TOTAL POLYPHENOLS CONTENT AND SENSORY ANALYSIS OF PLANT MACERATES OF HONEY INFUSED APPLE CIDER VINEGAR WITH APPLICATION IN DIABETES MANAGEMENT

Andra Nichitean[1,2], Florin Oancea[2,3], Cristina Mateescu [4], Oana Maria Bartas[5], Anca Becze[6]*

[1]ROM HONEY GROUP SRL, 1 Gradinari Str., 700390, Iasi, Romania; [2]Faculty of Biotechnologies, University of Agronomic Sciences and Veterinary Medicine of Bucharest, Bd. Mărăști nr. 59, 011464 Bucharest, Romania ; [3]Polymers and Bioresources Departments, National Institute for Research & Development in Chemistry and Petrochemistry—ICECHIM, Splaiul Independenței nr. 202, Sector 6, 060021 Bucharest, Romania; [4]National Institute for Research& Development in Food Bioresources National Service for Medicinal and Aromatic Plants and Bee Products, 6 Dinu Vintila Str, sector 2, 021102, Bucharest; [5]Economic College "Iulian Pop", 19 Emil Isac Str., 400023, Cluj-Napoca, Romania ; [6]INCDO-INOE 2000, Research Institute for Analytical Instrumentation Subsidiary, 67 Donath Street, 400293 Cluj-Napoca, Romania; **Corresponding author: anca.naghiu@icia.ro*

Abstract. This study investigates the total polyphenols content in plant macerates of honey-infused apple cider vinegar (ACV) and its potential implications for diabetes management. Five distinct recipes, comprising Lavender, Sage, Cinnamon, and Nigella individually and in combinations, were subjected to maceration for 24 and 48 hours. The samples were analyzed using the Folin-Ciocalteu method, and sensory attributes such as color, taste, smell, and acidity were evaluated by a panel of 10 evaluators. Notable variations in polyphenol content were observed, with Recipe 3 (Cinnamon) demonstrating the highest total polyphenols content of 407.95 ± 4.2 mg/ml Gallic Acid Equivalent (GAE) after 48 hours. Sensory analysis revealed that Recipe 2 (Sage) exhibited pleasant taste and smell attributes. The findings suggest the potential of these plant macerates as dietary adjuncts for diabetes management due to their substantial polyphenolic content and desirable sensory characteristics. Further exploration into their bioactive profiles and antidiabetic mechanisms is warranted.

Keywords: honey, apple cider vinegar, Folin-Ciocalteu, plant macerates, sensory analysis, polyphenols

INTRODUCTION

Polyphenols, a diverse group of naturally occurring compounds found in plants, have garnered substantial attention due to their potential health benefits. These compounds possess antioxidant, anti-inflammatory, and antidiabetic properties, making them promising candidates for applications in disease management and prevention (Williamson et al., 2018). Among various dietary sources, apple cider vinegar (ACV) has gained popularity for its potential health-promoting effects. ACV is rich in acetic acid and bioactive compounds like polyphenols, contributing to its physiological properties (Budak et al., 2014). Incorporating honey into ACV infusions further augments its therapeutic potential, as honey itself is recognized for its antioxidant and antidiabetic properties (Eteraf-Oskouei et al., 2013). Type 2 diabetes mellitus (T2DM) is a prevalent metabolic disorder characterized by hyperglycaemia

and insulin resistance. Management of T2DM often involves dietary interventions and natural remedies that can modulate blood glucose levels.

Plant-based interventions, including the use of herbal macerates, have shown promise in diabetes management due to their bioactive content. *Lavandula angustifolia* (lavender), *Salvia officinalis* (sage), *Cinamomum verum* (cinnamon), and *Nigella sativa* (black seed) are among the plant species renowned for their potential antidiabetic effects (Oboh et al., 2020; Karimi et al., 2020; Anderson et al., 2004; Randhawa et al., 2020). Lavender is characterized by purple flowers and narrow, linear leaves. The plant is rich in essential oils, which contain bioactive compounds such as linalool, linalyl acetate and 1,8-cineole (Buchbauer et al., 1993). Its therapeutic effects are largely attributed to these bioactive compounds. A growing body of evidence suggests that lavender may have beneficial effects in glycaemic control, thus constituting a potential option for diabetes management (Sebai et al., 2013). In vitro and animal studies have shown that lavender extract can improve insulin sensitivity and reduce blood glucose levels (Elshafie et al., 2017). Sage has been used in traditional medicine for its anti-inflammatory, antioxidant and antimicrobial properties. Moreover, an increasing number of studies suggests that the plant may have a role in the management of diabetes (Eidi et al., 2005). In animal experiments, sage extract showed potential in lowering blood glucose levels and improving lipid profile (Lima et al., 2006). *In vitro* studies have corroborated these effects, indicating that bioactive compounds can stimulate insulin secretion and improve insulin sensitivity (Behradmanesh et al., 2013). Cinnamon is known for its antioxidant, anti-inflammatory and antimicrobial properties. Recently, attention has also been directed towards its potential in the management of diabetes (Khan et al., 2003). Nigella sativa produces capsule fruits with numerous black seeds. These seeds are the ones that have been extensively studied for their medicinal properties and contain bioactive compounds, including thymoquinone, thymohydroquinone and alpha-hederin acid (Ahmad et al., 2013). Negrilica is used in traditional medicine for a variety of ailments, including asthma, hypertension, and inflammation. Recently, the attention of researchers has been directed towards its therapeutic potential in the management of diabetes (Fararh et al., 2002). *In vivo* and *in vitro* studies suggest that negrilica extract can have hypoglycemic, antidiabetic and antioxidant effects (Benhaddou-Andaloussi et al., 2010). Moreover, evening primrose seeds seem to improve pancreatic beta cell function, thus increasing insulin secretion (Alimohammadi et al., 2013).

This study aims to evaluate the content of total polyphenols in distinct plant macerates of honey-infused apple cider vinegar. The selected plant materials - *Lavandula angustifolia*, *Salvia officinalis*, *Cinamomum verum*, and *Nigella sativa* - have been chosen for their documented antidiabetic properties. Polyphenol-rich plant macerates, when combined with honey-infused ACV, may offer a synergistic effect, potentially enhancing their collective therapeutic potential in diabetes management.

MATERIALS AND METHODS

Materials used for maceration. Following the bibliographic study, raw materials were identified, which are also rich sources of polyphenols but which also

have diabetes management properties demonstrated by studies. The ingredients used to make the product are:

1. Apple vinegar obtained from apple cider;
2. Acacia honey;
3. Lavender - *Lavandula angustifolia* - in the form of flowers with a moisture content ≤ 85%;
4. Sage - *Salvia officinalis* - sage leaves with moisture content ≤ 85%;
5. Cinnamon – *Ceylon powder;*
6. Nigella - *Nigella sativa* - Nigella seed powder.

The recipes for the study used are presented in table 1.

Tabel 1

Recipes used for maceration

Recipe name	Ingredients	Quantity	Unit of measurement
Recipe 1	Apple vinegar	40	ml
	Acacia honey	2	g
	Lavender	2	g
Recipe 2	Apple vinegar	40	ml
	Acacia honey	2	g
	Sage	2	g
Recipe 3	Apple vinegar	40	ml
	Acacia honey	2	g
	Cinnamon	2	g
Recipe 4	Apple vinegar	40	ml
	Acacia honey	2	g
	Nigella	2	g
Recipe 5	Apple vinegar	40	ml
	Acacia honey	2	g
	Lavender	0.5	g
	Sage	0.5	g
	Cinnamon	0.5	g
	Nigella	0.5	g

Reagents. Folin-Ciocalteu solution from Sigma-Aldrich, ≥98.0% Gallic acid standard from Sigma-Aldrich, 10% sodium carbonate solution from Supelco, ultra-pure water obtained using the ULTRACLEAR UV UF EVOQUA Purification system (Pittsburgh, PA, USA).

Maceration. After weighing all the ingredients, they were homogenized with the help of the laboratory mixer and placed in brown glass containers with lids. Each recipe was left to macerate for 24 h and 48 h, at ambient temperature (21°C) protected from light.

Folin-Ciocalteu analysis. After the maceration period, the samples were centrifuged (Hettich, Germania) and the supernatant was analysed. 5 ml of distilled water, 1.5 ml of sodium carbonate solution (10%), 0.5 ml of sample and 0.5 ml of Folin-Ciocalteu solution were pipetted into a 10 ml graduated flask. The flasks filled with ultrapure water up to the 10 ml mark and then left for 45 minutes at room temperature, after which they were measured at a wavelength of 765 nm against a blank solution using the UV-VIS Spectrometer Lambda 25, Perking Elmer. The

measurements were compared with a gallic acid calibration curve (25, 50, 100, 250 ppm), and the results were expressed in gallic acid equivalent. All of the samples were analysed three times to ensure the reliability of the results.

Sensory analysis. A basic scale was used to define sensory characteristics (table 2).

Tabel 2

Scale used for sensory characteristics

Criterion name	Scala		
Color	Light	Medium	Dark
Taste	Unpleasant	Neutral	Pleasant
Smell	Unpleasant	Neutral	Pleasant
Acidity	Low	Medium	High

10 persons form the laboratory (5 males and 5 females, with ages between 26-40) have evaluate the recipes after the two maceration periods using the above mention scale. The most commune attribute from the 10 evaluations for each category was taken as the result.

RESULTS AND DISCUSSIONS

The total polyphenols contents in the samples are presented in table 3 and figure 1. The data shown are mean values ±SE.

Tabel 3

Total polyphenols content in the samples

Crt. No	Sample name	Total polyphenols mg /ml GAE	
		24 h	48 h
1.	Recipe 1	187.23±1.9	195.24±1.9
2.	Recipe 2	304.71±2.8	356.24±3.8
3.	Recipe 3	344.83±3.1	407.95±4.2
4.	Recipe 4	321.01±3.7	375.81±3.5
5.	Recipe 5	289.63±2.5	321.57±3.1

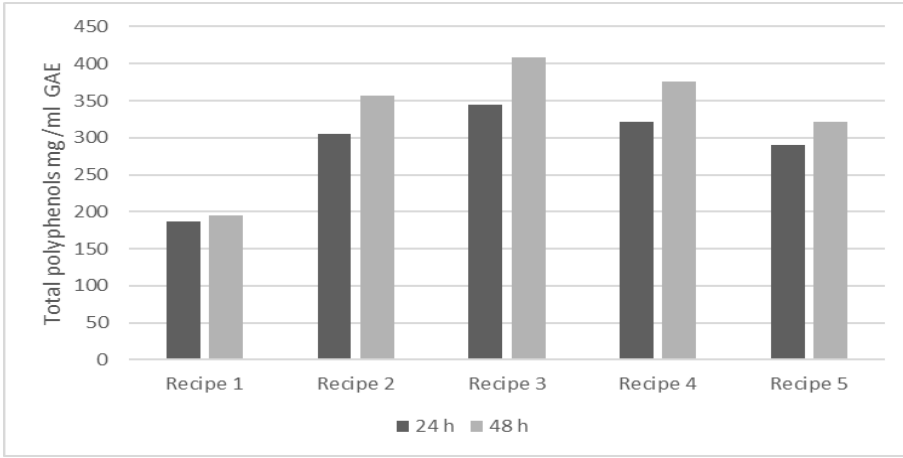

Figure 1. Total polyphenols content in the samples

The quantification of total polyphenols in the samples revealed notable variations between the different recipes and maceration durations. As shown in Table 2 and Figure 1, the total polyphenols content increased in most recipes over the 48-hour maceration period. Recipe 3 (Cinnamon) exhibited the highest total polyphenols content, with a mean of 407.95 ± 4.2 mg/ml of Gallic Acid Equivalent (GAE) after 48 hours. Notably, Recipe 2 (Sage) and Recipe 4 (Nigella) also demonstrated substantial polyphenolic content, with mean values of 356.24 ± 3.8 mg/ml GAE and 375.81 ± 3.5 mg/ml GAE, respectively, after 48 hours of maceration. These results suggest that the plant materials employed in these recipes contributed significantly to the polyphenolic content of the macerates.

The sensory analysis, conducted by a panel of 10 evaluators, aimed to discern the attributes of color, taste, smell, and acidity in the various recipes at both 24-hour and 48-hour maceration intervals. (Tabel 4)

Tabel 4

Sensorial analysis of samples

Crt. No.	Sample name	Maceration duration	Color	Taste	Smell	Acidity
1.	Recipe 1	24 h	Dark	Pleasant	Pleasant	Medium
		48 h	Dark	Unpleasant	Pleasant	Medium
2.	Recipe 2	24 h	Dark	Pleasant	Pleasant	Medium
		48 h	Dark	Pleasant	Pleasant	Medium
3.	Recipe 3	24 h	Medium	Pleasant	Pleasant	Medium
		48 h	Medium	Pleasant	Pleasant	Medium
4.	Recipe 4	24 h	Dark	Neutral	Neutral	Medium
		48 h	Dark	Unpleasant	Neutral	Medium
5.	Recipe 5	24 h	Dark	Pleasant	Pleasant	Medium
		48 h	Dark	Unpleasant	Pleasant	Medium

Notable trends emerged in terms of sensory characteristics:

- Color: Across all recipes, the macerates tended to exhibit a darker color after the 48-hour maceration period, suggesting increased extraction of bioactive compounds.
- Taste and Smell: Recipe 2 (Sage) was consistently rated as having a pleasant taste and smell after both maceration intervals, indicating its potential for enhanced palatability.
- Acidity: The acidity levels, represented by the attribute of acidity in the sensory analysis, remained relatively consistent across most recipes and maceration periods, with a medium level of acidity being dominant.

CONCLUSIONS

The findings of this study hold implications for diabetes management, as polyphenols have been associated with potential antidiabetic effects. The substantial content of total polyphenols in the plant macerates, especially in recipes involving Cinnamon, Sage, and Nigella, indicates that these formulations could contribute to modulating blood glucose levels and mitigating the effects of diabetes. Furthermore,

the sensory analysis results suggest that Recipe 2 (Sage) has the potential to offer a balance between desirable taste and smell attributes, which could enhance patient compliance in incorporating these formulations into their dietary routines. In conclusion, the study demonstrated the variation in total polyphenols content among different plant macerates of honey-infused apple cider vinegar. Recipes incorporating Cinnamon, Sage, and Nigella exhibited notable polyphenolic content after the maceration process. Sensory analysis indicated that Recipe 2 (Sage) stood out in terms of taste and smell attributes. These findings underscore the potential of these formulations as dietary adjuncts for diabetes management, warranting further investigation into their specific bioactive profiles and antidiabetic mechanisms.

Acknowledgments. Project SMIS code 151840, belonging to S.C. Rom Honey Group S.R.L., financed by the Regional Operational Program 2014-2020, Priority Axis 1 – Promotion of technological transfer, Investment Priority 1.1., Operation 1.1.C. The financing contract was concluded with the Ministry of Development, Public Works and Administration as the Management Authority and with the Intermediate Body South Muntenia Regional Development Agency, as the Intermediate Body.

REFERENCES

1. Williamson G, Kay CD, Crozier A. The bioavailability, transport, and bioactivity of dietary flavonoids: a review from a historical perspective. Comprehensive Reviews in Food Science and Food Safety. 2018;17(5):1054-1112.
2. Budak NH, Aykin E, Seydim AC, Greene AK, Guzel-Seydim ZB. Functional properties of vinegar. Journal of Food Science. 2014;79(5):R757-R764.
3. Eteraf-Oskouei T, Najafi M. Traditional and modern uses of natural honey in human diseases: a review. Iranian Journal of Basic Medical Sciences. 2013;16(6):731-742.
4. Oboh G, Olasehinde TA, Ademosun AO, et al. Essential oil from lavender (Lavandula angustifolia) attenuates haloperidol-induced orofacial dyskinesia: Possible mechanism of action. Metabolic Brain Disease. 2020;35(2):365-374.
5. Karimi J, Sheikh N, Makhdoomi K, et al. Salvia officinalis L. for diabetes: A double-blind, placebo-controlled clinical trial. Journal of Ethnopharmacology. 2020;248:112319.
6. Anderson RA, Broadhurst CL, Polansky MM, et al. Isolation and characterization of polyphenol type-A polymers from cinnamon with insulin-like biological activity. Journal of Agricultural and Food Chemistry. 2004;52(1):65-70.
7. Randhawa MA, Alenazi NA, Al Matroudi A, Alenazi MM, Alsabaani NA. Nigella sativa L. supplementation: Outcome in diabetes and functionality as a potential therapeutic option. Journal of Diabetes and Metabolic Disorders. 2020;19(2):1573-1581.
8. Buchbauer, G., et al. (1993). "Aromatherapy: evidence for sedative effects of the essential oil of lavender after inhalation." Zeitschrift für Naturforschung C, 48(11-12), 1067-1072.
9. Sebai, H., et al. (2013). "Lavender (Lavandula stoechas L.) essential oils attenuate hyperglycemia and protect against oxidative stress in alloxan-induced diabetic rats." Lipids in health and disease, 12(1), 189.
10. Elshafie, H. S., et al. (2017). "An Overview on the Antioxidant Activity of Lavender Species and Their Potential Role in Oxidative-Stress-Related Diseases." Medicina, 55(9), 453.

11. Eidi, A., Eidi, M., & Esmaeili, E. (2005). "Antidiabetic effect of garlic (Allium sativum L.) in normal and streptozotocin-induced diabetic rats." Phytomedicine, 12(10), 624-629.
12. Lima, C. F., et al. (2006). "Metformin-like effect of Salvia officinalis (common sage): is it useful in diabetes prevention?" British Journal of Nutrition, 96(2), 326-333.
13. Behradmanesh, S., Derees, F., & Rafieian-Kopaei, M. (2013). "Effect of Salvia officinalis on diabetic patients." Journal of Renal Injury Prevention, 2(2), 51-54.
14. Khan, A., et al. (2003). "Cinnamon improves glucose and lipids of people with type 2 diabetes." Diabetes Care, 26(12), 3215-3218.
15. Mang, B., et al. (2006). "Effects of a cinnamon extract on plasma glucose, HbA, and serum lipids in diabetes mellitus type 2." European Journal of Clinical Investigation, 36(5), 340-344.
16. Davis, P. A., & Yokoyama, W. (2011). "Cinnamon intake lowers fasting blood glucose: meta-analysis." Journal of Medicinal Food, 14(9), 884-889.
17. Ahmad, A., et al. (2013). "A review on therapeutic potential of Nigella sativa: A miracle herb." Asian Pacific Journal of Tropical Biomedicine, 3(5), 337-352.
18. Fararh, K. M., et al. (2002). "Thymoquinone reduces hepatic glucose production in diabetic hamsters." Research in Veterinary Science, 73(3), 279-282.
19. Benhaddou-Andaloussi, A., et al. (2010). "The in vivo antidiabetic activity of Nigella sativa is mediated through activation of the AMPK pathway and increased muscle Glut4 content." Evidence-Based Complementary and Alternative Medicine, 2011.
20. Alimohammadi, S., et al. (2013). "Effect of Nigella sativa oil on various clinical and biochemical parameters of the metabolic syndrome." International Journal of Diabetes in Developing Countries, 33(3), 190-196.