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ANALYSIS OF ORGANIC ACIDS IN HERBAL AND FRUIT SYRUPS BY LIQUID CHROMATOGRAPHY

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Abstract

Syrups are a pleasing balance betveen aromatic and medicinal herbs, an aromatic herb has a strong or bitter taste, an aromatic herb can be added for flavour, if ihe medicinal herb has a bitter or strong taste. The investigation of the research was to evalute the content of organic acids in the herbal and fruit syrups. Some of popular Latvian herbal syrups made from: *Plantago major, Chamaenerion angustifolium* flower, *Calluna vulgaris* flower, *Picea* young shoots, *Pinus* young shoots, *Pinus* cone, *Achillea millefolium, Syringa vulgaris* flower and fruit syrups made from: *Crataegus curvisepala* fruit, *Sorbus aucuparia* fruit, *Rosa canina* fruit, *Japanese quince* fruit, *Aronia melanocarpa* fruit, *Pyrus malus* and *Rheum rhabarbarum* were selected for analysis. The current research focuses on the evaluation of organic acid, pH content and dry matter in herbal and fruit syrups. The major organic acids (oxalic acid, tartaric acid, quinic acid, malic acid, ascorbic acid, citric acid, fumaric acid and succinic acid) were determined by applying the method of high-performance liquid chromatography (Schimadzu Prominence HPLC). In the present experiments it was found that there are significant differences in the organic acids content between different herbal and fruit syrups. In general, all samples tested in this study, demonstrated high content of organic acids. The highest content of organic acids was found in *Pinus* cone syrup 7.82 g 100 g^{-1}, *Rheum rhabarbarum* syrup 4.27 g 100 g^{-1} and *Picea* young shoots syrup 4.14 g 100 g^{-1}. Whereas, the lowest total organic acid content was in *Syringa vulgaris* syrup 1.24 g 100 g^{-1}, *Rosa canina* fruit syrup 1.40 g 100 g^{-1}, *Achillea millefolium* syrup 1.61 g 100 g^{-1} and *Sorbus aucuparia* fruit syrup 1.68 g 100 g^{-1}. Results of the present experiments demonstrated that pH in analysed herbal and fruit syrups was significantly different ($p<0.05$).

Keywords: herbal syrups, fruit syrups, organic acid, HPLC

Introduction

Fruits have been described to contain essential minerals, vitamins, organic acids, amino acids, which provides a wide spectrum for value addition of the juice for applications in the food industry. Often the value add products than be obtained from fruits is the syrup. That's it potential uses in the food industry. The fruit and herbal syrups can be used as an ingredient different bread products. Manufacturers in the food industry often prefer to use carbohydrates in the form of syrup mostly due to the ease and efficiency of solutions and to the favoured process economics (Willis et al., 2013). Scientist Kaushik (2016) from India reported that syrup is a concentrated mixture of sugar and purified water. He emphasizes that the high carbohydrates content distinguishes syrups from other types of liquids. Herbal and fruit syrups may or may not contain added flavouring agents or medication. Scientific literature explains that herbal syrups without a medication, but with a different flavouring agent are called flavoured or non-medicated syrups. Herbal flavoured syrups are often used for prevent unpleasant tasting medications – resulting is medicated syrup (Kaushik et al., 2016). Pleasant flavour, high content of organic acids and fibre makes fruits valuable and interesting raw material for the development of various, healthy food products. The fruit are suitable for juice, liqueur, wine, jam, puree production and also for pectin and aromatic compounds extraction. Syrups, carbonated soft drinks, liqueurs, caramels and marmalades are popular products on the market of the Baltic States and neighbouring countries (Rubinskienė et al., 2014). Some researchers found that fruit syrups contain biologically active compounds that help to create taste and appearance, improve storage time, and provide the body with energy (Willis et al., 2013). Kaushik et al. (2016) informed that in the last years plant derived products are increasingly being sought as the nutraceuticals, cosmetics and medicinal products and are available in health food shops and pharmacies over the counter as self-medication. Herbals are widely used in Phytomedicine, which has a good effect on human health, have anti-inflammatory activity, anti-digestive simulation, antimicrobial activity and antioxidant activity (Zhang et al., 2018; Carabajal et al., 2017).

In turn researcher Brewer (2011) reported that the consumption of foods containing antioxidants is now widely considered to be exert a beneficial effect on human health and an effective trategy to reduce oxidative damage. The food industry in the last years has shiffing focus to herbal products from natural sources as a replacement for synthetic antioxidants and also as nutraceuticals. In her study a scientist Augšpole et al. (2018) noticed that herbals and derived products have many beneficial properties, which are associated with the especially phenolic compounds and presence of secondary metabolites. Fruit and herbal syrup products have been declared to contain antioxidant and antiradical activities, and phenolic compounds (Thériault et al., 2006). Food scientists have proved that therapeutic effects of many herbal species including spring wild plans are attributed to be presence of antioxidative phenolics and organic acids in their tissues. In herbals they are involed in numerous roles from structural to protective (Vajic et al., 2015). In turn Robbins (2003) reported that Phenolic acids are aromatic secondary plant metabolites, widely spread throughout the plant vegetation. Group of scientists (Mahmood et al., 2012) from Pakistan University of Agriculture described that determination of organic acids is important to the food industry. They emhasize that organic acids play a significant role in influencing appearance, smell and flavour of foods beverages. Definitions on the type and concentration of organic acids are important to ensure the quality of food, especially beverages, juices and syrups. Scientists explain that analytical methods for the determination of organic acids include liquid and gas chromatography, capillary electrophoresis and enzymatic analysis. In turn the separation on short chain aliphatic acids has been determined by phase high performance liquid chromatography using a different column stationary phases (Mahmood et al., 2012). Literature data suggest that organic acids are can give the characteristic gustation and sour taste of the herbals and fruits. Due to organic acids nature, these compounds have found many applications in the beverages and food industry. They are amply used in the industry of beverages and juices as preservatives and pH regulators (Kucner et al., 2014). Researcher Kaushik et al. (2016) reported that advantages of herbal syrup is ability to disguise the bad gustation of medications. These syrups are thicker than water solutions, therefore only a portion of the medication substances dissolved in the syrup comes in contact with the taste buds. Researcher explain that the high carbohydrates content of herbal syrups gives them a sweet taste that helps conceal the bad taste of the medicine. This is why herbal and fruit syrups are recommending for paediatric medications. The thick character of herbal syrups also has a soothing effect on irritated tissues (Kausik et al., 2016). Fruits and herbs are used in many domains, including nutrition, beverages, cosmetics, medicine, fragrances, flavouring and dyeing (Augspole et al., 2018). Fruit and herbal are used by the most of the world's population. Herbal plants provide beneficial health effects due to the presence of antioxidant (Augspole et al., 2017). According to report of the World Health Organization (WHO), about 80% of the world population still uses fruits and herbs for their primary health care needs. Fruit and plants formulations have reached widespread acceptability as therapeutic agents. In the World Health Organization (WHO) definition is three kinds of fruit and herbal medicines – medicinal products, raw plant material and processed plant material (Kaushik et al, 2016). Selection of the most suitable plant cultivars and hybrids for industrial processing and expansion of product assortment is very important. The perennial plant breeding process is time-consuming and may last many years (Rubinskienė et al., 2014). Food scientists have proved that chemical composition of herbal is affected by different factors: climate, harvest time, variety, vegetative stage of the herbal, genotype, soil, storage, technological processes applied, treatment and processing (Marrelli et al., 2012; Meireles, 2009).

The aim of this investigation was to evaluate the concent of organic acids, as a potential source of biologically active compounds, in fruit and herbal syrups using high-performance liquid chromatography.

Materials and Methods

The research was carried out at the Department of Chemistry of the Faculty of Food Technology of the Latvian University of Life Sciences and Technologies. The object of the research was Latvian herbal syrups: Greater plantain (*Plantago major)*, Rosebay willowherb *(Chamaenerion angustifolium)* flower, Heather *(Calluna vulgaris)* flower, Spruce *(Picea)* young shoots, Pine *(Pinus)* young shoots, Pine *(Pinus)* cone, Yarrow *(Achillea millefolium),* Lilac *(Syringa vulgaris)* flower and fruit syrups: Hawthorn (*Crataegus curvisepala)* fruit, Rowan (*Sorbus aucuparia)* fruit, Rosehip (*Rosa canina)* fruit, Japanese quince (*Chaenomeles japonica)* fruit, Aronia (*Aronia melanocarpa)* fruit, Apple (*Pyrus malus)* and Rhubarb (*Rheum rhabarbarum).*

Preparation of herbal syrups

Preparation of herbal and fruit syrups - the fixed mass of certain plants or fruits (200 g fruits) were boiled in a certain volume of water (400 mL). After that, sugar has been added (400 g) and composition has been boiled for 20 min. The time of preparing as well as the amount of taken ingredients and other raw materials may vary depending on plant or fruit origin. Syrups were prepared by usual domestic preparation technique and precise technologies are confidential. After boiling syrups were filtered through cheesecloth and centrifuged for 5 minutes at 10 000 rpm.

Determination of pH

The pH level of herbal syrup samples was determined with potentiometric method WTW pH (pH538) meter. The electrode Sen Tix 97T was used.

Determination of organic acids by High Performance Liquid Chromatography

Organic acids in the syrups was analysed with high-performance liquid chromatography (HPLC), Schimadzu LC-20 Prominence, DAD M20A detector, LC-20A solvent delivery system, CBM-20A system controller and LCsolution data system software.

Preperation of the calibration solution: in the 50 mL volumetric flask with narrow neck weight 0.1 g oxalic, 0.1 g tartaric, 0.1 g quinic, 0.1 g malic, 0.1 g ascorbic, 0.1 g citric, 0.02 g fumaric and 0.1 g succinic acids. Fill the volumetric flask with HPLC grade methanol (CHROMASOLV®) till mark and mix.

Chromatography parameters for the separation of organic acids: YMC C18 analytical column, 5µm, 4.6 mm×250 mm, column temperature +35 °C, wavelength 210 nm, volume of the sample injection 10 µL, start flow rate 1.25 mL min^{-1}, mobile phase at the gradient conditions: Acetonitrile (A) : 0.05 *M* KH_2PO_4 (B) (1:99) was used. Each measuring was carried out several times and then the simple average was calculated (Cinkmanis et al., 2018). Figure 1 shown the calibration chromatogram of organic acids.

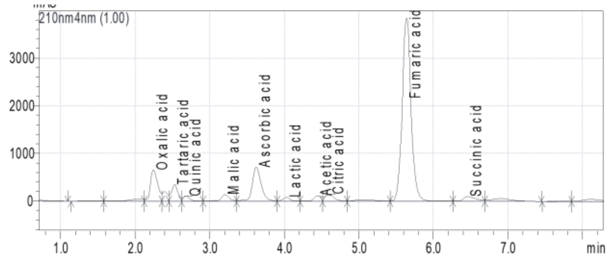

Figure 1. Chromatogram of the calibration solution of the organic acids

Statistical analysis of the research

The data of the research was analysed by the statistical and mathematical methods (standard deviation, mean). Data compared by the analysis of variance (ANOVA) and significance was defined at $p<0.05$. For the data analysis the Microsoft Excel software of the version 2016 was used.

Results and Discussion

Determination of organic acids

To compare herbal and fruit syrups, the content of organic acids was determined in the ascorbic, citric, oxalic, quinic, tartaric, succinic and fumaric acids (Tables 1, 2, 3, 4).

Table 1

Oxalic, tartaric, quinic and malic acids of herbal syrups, g 100 g^{-1}

Syrups	Oxalic acid	Tartaric acid	Quinic acid	Malic acid
Yarrow	0.01±0.003	0.60±0.03	0.08±0.005	0.22±0.02
Pine young shoots	0.06±0.002	0.50±0.02	0.87±0.06	0.08±0.01
Spruce young shoots	0.06±0.01	1.06±0.05	1.83±0.03	0.12±0.01
Heather flower	0.01±0.003	1.06±0.05	0.02±0.005	0.05±0.01
Rosebay willowherb flower	0.01±0.002	0.59±0.01	0.09±0.001	0.18±0.02
Greater plantain	nd	0.43±0.02	0.13±0.02	0.31±0.02
Pine cone	0.03±0.005	0.91±0.04	0.78±0.04	0.55±0.03
Lilac flower	0.01±0.004	0.36±0.02	0.35±0.01	0.21±0.01

nd – not detected

Table 2

Ascorbic, citric, fumaric and succinic acids of herbal syrups, g 100 g^{-1}

Syrups	Ascorbic acid	Citric acid	Fumaric acid	Succinic acid
Yarrow	0.03±0.001	0.49±±0.06	nd	0.17±0.03
Pine young shoots	1.07±0.02	0.01±0.001	nd	0.12±0.001
Spruce young shoots	0.81±0.04	0.02±0.001	nd	0.24±0.04
Heather flower	nd	0.42±0.02	nd	0.29±0.02
Rosebay willowherb flower	0.24±0.01	0.33±0.05	nd	1.00±0.04
Greater plantain	0.01±0.03	3.44±0.13	nd	0.15±0.01
Pine cone	5.52±0.12	0.01±0.001	nd	0.02±0.004
Lilac flower	0.03±0.01	0.13±0.007	nd	0.15±0.03

nd – not detected

Table 3

Oxalic, tartaric, quinic and malic acids of fruit syrups, g 100 g^{-1}

Syrups	Oxalic acid	Tartaric acid	Quinic acid	Malic acid
Rowan fruit	nd	1.00±0.06	0.01±0.005	0.44±0.02
Rhubarb	0.12±0.01	2.41±0.08	0.03±0.01	1.29±0.06
Rosehip fruit	0.01±0.005	0.44±0.02	0.36±0.01	0.15±0.01
Japanese quince fruit	nd	0.47±0.03	0.53±0.03	0.90±0.02
Aronia fruit	0.01±0.005	0.69±0.03	0.30±0.02	0.51±0.03
Apple	0.01±0.005	0.96±0.05	0.01±0.005	1.21±0.04
Hawthorn fruit	nd	0.8	0.36±0.02	0.24±0.02

nd – not detected

Table 4

Ascorbic, citric, fumaric and succinic acids of fruit syrups, g 100g^{-1}

Syrups	Ascorbic acid	Citric acid	Fumaric acid	Succinic acid
Rowan fruit	0.02±0.005	0.04±0.002	nd	0.17±0.01
Rhubarb	0.01±0.005	0.05±0.004	nd	0.36±0.03
Rosehip fruit	0.08±0.01	0.30±0.03	0.02	0.04±0.01
Japanese quince fruit	0.02±0.005	nd	nd	0.20±0.05
Aronia fruit	0.02±0.003	0.17±0.02	nd	0.09±0.01
Apple	0.02±0.002	0.15±0.03	nd	nd
Hawthorn fruit	0.04±0.002	0.37±0.04	nd	0.12±0.02

nd – not detected

The highest oxalic and tartaric acids content was found in Pine young shoots syrup and Spruce young shoots syrup 0.06 and 1.06 g 100 g^{-1}, respectively the highest quinic acid content was detected in Spruce young shoots syrup 1.83 g L^{-1}, and malic acid in Pine cone syrup 0.55 g 100 g^{-1} (Table 1).

The highest ascorbic acid content was detected in Pine cone syrup 5.52 g 100 g^{-1} and Pine young shoots syrup 1.07 g 100 g^{-1}, citric acid in Greater plantain syrup 3.44 g 100 g^{-1}, and succinic acid in Rosebay willow herb flower syrup 1.00 g 100 g^{-1}. Fumaric acid was not found in herbal syrups (Table 2).

The highest oxalic and tartaric acid content was found in Rhubarb syrup 0.12 and 2.41 g 100 g^{-1}, respectively, while quinic acid dominated in Japanese quince fruit syrup 0.53 g 100 g^{-1}, and malic acid in Rhubarb syrup 1.29 g 100 g^{-1} and Apple syrup 1.21 g 100 g^{-1} (Table 3). The highest ascorbic acid content was detected in Rosehip fruit syrup 0.08 g100 g^{-1}, whereas highest citric acid contend was determined in Hawthorn fruit syrup 0.37 g 100 g^{-1}, and succinic acid in Rhubarb syrup 0.36 g 100 g^{-1}. Fumaric acid was found only in one Rosehip fruit syrup 0.02 g 100 g^{-1} (Table 4).

The richest source of the total organic acids comparing all herbal and fruit syrups was Pine cone 7.82 g 100 g^{-1}, Rhubarb 4.27 g 100 g^{-1} and Spruce young shoots 4.14 g 100 g^{-1}. The lowest content of total organic acid was found in Lilac flower 1.24 g g^{-1}, Rosehip fruit 1.40 g 100 g^{-1}, Yarrow 1.61 g 100 g^{-1} and Rowan fruit 1.68 g 100 g^{-1} syrups.

Determination of pH

The results of pH level performed on the plant and herbal syrup formulation revealed that the pH varied in the range of 4.27–3.19, which indicates acidic environment. The difference between lowest and highest pH was significant – 1.08 units ($p<0.05$).

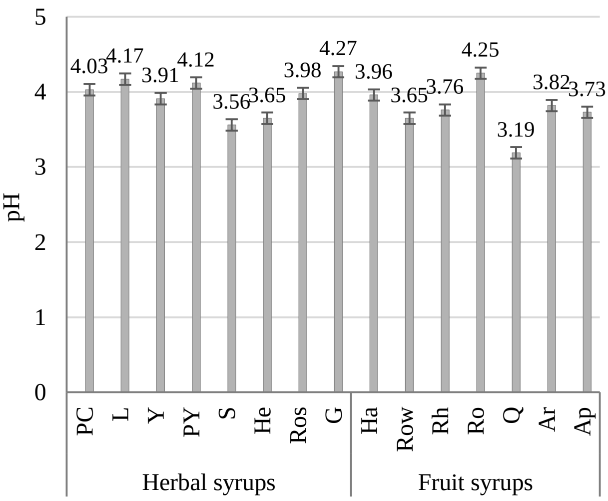

Figure 2. pH of herbal and fruit syrups

PC – Pine cone, L – Lilac flowers, Y – Yarrow, PY – Pine young shoots, S – Spruce young shoots, He – Heather flower, Ros – Rosebay willowherb flower, G – Greater plantain, Ha – Hawthorn fruit, Row – Rowan fruit, Rh – Rhubarb, Ro – Rosehip fruit, Q – Japanese quince fruit, Ar – Aronia fruit, Ap – Apple

The highest pH level was found in herbal and fruit syrups in descending order: Greater plantain 4.27, Lilac 4.17, Pine young shoots 4.12, Pine cone 4.03 and Rosehip fruit 4.25, the lowest pH is measured by Japanese quince fruit 3.19, Spruce young 3.56 and Heather flower 3.65.

Acidic environment of herbal and fruit syrups is resulting of concentration of individual organic acids in plants, large content of sugar sucrose, its hydrolysation rate with formation of inverted sugar and glucose oxidation to gluconic acid at a specific temperature (Farrokhi et al., 2012).

Conclusions

The results of the research indicate that fruits and herbal syrups are source of organic acid compounds.

The herbal syrups accumulated higher amounts of organic acid compounds (Pine cone syrup – ascorbic acid was 5.52±0.85 g 100g^{-1} and Plantain syrup Citric acid 3.44±0.74 g 100 g^{-1}, respectively) than the fruit syrups.

Furthermore, Fruit syrups (rhubarb) had higher content of tartaric acid (2.41±0.08 g 100 g^{-1}) and higher malic acid (rhubarb and apple syrups) (1.29±0.42 g 100 g^{-1} and 1.21±0.35 g 100 g^{-1}).

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