ANTIOXIDANT CAPACITY OF PLANT MACERATES OF HONEY INFUSED APPLE CIDER VINEGAR WITH APPLICATION IN WEIGHT LOST MANAGEMENT

Andra NICHITEAN[1,2], Carmen Daniela PETCU[1], Florin OANCEA[2,3], Cristina MATEESCU[4], Anca BECZE[5]

e-mail: carmenpetcufmvb@gmail.com

Abstract

The growing prevalence of obesity and chronic diseases has heightened interest in the potential health benefits of natural antioxidants. This study aims to explore the antioxidant capacities of various recipes of honey-infused apple cider vinegar macerates containing plant extracts. Eight different recipes were formulated using apple cider vinegar, acacia honey, and a variety of plant extracts including ginger, green tea, dandelion root, and chicory root. The mixtures were allowed to macerate for 24 and 48 hours at room temperature (21°C), followed by centrifugation and antioxidant capacity analysis using a specialized ACW kit and a PHOTOCHEM device. The antioxidant capacities varied significantly among the eight recipes, ranging from 0.25±0.001 to 1.88±0.008 µg/g (Equivalent Vit C). Generally, an increase in antioxidant capacity was observed from 24 to 48 hours of maceration. Recipes with multiple plant extracts showed remarkably higher antioxidant capacities, indicating potential synergistic effects. The study reveals substantial variations in antioxidant capacities among different recipes, influenced by both the type and combination of plant extracts used, as well as maceration time.

Key words: apple cider vinegar, antioxidant capacity, honey, plant macerates

The alarming increase in obesity and overweight cases worldwide has prompted an urgent call for effective weight loss management strategies (World Health Organization, 2000).

Among a plethora of natural remedies, apple cider vinegar (ACV) has gained attention for its potential weight loss benefits, such as appetite suppression and enhancing metabolism (Khezri *et al*, 2018). Similarly, honey has been credited for its antioxidant and anti-inflammatory properties (Savu *et. al*., 2002; Dobre I.R. *et. al.*, 2017; El-Seedi *et al,* 2022). The incorporation of these two elements with plant macerates can potentially amplify their individual beneficial properties, thus presenting a natural and potent solution for weight management (Talhouk *et al,* 2007).

In this context, the safety of the obtained products must be ensured (Mitrea I.S. *et. al*, 2003; Petcu C.D., 2006; Laslo C. *et. al*, 2008).

Traditional plant maceration involves steeping plant materials in a solvent, frequently ACV, to extract bioactive compounds (Abubakar *et al,* 2020; Brezeanu C. *et al,* 2022; Murariu O.C. *et al*, 2014). This process has been suggested to enhance the antioxidant capacity of the resulting mixture. However, limited studies have quantitatively assessed this effect or explored its potential applications in weight loss (Gonçalves *et al,* 2022). Moreover, foods and substances rich in antioxidants are increasingly being recognized for their role in supporting weight loss (Predescu C *et. al.*, 2020; Murariu O.C. *et al,* 2020). This is achieved by combating oxidative stress and inflammation, conditions often elevated in overweight or obese individuals (Speer *et al,* 2020).

Ginger has an underground rhizome with numerous bioactive compounds, including gingerol and shogaol, which have been the subject of

[1] University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania
[2] ROM HONEY GROUP SRL, Iasi, Romania
[3] Polymers and Bioresources Departments, National Institute for Research & Development in Chemistry and Petrochemistry—ICECHIM, Bucharest, Romania
[4] National Institute for Research& Development in Food Bioresources National Service for Medicinal and Aromatic Plants and Bee Products, Bucharest, Romania
[5] INCDO-INOE 2000, Research Institute for Analytical Instrumentation Subsidiary, Cluj-Napoca, Romania

numerous researches for their therapeutic potential (Ali *et al*, 2008). In addition to its uses in the treatment of gastrointestinal and anti-inflammatory problems, ginger has recently attracted attention for its potential in weight management (Mansour *et al*, 2012). The studies conducted indicated that ginger can play a role in inhibiting appetite, improving thermogenesis and stimulating lipolysis (Arablou *et al*, 2014). Thus, ginger extracts can be considered a potential adjunct in weight loss strategies (Maharlouei *et al*, 2019).

Green tea has been studied for a number of beneficial effects, including anti-cancer, cardioprotective and anti-inflammatory properties. In recent years, research has also focused on its role in weight management (Hursel *et al*, 2009). Studies suggest that the polyphenols in green tea can have a thermogenic effect, thus increasing the metabolic rate and favoring the oxidation of fats (Dulloo *et al*, 1999). Supplementation with green tea extract has proven effective in reducing body weight and fat mass, in combination with a diet and adequate physical exercises (Rains *et al*, 2011).

The dandelion plant is recognized for its content rich in bioactive compounds, including polyphenols, flavonoids and terpenoids, which have antioxidant and anti-inflammatory properties (Martinez *et al*, 2015). Dandelion extract has been studied for its diuretic, hepatoprotective and anti-inflammatory potential. In addition, recent research suggests that it can have a positive impact on regulating body weight and cholesterol levels (Moon *et al*, 2009). In vitro and animal studies have indicated that dandelion extract can inhibit fat accumulation and reduce LDL cholesterol levels, probably by modulating the activity of lipogenic enzymes and fatty acid transporters (Choi *et al*, 2010).

Among the studied effects of chicory are the ability to modulate the immune system, antioxidant effects and anti-inflammatory potential. Recent research suggests that chicory can play an important role in regulating lipid metabolism (Roberfroid *et al*, 2010). Studies indicate that inulin, a type of soluble fiber present in chicory, can promote satiety and slow down digestion, which contributes to weight management (Abrams *et al*, 2005).

This study seeks to explore the potential utility of plant macerates and determining their antioxidant capacity and utilizing honey-infused apple cider vinegar to produce them.

MATERIAL AND METHOD

Materials used for maceration

Following the bibliographic study, raw materials were identified which are also rich sources of polyphenols, but which also have body mass management properties demonstrated through clinical studies. The ingredients used to make the product are:

1. Apple vinegar obtained from apple cider;
2. Acacia honey;
3. Ginger - Zingiber officinale - powdered root, country of origin China;
4. Ginger extract 1:2.25 in hydroethanolic solution (alcohol ratio 96/water 45/55);
5. Green tea - Camellia sinensis – powder, country of origin China;
6. Green tea extract - green tea leaves, aqueous extract 4:1;
7. Dry extract of dandelion root - Taraxacum officinale - standardized min 5% polyphenols
8. Chicory - Cichorium intybus - chicory root powder

All products have been bought from the specialized stores in Cluj County or produced in the INCDO-INOE 2000, Research Institute for Analytical Instrumentation Subsidiary Laboratory.

The recipes developed for the study are presented in *table 1*.

Table 1

Recipes used for maceration

Recipe name	Ingredients	Quantity	Unit of measurement
Recipe 1	Apple vinegar	40	ml
	Acacia honey	2	g
	Ginger	2	g
Recipe 2	Apple vinegar	40	ml
	Acacia honey	2	g
	Green tea	2	g
Recipe 3	Apple vinegar	40	ml
	Acacia honey	2	g

Recipe name	Ingredients	Quantity	Unit of measurement
	Dry extract of dandelion root	2	g
Recipe 4	Apple vinegar	40	ml
	Acacia honey	2	g
	Chicory root powder	2	g
Recipe 5	Apple vinegar	40	ml
	Acacia honey	2	g
	Ginger extract	2	ml
Recipe 6	Apple vinegar	40	ml
	Acacia honey	2	g
	Green tea extract	2	g
Recipe 7	Apple vinegar	40	ml
	Acacia honey	2	g
	Ginger	0.5	g
	Green tea	0.5	g
	Dry extract of dandelion root	0.5	g
	Chicory root powder	0.5	g
Recipe 8	Apple vinegar	40	ml
	Acacia honey	2	g
	Ginger extract	0.5	g
	Green tea extract	0.5	ml
	Dry extract of dandelion root	0.5	g
	Chicory root powder	0.5	g

Reagents
ACW kit consisting of: standard solution of vitamin C, stock solution (Photo sensitizer and detection reagent), a buffer solution and a diluting solution, from Analitic Jena; Germany.

Maceration
After measuring out all the components, the ingredients were blended together using a lab mixer before being transferred into brown glass jars with lids. The mixtures were then allowed to macerate for periods of 24 and 48 hours at room temperature (21°C), shielded from exposure to light. The samples were then centrifuged (Hettich, Germany) at 3500 RPM for 5 minute and the supernatant was analyzed.

Antioxidant capacity analysis
The samples were analyzed using fallowing the ACW kit manufacturer's instructions and using PHOTOCHEM Analitic Jena; Germania. The instrument uses the photochemical excitation of radical formation combined with luminometric detection. Results are expressed in ascorbic acid equivalents. The following calculation method is applied:

Concentration [µg/ml] = (Q×D×M)/PV (1)

Concentration [µmol/ml] = (Q×D×)/PV (2)

where:
Q - nomol (ascorbic acid), read on the machine
D- dilution factor
M- molecular mass (ascorbic acid=176.13 ng/nmol)
PV- pipette volume in test tube

Each sample was analyzed in triplicate to ensure the accuracy of the measurement.

RESULTS AND DISCUSSIONS

The experiment used a variety of macerated recipes with different proportions of acacia honey, apple vinegar, and other plant extracts like ginger, green tea, dandelion root, and chicory root powder. The maceration process was standardized, with each recipe containing 40 ml of apple vinegar and 2 g of acacia honey, combined with various other ingredients. The total antioxidant capacity of the samples is presented in *table 2* and *figure 1*.

Table 2

Total polyphenols content in the samples*

No	Sample name	Total antioxidant capacity Equiv. Vit C µg/g	
		24 h	48 h
1.	Recipe 1	0.83±0.012	0.92±0.009
2.	Recipe 2	0.49±0.005	0.53±0.004
3.	Recipe 3	1.29±0.015	1.33±0.011
4.	Recipe 4	0.84±0.008	0.96±0.009
5.	Recipe 5	0.25±0.001	0.26±0.001
6.	Recipe 6	1.17±0.009	1.29±0.012
7.	Recipe 7	1.77±0.009	2.30±0.018
8.	Recipe 8	1.88±0.008	1.80±0.014

* The data shown are mean values ± Standard Error

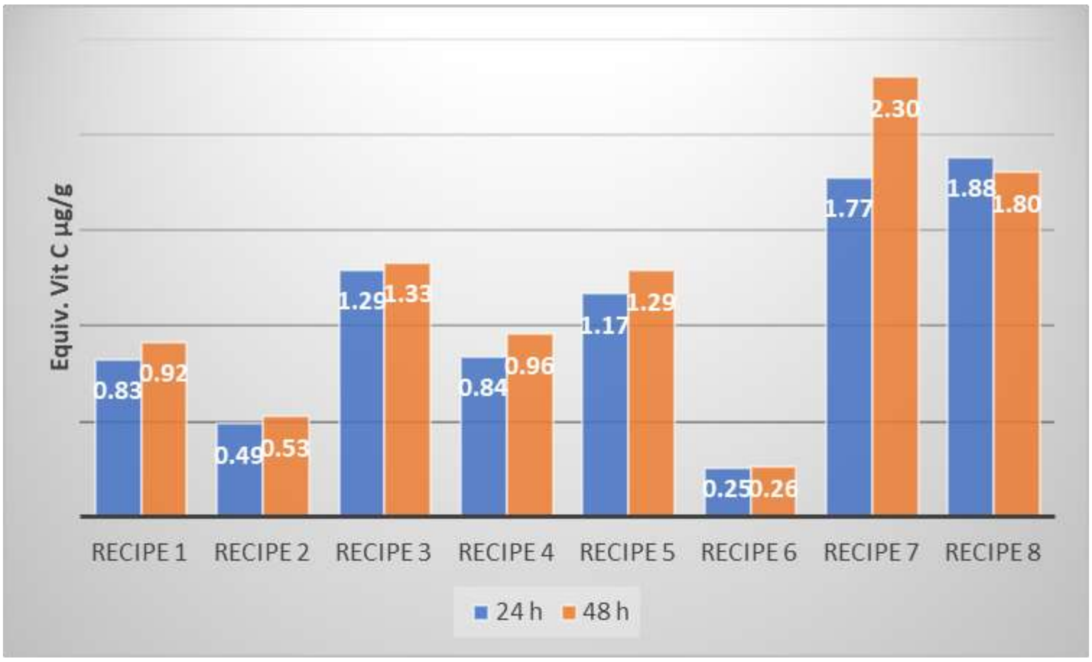

Figure 1 **Total antioxidant capacity of the samples**

The data suggests a wide range of antioxidant capacities across the eight recipes, varying from 0.25±0.001 to 1.88±0.008 µg/g. In general, there is an increase in antioxidant capacity from 24-hour to 48-hour maceration for almost all recipes except Recipe 8, which shows a slight decrease. This suggests that longer steeping time generally enhances antioxidant activity, a finding that could be due to the increased extraction of bioactive compounds over time. The highest antioxidant capacities were observed for Recipes 7 and 8, which combine multiple plant extracts. This could indicate a synergistic effect among the various bioactive compounds. Recipe 3, containing dry extract of dandelion root, also showed notably high antioxidant activity, suggesting this ingredient could be particularly rich in antioxidants. The lowest antioxidant capacity was observed in Recipe 5, containing ginger extract. That raises questions about the efficacy of ginger extract in this formulation, especially when compared to Recipe 1, which uses whole ginger and has a higher antioxidant capacity.

The standard errors associated with each antioxidant measurement are low (ranging from ±0.001 to ±0.018), which adds confidence to the reproducibility of the findings, suggesting that the method employed is robust and reliable for analyzing antioxidant capacities.

It is evident that the inclusion of different plant extracts has a profound influence on the antioxidant capacity.

CONCLUSIONS

From the studies it was found that there are significant differences between the antioxidant capacity of the products obtained using 8 recipes of macerated products that include different combinations of apple vinegar, acacia honey and plant extracts, represented by ginger, green tea, dandelion root and powder of chicory root.

The antioxidant capacity of the obtained extracts varies from 0.25±0.001 to 1.88±0.008 µg/g. In general, there is an increase in antioxidant capacity from 24-hour to 48-hour maceration for almost all recipes except recipe 8, where ginger extract and green tea extract were used, which shows a slight decrease.

The results indicate that the choice of plant extracts and the time allowed for maceration can significantly influence the antioxidant capacity of honey-infused apple vinegar mixtures. Future studies may focus on the individual bioactive compounds responsible for the observed antioxidant activity and their specific roles in weight loss management.

ACKNOWLEGMENTS

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

Abrams S.A., Griffin I.J., Liang L., Gunn S.K., Darlington G., Ellis K.J. 2005 - *A combination of prebiotic short- and long-chain inulin-type fructans enhances calcium absorption and bone mineralization in young adolescents.* The American Journal of Clinical Nutrition, 86(2), 471-476.

Abubakar A.R. & Haque, M., 2020 - *Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes.* Journal of pharmacy & bioallied sciences, 12(1), 1.

Ali B.H., Blunden G., Tanira M.O., Nemmar A., 2008 - *Some phytochemical, pharmacological and toxicological properties of ginger (Zingiber officinale Roscoe): A review of recent research.* Food and Chemical Toxicology, 46(2), 409-420.

Arablou T., Aryaeian N., Valizadeh M., Sharifi F., Hosseini A.F. & Djalali M., 2014 - *The effect of ginger consumption on glycemic status, lipid profile and some inflammatory markers in patients with type 2 diabetes mellitus.* International Journal of Food Sciences and Nutrition, 68(4), 391-398.

Choi U.K., Lee O.H. Yim J.H., Rhee Y.K., Lim S.I., Kim Y.C., 2010 - *Hypolipidemic and antioxidant effects of dandelion (Taraxacum officinale) root and leaf on cholesterol-fed rabbits*. International Journal of Molecular Sciences, 11(1), 67-78.

Dobre I. R., Marmandiu, A., Gajaila, G., Cotor, G., Ghiță, M., Fernoagă, C., 2017 - *Prevention of diseases in the beekeeping holdings–An essential condition for obtaining organic honey*. Journal of Biotechnology, Volume 256, Page S68, ISSN 0168-1656.

Dulloo A.G., Duret C., Rohrer D., Girardier L., Mensi N, Fathi M, Chantre P., Vandermanderet J., 1999 - *Efficacy of a green tea extract rich in catechin polyphenols and caffeine in increasing 24-h energy expenditure and fat oxidation in humans.* The American Journal of Clinical Nutrition, 70(6), 1040-1045.

El-Seedi H.R., Eid N., El-Wahed A.A.A., Rateb M.E, Afifi H.S, Alggethami A.F. Zhao C., 2022 - *Honey bee products: Preclinical and clinical studies of their anti-inflammatory and immunomodulatory properties.* Frontiers in Nutrition, 8, 761267.

Gonçalves L.B., Pessota C.A., Fortes T.A., 2022 - *Phytochemical evaluation and antioxidant and toxicity activities of compounded teas indicated for weight loss*. Rev Bras Plantas Med/Braz J Med Plants, 24, 81-90.

Hursel R., Viechtbauer W., Westerterp-Plantenga M.S., 2009 - *The effects of green tea on weight loss and weight maintenance: A meta-analysis.* International Journal of Obesity, 33(9), 956-961.

Moon H.S., Lee H.G., Chung C.S., Kim T.G., 2009 - *Antiobesity effect of PEGylated conjugated linoleic acid on high-fat diet-induced obese C57BL/6J (ob/ob) mice: attenuation of insulin resistance and enhancement of antioxidant defenses.* Journal of Nutritional Biochemistry, 19(4), 249-256.

Khezri S.S., Saidpour A., Hosseinzadeh N., Amiri Z., 2018 - *Beneficial effects of Apple Cider Vinegar on weight management, Visceral Adiposity Index and lipid profile in overweight or obese subjects receiving restricted calorie diet: A randomized clinical trial*. Journal of Functional Foods, 43, 95-102.

Laslo C., Ştețca G., Suharoschi R., Mureșan C, 2008 - *Controlul calității şi igiena produselor alimentare de origine animală*, Editura Risoprint, Cluj-Napoca, pp. 28-30.

Maharlouei N., Tabrizi R., Lankarani K.B., Rezaianzadeh A., Akbari M., Kolahdooz F., Rahimi M., Keneshlou F., Asemi Z., 2019 - *The effects of ginger intake on weight loss and metabolic profiles among overweight and obese subjects: A systematic review and meta-analysis of randomized controlled trials.* Critical Reviews in Food Science and Nutrition, 59(11), 1753-1766.

Mansour M.S., Ni Y.M., Roberts A.L., Kelleman M., RoyChoudhury A., St-Onge M.P., 2012 - *Ginger consumption enhances the thermic effect of food and promotes feelings of satiety without affecting metabolic and hormonal parameters in overweight men: A pilot study.* Metabolism, 61(10), 1347-1352.

Martinez M., Poirrier P., Chamy R., D. Prüfer D., Schulze-Gronover C., Jorquera L., Ruiz G., 2015 - *Taraxacum officinale and related* *species—An ethnopharmacological review and its potential as a commercial medicinal plant.* Journal of Ethnopharmacology, *169, 244-262.*

Mitrea I.S., Petcu C.D., Savu G. 2003 - *Siguranța alimentelor prin aplicarea sistemului HACCP,* Editura Bogdana, ISBN: 973-9127-18-5: 1-85 Bucureşti.

Murariu O.C., Petcu Carmen Daniela, Ghimpețeanu O.M., Irimia L.M., 2020 – The influence of the storage interval on quality of the apple stored under controlled storage condition, Lucrări Științifice, seria Agronomie, 63(1), 121 - 126.

Murariu Otilia Cristina, Irimia Liviu, Murariu Florin, Anghel Roxana, 2014 – Research on the apples quality marketed in the Moldova area from the physico- chemical and sensorial point of view, Lucrări științifice, Seria Horticultură, 57, (1), 195 – 198.

Brezeanu C., Brezeanu P.M., Stoleru V., Irimia L.M., Lipșa F.D., Teliban G.C., Ciobanu M.M., Murariu F., Puiu I., Branca F., Murariu O.C., 2022 – Nutritional value of new sweet pepper genotypes grown in organic system, Agriculture, 12(11):1863, 1-19.

Petcu Carmen Daniela, 2006 - *HACCP-Food safety guarantor***,** Idea Design, București.

Predescu C., Papuc C., Ștefan G., Petcu C., 2020 - *Phenolics content, antioxidant and antimicrobial activities of some extracts obtained from Romanian summer savory and Lebanon wild thyme.* Sci. Works Ser. C Vet. Med., LXVI, 17–22

Rains T.M., Agarwal S., Maki K.C., 2011 - *Antiobesity effects of green tea catechins: A mechanistic review.* The Journal of Nutritional Biochemistry, 22(1), 1-7.

Roberfroid M., Gibson G.R., Hoyles L., McCartney A.L., Rastall R., Rowland I., Wolvers D., Watzl B., Szajewska H., Stahl B., Guarner F., Respondek F., Whelan K., Coxam V., Davicco M.J., Léotoing L., Wittrant Y., Delzenne N.M., Cani P.D., Neyrinck A.M., Meheust A., 2010 - *Prebiotic effects: metabolic and health benefits.* British Journal of Nutrition, 104(S2), S1-S63.

Savu C. & Petcu C.D., 2002 - *Hygiene and control of products of animal origin.* Bucharest, RO: Semne Publishing House.

Speer H., D'Cunha N.M., Alexopoulos N.I., McKune A.J.,Naumovski N., 2020 - *Anthocyanins and Human Health—A Focus on Oxidative Stress, Inflammation and Disease,* Antioxidants, 9(5), 366, doi: 10.3390/antiox9050366

Talhouk R.S., Karam C., Fostok S., El-Jouni W., Barbour E.K., 2007 - *Anti-inflammatory bioactivities in plant extracts.* Journal of medicinal food, 10(1), 1-10.

https://www.who.int/activities/controlling-the-global-obesity-epidemic.