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Chemical analysis of selected meads produced in Poland

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Abstract

The aim of this study was the analysis of 25 commercially available meads obtained from three leading producers in Poland. In the course of the analyses, the concentration of nine organic acids was determined using the capillary isotachophoresis technique, and the total polyphenol content (58–699 mg/L GAE) and the antioxidant activity were expressed as FRAP (234–6422 µmol/L Fe^{2+}) using spectrophotometric methods. We were able to indicate the acids whose main source was honey—gluconic acid (561–2287 mg/L) and formic acid (35–176 mg/L), the one that was formed during alcoholic fermentation—succinic acid (280–845 mg/L), and also those originating from the additives in the form of fruit juices, or as a result of acidification—tartaric acid (<LOD–159 mg/L), malic acid (135–1611 mg/L) or citric acid (125–4576 mg/L). Our results provide a further contribution to the general knowledge of the chemical composition of meads, and, in particular, these are the first results of this kind for meads commercially available in Poland. The analysis of principal components showed the correlation structure of the examined parameters and the existence of two clusters containing specific meads.

Keywords Mead · Organic acids · Antioxidant activity · Total polyphenol content · Alcoholic fermentation

Introduction

Meads are one of the oldest alcoholic beverages produced by humans [1], with the alcohol concentration varying between 9 and 18% by volume. Traditional meads are fermented alcoholic beverages produced from a mixture of honey and water, in which the water may be partially replaced by fruit juice. In Poland, there are four basic types of meads, classified based on the proportions of honey and water, namely, "półtorak", "dwójniak", "trójniak", and "czwórniak", with the v:v ratio 1:0.5, 1:1, 1:2, and 1:3, respectively [2, 3]. According to Polish directives, mead makers at the stage of preparing mead wort are allowed to use concentrated fruit juice, herbs, spices, nutrients, or food acids. In this way, producers can expand their sales offerings by creating alcoholic beverages based on honey, but with different bouquets of flavours and aromas.

Currently, depending on where honey is produced, local tradition, and specific recipes, there are many variations of this drink [4]. The main factors that affect the flavour of mead are the honey from which the drink was made (with botanical and geographical origins providing lots of varieties), the strain of yeast that was used to carry out alcohol fermentation, and the technological process, such as fermentation, heat treatment, or storage conditions [5]. Moreover, the final taste of the mead is influenced by the residual sugar, the concentration of ethanol, and the acidic value [6]. The complex chemical composition of mead comprises different groups of compounds, including carbohydrates, nitrogenous derivatives, vitamins, volatile compounds, minerals, enzymes, organic acids, and polyphenols. The two latter groups attract special attention, as they contribute to some important features of meads. Organic acids in meads are represented mainly by gluconic, acetic, succinic, acetic, malic, citric, or formic acids [7, 8]. The main sources of these compounds are bee honey, fruit juices, and food organic acids,

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which are added to ensure the optimal acidity of the beverage. In addition, some organic acids (e.g. succinic acid) are formed as by-products of ethanol fermentation. Organic acids not only affect the organoleptic properties of the final product, but also provide an acidic environment, which ensures stability and microbiological purity of the mead. Moreover, as sequestrants, they can additionally support the action of mead's antioxidants [9].

Polyphenols, represented by flavonoids and phenolic acids, provide, among other things, the antioxidant properties of mead [10]. The source of these compounds is mainly honey, but also some additives such as herbal spices or fruit juices used in the production of this beverage [5]. Studies indicate that the profile of polyphenolic compounds can indicate the floral origin of a particular honey [11] and some researchers suggest that the compounds could also serve as indicators of the quality and composition of mead [12, 13]. So far, few papers have been published on the analysis of the chemical composition of meads, especially Polish ones. The aim of the presented research was to analyse and compare Polish meads obtained from three leading producers. The analysis was focused on the profile of organic acids, total content of polyphenols, and antioxidant activity of the meads. In addition, the results obtained were analysed using the PCA method, to reveal the relationships between the examined parameters.

Materials and methods

Mead samples

25 varieties of mead were obtained from the three leading producers in Poland, namely, Pasieka Maciej Jaros (PMJ) from Łazisko, Apis (AP) from Lublin, and TiM located in Bielsko-Biała. The samples were denoted using the acronym of the producer, with consecutive number of the mead analysed and the indicator of the honey/water ratio (e.g. AP2/1.5° means the product no 2 from producer AP, with honey:water ratio (v:v) 1:0.5), and had the following meaning: 1:0.5, 1.5° mead; 1:1, 2° mead;, 1:2, 3° mead; and 1:3, 4° mead. The characteristics of the meads are shown in Table 1. The meads were diluted 20 times with distilled water immediately before the analysis. In some cases, the

Table 1 Characteristics of the studied meads ([+]—present; [−]—absent)

Producer	Symbol	Dark/buckwheat honey	Pale/multi-flower honey	Additives
AP	AP1/1.5°	ND[a]	ND	Raspberry fruit, rosehip
	AP2/1.5°	ND	ND	Wild berries must
	AP3/2°	ND	ND	Multi fruit juice
	AP4/2°	ND	ND	Blackcurrant juice
	AP5/2°	ND	ND	ND
	AP6/3°	ND	ND	Rowanberry juice
	AP7/3°	ND	ND	Spices, chokeberry juice
	AP8/3°	+	ND	Alpine herbs
	AP9/3°	ND	ND	Spices, cranberry juice
	AP10/3°	ND	+	Spices, cherry juice
	AP11/3°	ND	ND	ND
	AP12/3°	ND	+	Spices, plum must
	AP13/4°	ND	+	ND
PMJ	PMJ1/1.5°	+	+	Red grape juice
	PMJ2/2°	+	+	–
	PMJ3/2°	–	+	Apple juice
	PMJ4/2°	+	+	Blended mead
	PMJ5/2°	+	+	Chokeberry, elderberry juice
	PMJ6/2°	–	+	–
	PMJ7/2°	–	+	Raspberry juice
	PMJ8	+	+	Chokeberry, elderberry juice
TiM	TiM1/1.5°	+	ND	ND
	TiM2/2°	+	ND	ND
	TiM3/3°	+	ND	ND
	TiM4/4°	ND	ND	ND

[a]*ND* no data available from the producer

sample was diluted using two different dilutions so that all the analytes determined were within the concentration range of the standard solutions.

Reagents

Deionised water of 18 MΩ × cm was obtained from Milli Ro & Q water purification system (Merck-Millipore, Billerica, MA, USA). Caproic acid, Folin–Ciocalteu reagent, and ferric chloride ($FeCl_3$) were obtained from Fluka (Steinheim, Germany); malonic acid from Supelco (Bellefonte, USA); monohydrate citric acid from Chempur (Piekary Śląskie, Poland); D,L-malic acid and succinic acid from Lancaster (Morecambe, England); 95% formic acid, sodium salt fumaric acid, D-gluconic acid, 99.5% acetic acid, 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), and gallic acid from Sigma-Aldrich (France), and L-tartaric acid, sodium carbonate, and 80% D,L-lactic acid from Avantor Performance Materials Poland S.A. (Gliwice, Poland). 36–38% hydrochloric acid was from Baker Analyzed, methyl hydroxyethyl cellulose (M-HEC) from HERCULES (Prague, Czech Republic), β-alanine from Merck (Darmstadt, Germany), and L-histidine from Serva (Heidelberg, Germany). All the chemicals were of analytical purity.

Instrumentation

Determination of organic acids in mead

The isotachophoretic separations were performed using the electrophoretic analyser EA 202 M (Villa Labeco, Spisska Nova Ves, Slovakia) with a conductivity detector. The system was equipped with a sample valve of 30 μL fixed volume and two capillaries, namely, the pre-separation capillary (160 mm × 800 μm I.D.) and the analytical capillary (160 mm × 300 μm I.D.). A previously developed and validated method with some modifications was used to analyse the meads [8]. The pre-separation involved a current of 250 μA, while the actual separation in the analytical capillary column was performed at 40 μA during the initialisation phase and 50 μA during the detection phase. The following solution (with pH = 3.1) was used as the leading one: 10 mmol/L hydrochloric acid solution, including 0.1% methyl hydroxyethyl cellulose (M-HEC), 15% ethanol, and 10 mmol/L β-alanine. The terminating electrolyte contained 5 mmol/L of caproic acid solution including 30% ethanol.

The concentrations of the individual organic acids in the mead samples were determined using calibration curves drawn up (standard curve method) based on the measurement results of standard samples, which were the mixtures of acids of known concentrations: 50 mg/L, 25 mg/L, 12.5 mg/L, 6.25 mg/L, 3.125 mg/L, and 1.56 mg/L.

Determination of total polyphenols using the Folin–Ciocalteu reagent

The total phenolic compounds (TPC) of the mead samples were assessed using the Folin–Ciocalteu reagent method. A slightly modified analytical procedure, reported earlier [14], was performed. To 540 μL of deionised water were added, successively: 60 μL of standard solution or mead sample, 60 μL of 7% Na_2CO_3, and 30 μL of a doubly diluted Folin-Ciocalteu solution. The blank sample was a mixture of water and reagents. Absorbance was measured 30 min after the last reagent was added, at a wavelength of 720 nm using a Synergy 2 Multi-Mode Microplate reader spectrophotometer from BioTek Instruments. The total phenol content was determined using the standard gallic acid (GA) calibration curve (0.05–0.30 g/L GA). The mead samples were diluted to obtain concentrations within the range of concentrations of the standards used to prepare the calibration curve. The final results were expressed as GA equivalents (GAE).

Determination of antioxidant activity by the method of FRAP

The FRAP (ferric reducing antioxidant power) assay was performed according to Benzie's method [15], with modifications as described by Paśko et al. [13]. The FRAP method is based on the measurement of the degree of reduction of Fe^{3+} ions to Fe^{2+} by the components with the antioxidant potential present in the sample. Fe^{2+} ions are complexed with TPTZ (2,4,6-tris(2-pyridyl)-1,3,5-triazine), the product of which is a blue-coloured compound. In summary, the analytical procedure consisted of adding the following solutions to 400 mL of acetate buffer in the sequence: 60 μL of standard solution or mead sample and 200 μL of mixture for FRAP measurement (10 mL $FeCl_3$ solution (20 mmol/L), 10 mL TPTZ solution (10 mmol/L) and 20 mL of acetate buffer, pH = 3.6). The blank sample was a mixture of water and reagents. The absorbance was determined at 593 nm after 30 min and was proportional to the antioxidant capacity of the antioxidants in the mead. The standard curve was linear within the range from 271 to 1626 μmol/L $FeSO_4$. The final results were given as μmol/L Fe^{2+}.

Statistical approach

Descriptive statistics were calculated for all parameters. The differences between groups of meads due to their quality characteristics were tested using *t* Student's or Mann–Whitney tests, applied when appropriate. Levene's

test was used to assess the equality of variances in groups being compared. A probability level of $p < 0.05$ was considered to be statistically significant. The principal component analysis (PCA) model was used to describe the correlation structure between parameters in the whole group of meads. The parameters with large absolute values of their coordinates (> 0.3) on the first two principal components in the PCA model were assumed to determine the axes of the new coordinate system in PCA to the greatest extent. To express the strength of bivariate associations between such parameters, the cosines of the corresponding angles (i.e. correlation coefficients) were calculated. The "corresponding angle" means the angle determined by the two lines connecting the origin with coordinates of both parameters on the PCA loadings plot. The PCA approach was also applied to check whether the clusters of meads appear in the PCA score plot, and if so, what is the cause. Statistical analysis was conducted using packages: STATISTICA v.13 (TIBCO Software Inc., Palo Alto, CA, USA) and SIMCA-P v.9 (Umetrics, Umeå, Sweden).

Results and discussion

The results of the mead analyses are shown in Tables 2 and 3.

Tartaric acid

Tartaric acid was quantified in only six meads, namely, PMJ1/1.5° (159 ± 1 mg/L), PMJ4/2° (51 ± 1 mg/L), and in all meads from the producer TiM (99–137 mg/L). In case of PMJ1/1.5° mead, the producer declared that some of the water used in the preparation of these alcoholic beverages had been replaced by red grape juice. As it is well known, grapes and their products are one of the richest sources of tartaric acid [16]; thus, the high amount of the compound detected in PMJ1/1.5° mead, in comparison to other products examined, is highly justified. Mead PMJ4/2°, on the other hand, was created from a blend of meads, including mead PMJ1/1.5°. The presence of tartaric acid in mead from TiM company, in turn, can be explained by using grape juice as rehydration media for dry yeast activating (during its production), according to the manufacturer's declaration. Usually, dry yeast is activated by dissolving in water or a 3–5% sugar solution before proceeding. Thus, it appears that the tartaric acid present in the analysed meads came rather from the additives used in their production than from the honey itself. However, Sroka et al. [17] determined tartaric acid in experimental 2° and 3° meads before and after fermentation (the process took 28 days) and found that its concentration did not change significantly during the process, being in the range of 40–50 mg/L, which suggests that tartaric acid occurs naturally in the honey from which mead is made.

Kružík et al. [7] examined 11 different honey wines (made only from water and honey) and 6 dessert meads from Czech Republic, with possible additives such as sugar, alcohol, herbal extracts, wine, hops, caramel, and citric acid. The results indicated the presence of tartaric acid in eight honey wines and only in one dessert mead, with the concentration ranging from 40 to 510 mg/L.

However, it should be emphasised that there are studies in which the presence of this component was found in the honey itself. Thus, Dezmirean et al. [18] found the presence of tartaric acid in all samples of monofloral (black locust, linden, raspberry, canola, heather) and multifloral honey tested. The concentration of this substance fell within a wide range: 59.7–362.5 mg/kg. On the other hand, Suto et al. [19] studied 25 kinds of honey from different countries of the world, which they obtained directly on the market. Only six honey samples showed the presence of tartaric acid and at much lower concentrations (2.5–15.0 mg/kg) than that in Dezmirean et al.'s [18] studies. Thus, it is not clear if tartaric acid can be the indicator of additives present in the meads.

Gluconic acid

In most of the meads tested, this acid was present in the highest concentration among the examined organic acids. This was somewhat expected, as this is the predominant organic acid in honey regardless of its geographical and floral origin [18–20]. Interestingly, it was observed that the concentrations of gluconic acid in all meads obtained from producer PMJ were higher (1747–2287 mg/L) than those determined in the meads from other producers (TiM: 565–1039 mg/L; AP: 561–1337 mg/L), regardless of the honey-to-water ratio.

It should be stressed that gluconic acid is mainly formed in honey as a result of the oxidation of glucose by the enzyme glucose oxidase [21], but it can also be formed during fermentation.

The obtained results for gluconic acid are in good agreement with those obtained by Kružík et al. [7] and were lower by an order of magnitude than those determined in the mead by Švecová et al. [5]: 14,270–49,510 mg/L. The latter research group analysed 22 meads obtained from the trade network, beekeepers, or manufacturers from the Czech Republic.

The average concentration of gluconic acid turned out to be significantly different depending on the type of the mead (Fig. 1). Its average concentration in 3° meads was significantly lower than that determined in the groups of 1.5° and 2° meads. Interestingly, the highest average gluconic acid concentration was characterised by the group of 2° meads

Table 2 Results of chemical analysis of 1.5° meads and 2° meads

Mead	Concentration [mg/L]									Total phenols [mg/L GAE]	FRAP [μmol/L Fe^{2+}]
	Tartaric acid	Malonic acid	Formic acid	Citric acid	Malic acid	Lactic acid	Gluconic acid	Succinic acid	Acetic acid		
PMJ1/1.5°	159±1	378±5	93±14	633±16	403±6	359±11	1829±37	705±2	670±15	214±12	1347±36
TiM1/1.5°	99±5	187±0	166±15	1680±5	145±9	311±15	1039±82	280±2	377±7	339±19	2517±13
AP1/1.5°	<LOD[a]	277±4	104±4	1917±17	542±11	497±22	1125±66	450±1	482±2	699±12	6422±80
PMJ2/2°	<LOD	394±8	144±25	308±5	309±2	360±15	1917±26	477±0	786±24	206±2	827±4
PMJ3/2°	<LOD	593±11	157±6	133±3	1611±20	654±30	1786±49	569±11	529±27	233±6	1224±10
PMJ4/2°	51±1	415±4	128±20	257±14	186±3	446±3	2110±88	472±5	846±20	231±7	1083±32
PMJ5/2°	<LOD	313±9	115±25	125±6	483±9	538±28	1893±16	770±5	672±35	502±7	3478±20
PMJ6/2°	<LOD	291±1	76±16	443±9	135±5	382±6	2287±22	608±8	596±16	66±0	234±2
PMJ7/2°	<LOD	438±21	81±3	4576±151	172±10	604±19	1747±78	616±31	754±49	386±19	3628±40
TiM2/2°	95±1	140±7	89±3	1471±20	294±16	254±23	733±56	658±6	283±2	99±1	735±1
AP2/2°	<LOD	216±16	140±10	1066±25	383±0	474±1	1337±11	547±1	438±13	196±8	2390±55
AP3/2°	<LOD	248±1	57±13	1357±10	264±16	284±13	1032±33	475±1	390±8	108±8	686±5
AP4/2°	<LOD	292±5	107±18	1648±10	277±3	360±3	974±23	559±3	543±15	143±1	1203±12
AP5/2°	<LOD	286±5	117±20	1310±10	243±0	332±5	1080±31	580±3	517±6	97±4	567±7

[a]Below the limit of detection (3.4 mg/L for tartaric acid)

Table 3 Results of chemical analysis of 3° meads and 4° meads

Mead	Concentration [mg/L]									Total phenols [mg/L GAE]	FRAP [μmol/L Fe^{2+}]
	Tartaric acid	Malonic acid	Formic acid	Citric acid	Malic acid	Lactic acid	Gluconic acid	Succinic acid	Acetic acid		
PMJ8/3°	<LOD[a]	421±3	176±33	144±6	569±3	556±30	1975±42	695±4	657±24	458±10	4018±70
TiM3/3°	123±3	91±3	43±7	1491±4	436±18	291±5	587±3	843±13	508±11	96±4	926±2
AP6/3°	<LOD	271±1	60±21	707±11	308±10	229±10	787±25	532±8	244±5	85±2	705±14
AP7/3°	<LOD	284±5	49±11	1072±12	344±12	364±17	561±51	694±3	424±5	86±1	520±9
AP8/3°	<LOD	275±2	35±9	1477±33	285±2	287±12	761±3	653±10	393±11	60±2	341±13
AP9/3°	<LOD	252±3	48±9	1410±32	274±7	284±5	708±17	613±3	532±20	58±3	283±12
AP10/3°	<LOD	243±19	38±4	965±13	333±9	293±1	672±29	623±10	353±26	105±1	1023±15
AP11/3°	<LOD	275±2	40±13	1104±0	318±5	256±5	729±43	678±0	644±11	79±7	578±24
AP12/3°	<LOD	343±26	37±3	1246±19	339±14	265±13	1025±34	625±12	466±3	72±3	392±7
TiM4/4°	137±1	81±5	49±13	1184±17	444±6	232±4	565±25	845±1	355±6	138±0	2082±80
AP13/4°	<LOD	268±8	51±7	1052±19	302±5	329±20	1273±18	488±2	609±55	73±4	419±17

[a]Below the limit of detection (3.4 mg/L for tartaric acid)
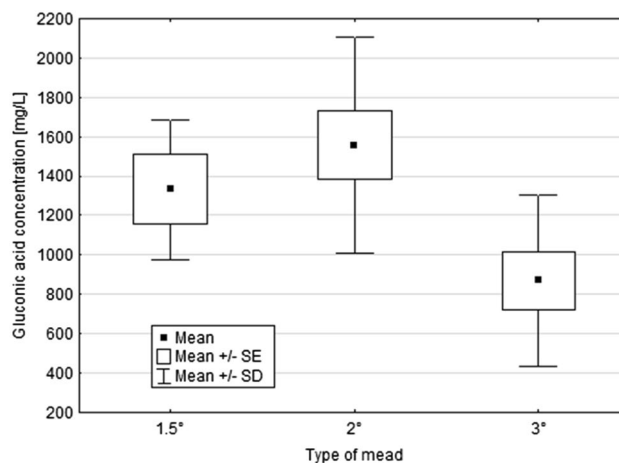
Fig. 1 Average concentration of gluconic acid in 1.5° mead, 2° mead, and 3° mead

(which may be caused by the overestimation of the average by the high concentration of acid in the 2° meads from TiM company); however, compared to the group of 1.5° meads, this difference was not statistically significant. The observed differences probably resulted from the different degree of honey dilution in different types of mead.

Lactic acid

Lactic acid was present in all the tested meads in the concentration range 229–654 mg/L. The obtained values for the concentrations of this acid were within the wide range determined by Kružík et al. [7], 260–4500 mg/L, and turned out in most cases to be lower than those determined by Sroka et al. [17], 620–1130 mg/L. No statistically significant differences were observed between the average concentration of this acid in the different groups of honey.

Succinic and acetic acids

Previous research indicates that succinic acid and acetic acid are mainly formed as by-products during the ethanol fermentation process [17, 18]. Synthesis of succinic and acetic acids strongly depends on the strain of yeast, the concentration of carbohydrates, the presence of nitrogen compounds [22], and pH [18, 22]. The concentration of succinic acid in the examined meads ranged from 281 to 845 mg/L, whereas in the course of analyses of commercially available honey wines, Kružík et al. [7] obtained a range of < 1–760 mg/L. In contrast, Švecová et al. [5] obtained the values for the concentration of this acid falling within a much wider range of 370—3980 mg/L. It should be emphasised here that last mentioned authors studied honey presumably of very different compositions, in terms

of the additives used: sherry mead, nut mead, almond mead, herbal mead, bitter mead, or raspberry mead.

The acetic acid was determined in a concentration range of 244–846 mg/L. The Czech meads analysed by Švecová et al. [5], as in the case of succinic acid, were characterised by varied acetic acid concentrations, 620–3110 mg/L. The concentration of this component for one mead deviated significantly from the upper limit of this range and was $16,611 \pm 60$ mg/L. The authors suggested that this beverage may have been spoiled by ongoing acetic fermentation. Other studies of Czech honey wines showed that the concentration of acetic acid did not exceed 960 ± 10 mg/L [7].

Comparing the average concentration of succinic acid in the three groups of the examined meads, it was observed that the average amount of succinic acid increased with the dilution of honey during the production of this beverage (Fig. 2). A statistically significant difference was found between the average concentrations of this acid in 1.5° and 3° meads. These observations are consistent with those obtained by Sroka et al. [17], where it was found that the concentration of succinic acid in the 4° meads was four times higher than that in 3° meads. No such consistencies were observed for acetic acid.

Citric and malic acids

Citric and malic acids are naturally occurring compounds in various types of honey [18, 19]. During alcoholic fermentation, citric acid concentration does not change significantly [17, 18]. Citric and malic acids are often used to regulate the acidity of the mead, but their source can also be fruit additives. The resulting concentration of citric acid in the tested honey ranged from 125 to 4576 mg/L. The highest concentration of this acid was noted in mead
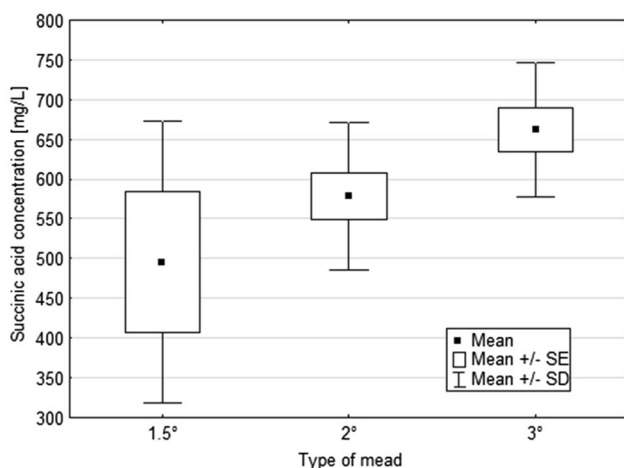

Fig. 2 Average concentration of succinic acid in 1.5° mead, 2° mead, and 3° mead

PMJ7/2°, which was probably the result of replacing some of the water with raspberry juice in the production of the wort (as declared by the producer). Concentrations of citric acid in other meads were below 2000 mg/L.

The concentration of citric acid, determined above 500 mg/L in 19 meads, may be a consequence of the rather high concentration of this acid in the honey used to produce the beverage [23], the addition of fruit juices and extracts during production, and the possible correction of the acidity of the mead by the addition of citric acid to the final product. The producer, labelled PMJ, declared no addition of citric acid at the production stage of the meads, so it can be concluded that in meads PMJ2/2° and PMJ6/2°, with no additives and citric acid content 308 ± 5 mg/L and 443 ± 9 mg/L, respectively, the only source of the compound was the honey used in their production. In a study by Dezmirean et al. [18], the concentration of citric acid determined in the meads produced without additives, e.g. in the form of fruit juices, did not exceed a concentration of 211 µg/g. In the case of the analyses conducted by Kružík et al. [7] in three dessert meads in which the producers declared the addition of citric acid, its concentration was in the range of 700–1100 mg/L. According to Švecová et al. [5], the citric acid content of Czech meads was in the range of 120–3130 mg/L, with the highest concentration determined in cherry mead, which was probably enriched with cherry juice, a good source of this acid [24].

The malic acid content in the meads ranged from 135 to 1611 mg/L. The highest concentration was observed in sample PMJ3/2°, which was probably due to the use of apple juice, rich in this acid, instead of a portion of water in making the wort [25]. The concentration of malic acid in the remaining 24 meads was below 600 mg/L. Again, producer PMJ declared that no malic acid was added to regulate the acidity of the final products. In honey PMJ2/2°, the concentration of this acid was 309 ± 2 mg/L and in PMJ6/2° 135 ± 5 mg/L. The values of the concentrations of malic acid obtained in the present study were similar to those analysed by Kružík et al. [7] (< 0.01–620 mg/L) and partially overlapped the lower range obtained for the Czech meads by Švecová et al. [5] (290–2860 mg/L).

Formic acid

Formic acid was identified in all examined meads, its concentration was much lower compared to other acids, and ranged from 35 to 176 mg/L. This acid occurs naturally in honey and its concentration can decrease during fermentation as a result of the partial metabolism of this component by yeast [17]. The obtained concentration
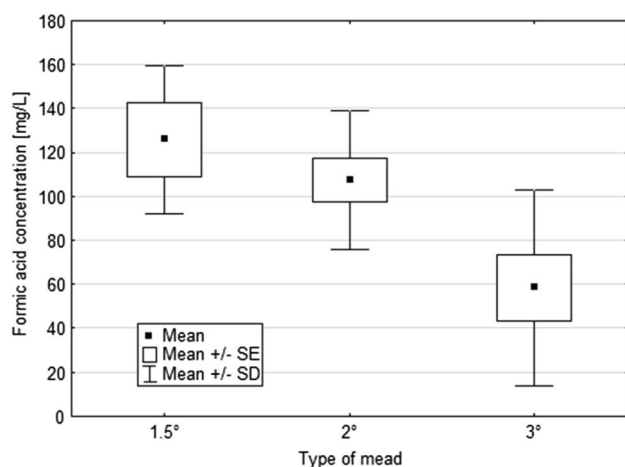
Fig. 3 Average concentration of formic acid in 1.5° mead, 2° mead, and 3° mead

values were mostly lower than those obtained by the Švecová et al. [5] (80–1460 mg/L) and Kružík et al. [7] (90–1060 mg/L).

The average concentration of formic acid was significantly lower in 3° meads than in 2° and 1.5° meads, which was probably related to the increasing dilution of honey in the production of each type of meads (Fig. 3). This acid is not a characteristic substance for fruits or spices.

Malonic acid

To the authors' knowledge, malonic acid has not been determined in mead to date. Also, information on the content of this acid in honey is lacking. Its source can be fruits, vegetables or spices, although its concentration is relatively low compared to other organic acids characteristic of these foods [26]. Malonic acid concentrations ranged from 81 to 593 mg/L.

Total polyphenol content and antioxidant activity

The antioxidant activity of the meads varied widely and was in the range between 233 and 6422 µmol/L Fe^{2+} The total polyphenol content (TCP) of these alcoholic beverages ranged from 58 to 699 mg/L GAE. Statistical analysis showed a positive correlation between TCP, the concentration of citric acid, and the antioxidant potential of meads, indicating that these compounds may contribute to this property of the beverages.

Interesting observations were made by Šmogrovičová et al. [27], examining Czech meads produced by batch fermentation of acacia, cherry floral, and honeydew forest apian honey, and from South Africa, obtained by continuous fermentation of honey derived from wild natural plants

Eastern Cape apian. The results of the study indicated similar total polyphenol content in the alcoholic beverages tested, which fell within a narrow range: 177.8–241.4 mg/L of gallic acid equivalents. Similar results in the course of studies on mead were obtained by Wintersteen et al. [28] (116–241 mg/L GAE) and Kružík et al. [7] (151.9–385.3 mg/L GAE). The main source of this group of compounds in the meads is the raw material from which they are made. Wilczyńska et al. [29] analysed 32 samples of the meads produced in Poland, of different origins, and, consequently, of different colours, and indicated that the content of polyphenols and their antioxidant potential are related to the colour of the honey. The highest total phenolic content and antioxidant activity were characterised by dark honey: buckwheat, honeydew, and heather honey, while light honey showed lower antioxidant activity and total phenolic content. In the present study, it is difficult to make similar observations, as in most cases, no information was provided from the producers about the type of honey was used to prepare the mead. Moreover, some of the products tested were the mixtures of dark and pale honey of unknown proportions (PMJ1/1.5°, PMJ2/2°, PMJ4/2°, PMJ5/2°, and PMJ8/3°).

Although honey is a source of phenolic compounds and exhibits antioxidant potential, no significant differences were observed between the different types of mead. This is probably due to the additives that were used in the production of meads such as fruit juices and musts, and spices, which provided an additional source of these compounds. This can be observed in the analysis of PMJ2/2° vs PMJ5/2° meads, made from the same honeys (mixture of dark and pale honey), with no additives in case of PMJ2/2°, and the additional chokeberry and elderberry juices in PMJ5/2° mead, where the values for TP and FRAP increased two and four times, respectively, for the latter. A similar increase was also noted for PMJ7/2° meads, when compared to PMJ6/2° mead—as both were produced from pale honey, the addition of raspberry juice to PMJ7/2° was the probable reason of its higher antioxidant potential.

The above-mentioned observations and relationships illustrate a broader phenomenon—a large variation in the chemical composition of meads, regardless of their type. In meads of the same type, from different producers, the concentrations of some ingredients may differ. In our study, we found a statistically significant difference in the concentrations of gluconic (1957 ± 206 vs 1029 ± 54 mg/L, $p = 0.000$) and acetic (697 ± 221 vs 483 ± 82 mg/L, $p = 0.021$) acids in 2° meads delivered by producers PMJ and AP. Therefore, for these two acids in 2° meads, we calculated weighted arithmetic means and standard deviations, which were equalled 1376 ± 880 mg/L and 550 ± 106 mg/L, respectively. A small number of samples of other types of meads precluded similar analyses for them.

The PCA model fulfilling cross-validation criteria was constructed for the following parameters: acetic acid, formic acid, FRAP, gluconic acid, lactic acid, malonic acid, TPC. Other parameters (malic acid, succinic acid) were not included in the model as they were considered noninformative, and citric acid was added in arbitrary amounts. One sample (AP1/1.5°) was excluded as being a clear outlier for two parameters—TPC and FRAP (Table 2). The model had two significant components, with eigenvalues of 4.43 and 1.25, which explained 63.3% and 17.9% of variance of the predictive parameters, respectively. The loadings for first two principal components are shown in Fig. 4. The first principal component in this model had positive weights predominantly for lactic acid, TPC, gluconic acid, and formic
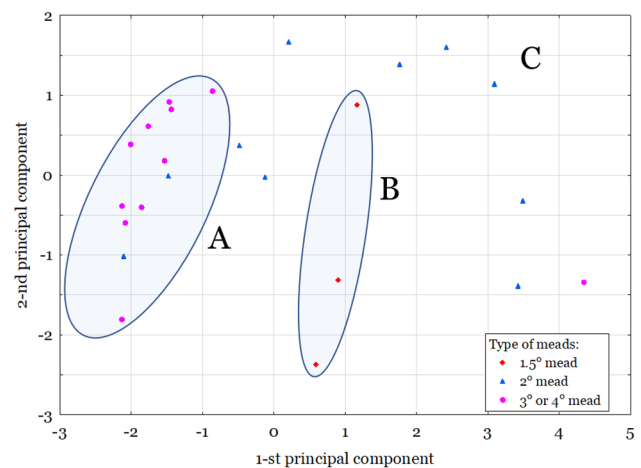

Fig. 5 Score scatter plot of mead samples in the PCA model (different types of meads are depicted by different colours; **A**, **B**, **C** distinguished clusters of samples)

acid. These parameters, being in one cluster, had high correlation coefficients with each other (Table 4). Malonic acid and acetic acid were the parameters which loaded mainly (and positively) on the second component, while FRAP loaded negatively the most on this component. Therefore, malonic acid and acetic acid were strongly positively correlated with each other, and strongly negatively with FRAP. Correlation coefficients for the pairs of parameters based on the PCA model (only the first ten positive coefficients with the highest values and only the two biggest negative ones) are shown in Table 4. Score scatter plot of the PCA model (Fig. 5) shows all mead samples in the space determined by the first two principal components. Visual inspection of it disclosed one cluster containing almost all samples of 3° meads or 4° meads types (with only one sample deviating from this group; true positive rate = 91%, false positive rate = 14%, cluster A) and the second cluster containing all three 1.5°meads and no other samples (cluster B), while the remaining samples of 2°meads did not form any clearly separated cluster (cluster C overlapping clusters A and B, not enclosed by an ellipse).

Fig. 4 Loadings of the first and second principal components in the PCA model (meaning of symbols: FRAP ferric reducing antioxidant power, TPC total phenolic compounds; 'a' stands for acid)

Table 4 Correlation coefficients for the pairs of parameters based on the PCA model

Pairs of correlated parameters		Correlation coefficients
Malonic acid	Acetic acid	1.000
Gluconic acid	Acetic acid	0.962
FRAP	TPC	0.961
Formic acid	TPC	0.957
Malonic acid	Gluconic acid	0.954
Formic acid	Lactic acid	0.877
Formic acid	FRAP	0.840
Lactic acid	Gluconic acid	0.831
Lactic acid	TPC	0.699
Lactic acid	Acetic acid	0.646
Acetic acid	FRAP	− 0.364
Malonic acid	FRAP	− 0.387

Conclusions

This study presents for the first time the results of chemical analyses of 25 meads available on the market, from the largest producers of this beverage in Poland. Meads from four groups were analysed, depending on the ratio of honey to water in the preparation of the wort, with the different content of various additives in the form of fruit juices and spices. Chemometric approach (PCA) showed the correlation structure of the examined parameters and the existence of two clusters containing specific meads.

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Data availability We declare transparency for its data for publication. The datasets are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval Ethics approval was not required for this research. This study does not involve any human or animal testing.

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