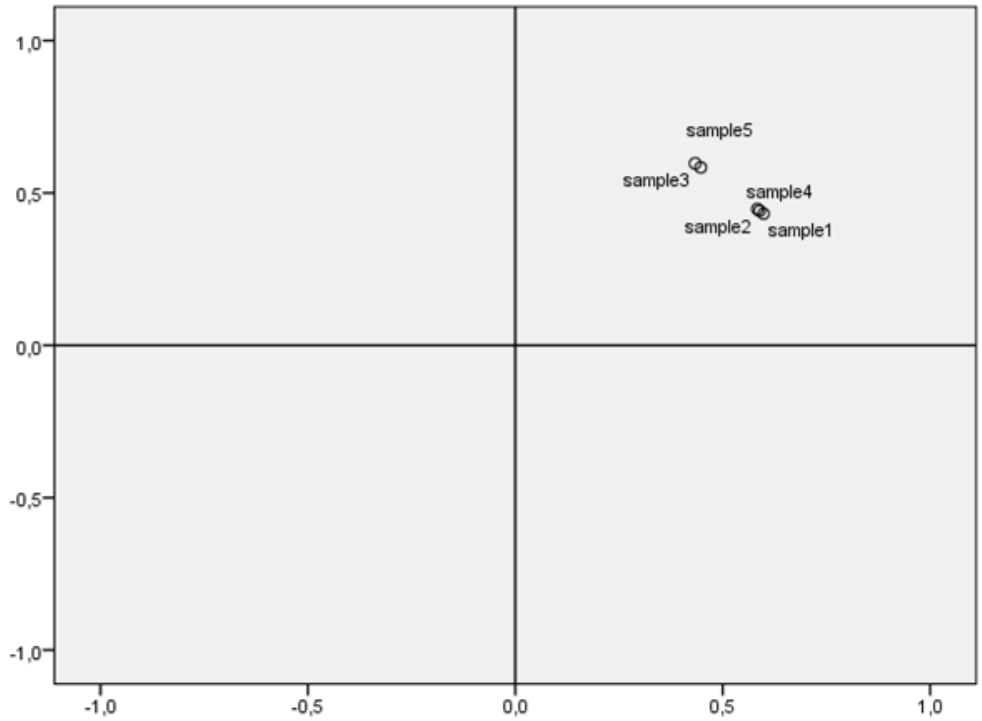

Figure 1 Principal component analysis (PCA) of wine and mulled wine samples.

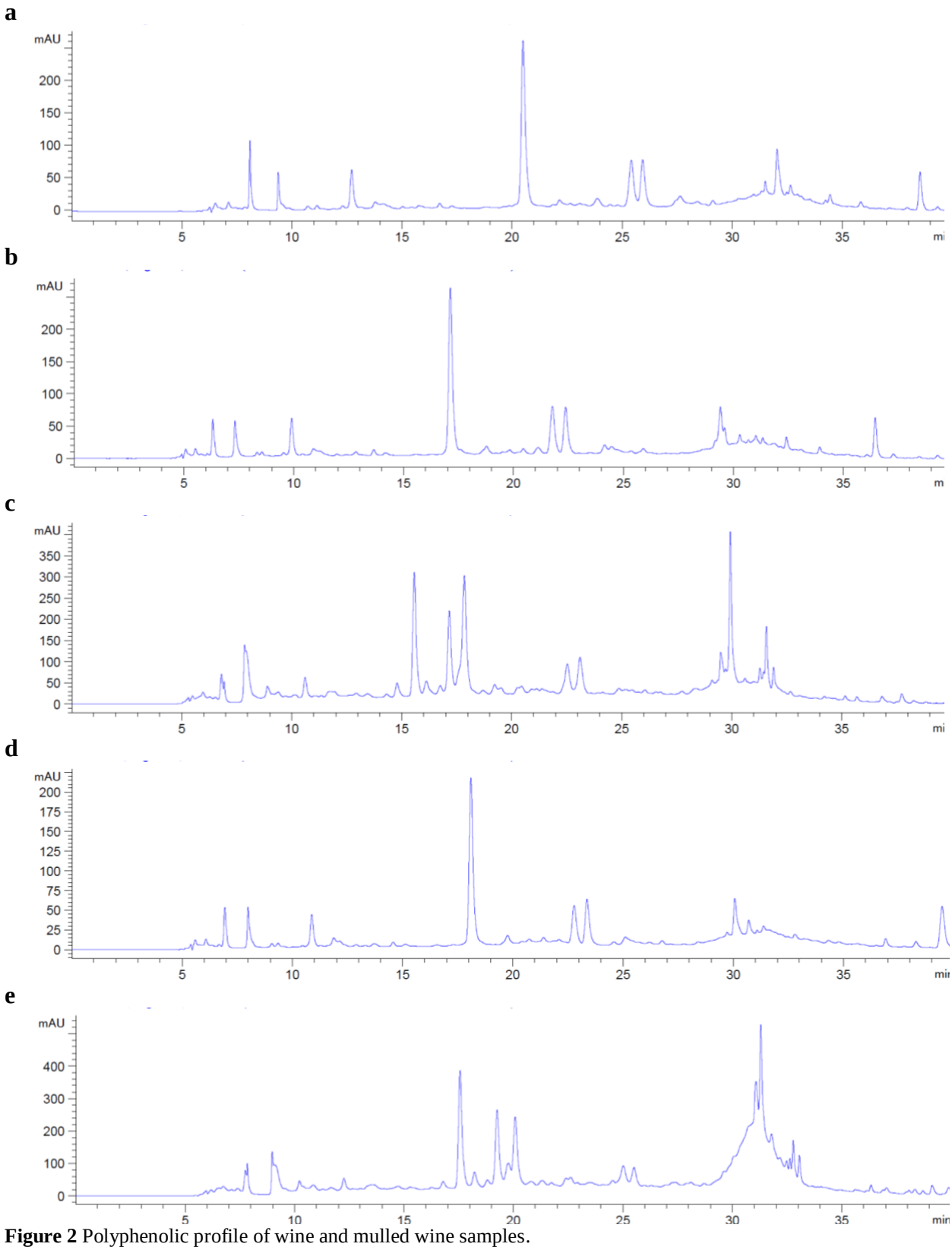

Figure 2 Polyphenolic profile of wine and mulled wine samples.
Note: *a: sample 1; b: sample 2; c: sample 3; d: sample 4; e: sample 5. According to Table 1.

Table 1 The samples used in the experiment.

Samples	Composition	Thermal treatment	Wine + spices
1	Wine	No	Wine (only wine)
2	Mulled wine without spices	Yes	Mulled wine (only wine)
3	Mulled wine + cloves	Yes	600 mL + 18 g
4	Mulled wine + cinnamon	Yes	600 mL + 17 g
5	Mulled wine + cinnamon + clove	Yes	600 mL + 17 g + 18 g

Table 2 PH, relative density, titrated acids and alcohol content in mulled wines.

	pH	Titrated acids (tartaric acid g.L^{-1})	Alcohol (vol.%)	The relative density (g.cm^{-3})
1	3.62 ±0.02	7.00 ±0.00	11.96 ±0.18^{a}	0.99 ±0.00
2	3.55 ±0.01	7.35 ±0.21	9.03 ±0.06^{c}	0.99 ±0.00
3	3.58 ±0.02	8.20 ±0.14	8.27 ±0.04^{b}	1.00 ±0.00
4	3.53 ±0.03	7.35 ±0.21	9.01 ±0.19^{bc}	0.99 ±0.00
5	3.63 ±0.02	7.65 ±0.07	9.42 ±0.18^{bc}	1.00 ±0.00

Note: *different letters (a, b, c) indicate statistically significant (p <0.05) differences.

Table 3 Total phenol content and antioxidant capacity of wine and mulled wine samples.

	Total phenol content (gallic acid mg.L^{-1})	Antioxidant capacity FRAP (μmol.L^{-1})
1	2 375.04 ±0.00^{a}	238.69 ±2.73^{a}
2	2 499.69 ±0.00^{b}	268.76 ±1.87^{c}
3	4 019.90 ±0.00^{c}	718.23 ±1.98^{d}
4	2 284.47 ±0.00^{d}	239.85 ±0,61^{acb}
5	4 165.99 ±0.00^{e}	717.61 ±1.71^{d}

Note: *different letters (a, b, c, d, e) indicate statistically significant (p <0.05) differences.

Temperature and pH affect the most degradation of phenolic compounds, though the degradation was found to be not so intense since the temperature of 111 °C degrades gallic acid during 30 minutes only by 1.3% **(Tanchev et al., 1997)**. The overall differences between wine and mulled wine samples can be seen in Figure 1. Principal component analysis found 2 seperate groups: group 1 – samples 1; 2 and 4; group 2 – samples 3 and 5 **(**Figure 1**)**.

CONCLUSION

The study clearly gives the picture about differences between wine and experimentally produced mulled wine. The health benefits of wine consumption are almost exclusively connected with high phenolic content and consequently high antioxidant properties. The sharing of this fact in many countries has raised the consumption of wine significantly. On the other hand, alcohol content of wine is the subject of constant evaluation of medical studies. The results gained by our research are indicating that during heating process of mulled wine consumption phenolic profile is not interrupted and can be even improved by the addition of regular spices the most often used to produce mulled wine, such as cinnamon and cloves. Concurrently, the heating process is significantly reducing alcohol content. Certainly, the effects of heating on wine, as representative of highly complex polyphenolic matrix, will be the subject of future studies since interection between polyphenolic compounds between them and also between other compounds present in wine has not been still explained enough.

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