Impact of tocopheryl and retinyl acetates on oxidative stability of *Hypericum perforatum* L. and *Matricaria recutita* L. macerates in corn oil during storage

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Keywords:

Corn
Oil
Herb
Macerates
Tocopherol
Retinol
Storage
Oxidative stability

Article history:

Received 19.05.2025
Received in revised form 16.09.2025
Accepted 30.09.2025

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DOI:
10.24263/2304-974X-2025-14-3-5

Abstract

Introduction. This study examined the influence of tocopheryl and retinyl acetates on the oxidative stability of *Hypericum perforatum* L. and *Matricaria recutita* L. macerates in corn oil during storage.

Materials and methods. Refined corn oil macerated with *H. perforatum* and *M. recutita* were supplemented with tocopheryl acetate (500–1500 mg/kg) or retinyl acetate (170–690 mg/kg) and stored for six weeks at 20±2 °C and 4±1 °C. Oxidative stability was evaluated by acid value (AV), radical scavenging activity (RSA) by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay; vitamin retention by high-performance liquid chromatography, and fatty acid profile by gas chromatography.

Results and discussion. Both macerates contained ~60–62% linoleic acid, reflecting high oxidisability. Initial RSA was slightly higher in *H. perforatum* (≈40%) than in *M. recutita* (≈36%), consistent with its detectable vitamin A and higher β-carotene content. Baseline AV was 0.40 mg KOH/g. After six weeks at 20 °C, AV in untreated controls exceeded the deterioration threshold (~1.0 mg KOH/g), reaching 1.05 (*H. perforatum*) and 1.12 (*M. recutita*) mg KOH/g. Storage at 4 °C reduced AV increase by ~25–35% (controls: 0.72 and 0.79 mg KOH/g, respectively), confirming temperature as a key factor in hydrolytic/oxidative change.

Antioxidant addition produced clear, dose-dependent protection. Tocopheryl acetate was most effective across both macerates and temperatures. At 1500 mg/kg, final AVs were limited to 0.65 (*H. perforatum*) and 0.68 (*M. recutita*) at 20 °C (~35–40% lower than controls), and to 0.50–0.51 mg KOH/g at 4 °C, i.e., close to baseline and well below sensory-risk thresholds. Intermediate doses (500–1000 mg/kg) also reduced AV, with effects scaling by concentration, suggesting that chain-breaking activity of α-tocopherol esters predominated over any pro-oxidant side effects at tested levels. Retinyl acetate conferred a weaker, temperature-dependent effect. At its highest dose, AVs approached ~1.00 mg KOH/g in both macerates at 20 °C, which is better than controls but near the acceptability threshold, while at 4 °C values were 0.72 (*H. perforatum*) and 0.75 mg KOH/g (*M. recutita*). Thus, retinyl acetate alone does not ensure stability under ambient storage but can complement temperature control.

Conclusion. The combination of tocopheryl acetate at 1500 mg/kg with storage at 4 °C was most effective in preserving oxidative stability in both macerates. *H. perforatum* showed inherently greater resistance to oxidation than *M. recutita*, indicating its superior suitability as a lipid carrier in cosmetic formulations.

Introduction

Lipid oxidation is a key factor influencing the quality, safety, and shelf life of vegetable oils. The accumulation of primary (hydroperoxides) and secondary (aldehydes, ketones) oxidation products impairs sensory properties, nutritional value, and functional properties of lipid raw materials and fat-containing products (Loganathan et al., 2022; Martín-Torres et al., 2023). Recent reviews highlight the multifactorial nature of this process driven by unsaturation degree, free fatty acid content, trace metals, temperature, and oxygen exposure, and the need for validated stability assessments using peroxide value, acid value, thiobarbituric acid reactive substances, and induction period (Chabni et al., 2024; Li et al., 2019).

In cosmetic technologies, edible vegetable oils are increasingly used as emulsion components due to their biocompatibility, protective barrier properties, and the content of lipophilic bioactive substances (Hantikainen and Lagerros, 2023; Kunik et al., 2022). Recent reviews in cosmetic dermatology highlight their role in skin barrier restoration and antioxidant protection (Liu et al., 2025; McMullen, 2024), which align the quality requirements of food-grade lipids with those essential for cosmetic stability (Abdalla et al., 2024).

A particular area of interest is oil macerates obtained by infusing plant material in refined edible oils (Arellano et al., 2019). More often refined corn oil is used as the base for macerates. However, the removal of natural antioxidants during refining, together with a possible increase in free fatty acid content (introduced by plant residues), can compromise oxidative stability, thereby increasing the need for antioxidant protection (Ma et al., 2023). This correlates with data on differences in endogenous antioxidants (types and levels of tocopherols) across oils and their contribution to stability (Kim et al., 2019).

Among strategies to improve the stability of lipid systems in food and cosmetic matrices, supplementation with lipophilic antioxidants, in particular fat-soluble vitamins, plays an important role (Niki, 2014; Shahidi and Zhong, 2010; Thiele et al., 2005). Recent studies highlight the chain-breaking mechanisms of tocopherols (vitamin E) and their synergy with other antioxidants, with attention given to optimal concentrations and matrices (Athanasiadis et al., 2023; Bayram and Decker, 2023; Martínez-Senra et al., 2024). For applied purposes, accelerated antioxidant screening tests in real oil systems (Rancimat/Oxitest) remain relevant (Chabni et al., 2024).

In light of this, the present study investigates the potential to improve the oxidative stability of *Hypericum perforatum* (common name klamathweed) and *Matricaria recutita* (common name chamomile) macerates prepared in refined corn oil by supplementing them with fat-soluble vitamins. The objectives are: (i) to quantitatively assess the effect of vitamin addition on acid value dynamics and antioxidant capacity during 6 weeks of storage at 4°C and 20°C; and (ii) to establish practical parameters (concentration, storage conditions) ensuring technological reliability when incorporated into cosmetic emulsions. This approach aligns with current trends in using natural antioxidants and combines food lipid quality requirements with cosmetic stability criteria.

Materials and methods

Materials

Oil samples. Vegetable oils in the form of macerates of klamathweed (*Hypericum perforatum* L.) and chamomile (*Matricaria chamomilla* L.) were used in the study. They were prepared by infusing the plant raw material in refined, deodorised corn oil (Leko Style Ltd., Kyiv, Ukraine) (Figure 1).

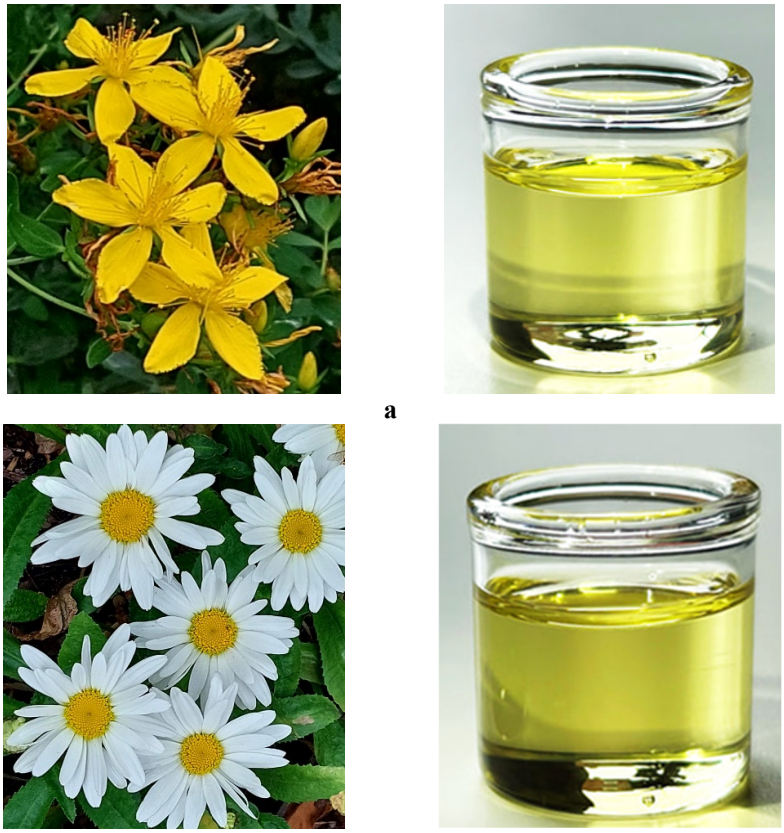

Figure 1. Images of herbs and their oil macerates:
a, ***Hypericum perforatum;*** **b,** ***Matricaria recutita***

The basic characteristics of macerates provided by the manufacturer is shown in Table 1.

Table 1

Characteristics of the herb oil macerates

Characteristics	***H. perforatum***	***M. recutita***
Acid value, mg KOH/g	0.4	0.4
Refractive index (at 20°C)	1.4730	1.4720
Density (at 20°C)	0.921	0.920
The number of KMAFAnM, CFU/mL	25	22

Note: KMAFAnM, the total number of mesophilic aerobic and facultative anaerobic microorganisms; CFU, colony-forming unit

According to the manufacturer's certificate, both macerates showed no acute skin toxicity, irritation, or sensitisation. Tocopheryl acetate (vitamin E, $C_{31}H_{52}O_3$; PrJSC Tekhnolog, Uman, Ukraine) and retinyl acetate (vitamin A, $C_{20}H_{30}O$; PJSC Vitaminy, Uman, Ukraine) were used as antioxidant additives, both meeting pharmacopeia quality standards.

Determination of oxidative stability of herbal oil macerates

Determination of acid value. Acid value (AV) was determined by titration according to ISO 660:2020 with minor modifications. An oil sample (4.0–5.0 g, ±0.01 g) was dissolved in 50 mL of ethanol–diethyl ether (1:1, v/v), and 3–5 drops of 1% phenolphthalein were added as an indicator. The mixture was titrated with 0.1 N potassium hydroxide solution (KOH) under constant stirring until a stable pale pink colour persisted for ≥30 s. AV (mg KOH/g) was calculated as**:**

$$\mathrm{AV} = 5.61\frac{V \cdot K}{m},$$

where 5.61 is titre of 0.1 N KOH solution, mg/mL;

V is volume of 0.1 N KOH solution used for titration, mL;

K is correction factor of the titre;

m is mass of the oil sample, g.

Determination of antioxidant activity. Radical scavenging activity (RSA) was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. A stock solution was prepared by dissolving 24 mg DPPH in 100 mL methanol and filtered to obtain a working solution with absorbance ~0.973 at 517 nm. For analysis, 3 mL of the DPPH working solution was mixed with 100 µL of oil (control: 100 µL methanol). Samples were incubated in darkness for 30 min, and absorbance was measured at 517 nm. Replicates (n = 4) showed a standard deviation ≤0.15. RSA (%) was calculated as:

$$\mathrm{RSA} = \left(\frac{\mathrm{A_c} - A_s}{A_c}\right) \times 100\%$$

where Ac is control reaction absorbance; As is testing specimen absorbance.

Determination of qquantitative composition of fatty acids. Fatty acid composition was analysed by gas chromatography (ISO 12966-1:2014) using a Crystal-2000M system with an Agilent DB-FFAP column (50 m × 0.32 mm × 0.50 µm). Sample volume was 1 µL; helium was used as the carrier gas. Conditions: injector 220 °C; evaporator 230 °C; detector 270 °C; isothermal programme: 60 °C (1 min), ramp to 160 °C at 20 °C/min (hold 1 min), then to 250 °C at 5 °C/min (hold 15 min). Fatty acid methyl esters were identified with a 37-component FAME Mix (Supelco), and chromatograms were processed using HP ChemStation software.

Determination of vitamin A, vitamin E and β-carotene content. The content of vitamin A (retinol and its esters), vitamin E (tocopherols), and β-carotene was determined by high-performance liquid chromatography (HPLC). Sample preparation: 0.50±0.01 g of oil sample was dissolved in 10 mL of n-hexane and filtered through a 0.45 µm membrane filter. Chromatographic conditions: Agilent 1260 Infinity system (Agilent Technologies, USA); Zorbax Eclipse XDB-C18 column, 250 × 4.6 mm, 5 µm; mobile phase: methanol: water (95:5, v/v); isocratic elution; flow rate: 1.0 mL/min; column temperature: 25°C; injection volume: 20 µL. Detection: 325 nm for retinol (vitamin A); 292 nm for α-tocopherol (vitamin E); 450 nm for β-carotene.

Quantitative determination was carried out by the external standard method using standard samples of retinyl acetate, α-tocopheryl acetate, and β-carotene (Sigma-Aldrich, USA, purity ≥ 97%). Results were expressed in mg/100 g of product.

Statistical analysis. The statistical analysis of the results was carried out using Microsoft Excel. All determinations were performed in triplicate. Values are expressed as mean±standard deviation (SD).

Results and discussion

Fatty acid composition of the corn oil macerates with *Hypericum perforatum* and *Matricaria recutita*

Fatty acid composition is a key determinant of both the nutritional and technological value of vegetable oils, as well as their oxidative stability during storage (Kim et al., 2022). Table 2 summarises the content of individual fatty acids in the corn oil macerates with *Hypericum perforatum* and *Matricaria recutita.*

Table 2

Fatty acid composition of the corn oil macerates

Fatty acid	Content,% of total amount, in corn oil macerates with	
	H. perforatum	*M. recutita*
C14:0 Myristic	0.07±0.01	0.07±0.01
C16:0 Palmitic	6.46±0.03	6.57±0.03
C16:1 Palmitoleic	0.13±0.01	0.11±0.01
C17:0 Margaric	0.03±0.01	0.04±0.01
C17:1 Margaroleic	0.02±0.01	0.02±0.01
C18:0 Stearic	3.40±0.02	3.36±0.02
C18:1 Oleic	26.24±0.10	27.67±0.10
C18:2 Linoleic	61.99±0.15	60.11±0.15
C18:3 Linolenic	0.09±0.01	0.26±0.01
C20:0 Arachidic	0.25±0.01	0.36±0.01
C20:1n11 Gadoleic	0.20±0.01	0.32±0.01
C22:0 Behenic	0.71±0.01	0.78±0.01
C22:2 Docosadienoic	–	0.08±0.01
C24:1 Nervonic	0.26±0.01	0.26±0.01
Unidentified	0.41	0.25
SFA	10.92	11.18
USFA	88.67	88.57
USFA/SFA	8.12	7.92

Note: Indicates the standard deviation of the data, n = 3; SFA, saturated fatty acid; USFA, unsaturated fatty acid.

According to the obtained data (Table 2), both macerates were dominated by polyunsaturated fatty acids, particularly linoleic acid (C18:2), which accounted for 61.99% in *Hypericum* macerate and 60.11% in *Matricaria* macerate. A considerable proportion was also represented by monounsaturated oleic acid (C18:1) – 26.24% and 27.67%, respectively. The content of saturated fatty acids was relatively low; among them, palmitic acid (C16:0) prevailed at 6.46 – 6.57%, followed by stearic acid (C18:0) at 3.36 – 3.40%. Other saturated acids (myristic, margaric, arachidic, and behenic) were present in amounts below 1%. The *Matricaria* macerate contained a slightly higher proportion of linolenic acid (C18:3) – 0.26% compared with 0.09% in *Hypericum* macerate, and uniquely included docosadienoic acid (C22:2) at 0.08%.

Thus, both samples were characterised by a high proportion of polyunsaturated fatty acids, dominated by linoleic acid, which corresponds to the fatty acid profile of corn oil used as the maceration base (Arellano et al., 2019). The high polyunsaturated fatty acids (PUFA) content, particularly linoleic acid (≈60 – 62%) together with the presence of linolenic acid, indicates increased susceptibility of the studied macerates to oxidative degradation during storage (Shahidi and Zhong, 2010). This profile underlines the need for protective measures, including antioxidant supplementation and optimal storage conditions, which became the focus of the present study.

The predominance of linoleic acid confirms that both macerates inherit the lipid profile of corn oil, the solvent used for maceration. However, the presence of minor differences, such as the higher linolenic acid content and unique docosadienoic acid fraction in *Matricaria* macerate, may explain its relatively lower oxidative stability observed later in storage experiments. Similar relationships between PUFA enrichment and reduced stability have been reported for soybean and sunflower oils, where linolenic acid, even at concentrations below 1%, acts as a strong pro-oxidant trigger (Shahidi and Zhong, 2010). From a formulation perspective, this suggests that *Hypericum* macerates may serve as a more robust lipid carrier in cosmetic emulsions than *Matricaria*, unless additional antioxidant measures are applied.

Antioxidant capacity of the corn oil macerates with *Hypericum perforatum* and *Matricaria recutita*

Antioxidant activity of oil systems is an important indicator of their potential to slow oxidative processes during storage and in finished products (Abdalla et al., 2024). It reflects the ability of the lipid fraction to neutralize free radicals generated through auto-oxidation, thereby preserving the functional and sensory properties of the product (Wang et al., 2023). For the *H. perforatum* macerates, radical scavenging activity (RSA) determined by the DPPH assay was 40%, whereas the M. recutita macerate showed a lower value, approximately 36%. This difference can be attributed to variations in the composition of biologically active compounds extracted into the oil phase during maceration, particularly phenolic constituents and pigments with antioxidant properties (Harhaun et al., 2020). The higher RSA observed in the *H. perforatum* macerate indicates its greater potential to stabilize the lipid phase, making it more promising for extending the shelf life of products formulated on its basis.

This finding is consistent with the broader evidence that plant-derived antioxidants, particularly phenolic compounds and carotenoids, contribute significantly to the radical scavenging activity of herbal oils. The slightly higher RSA values in *H. perforatum* macerate suggest that its natural antioxidant profile may complement the effect of added antioxidants during storage. In contrast, the lower intrinsic RSA of *M. recutita* macerate underlines its greater susceptibility to oxidative changes and highlights the need for additional stabilisation strategies.

Vitamin A and β-carotene content in of the corn oil macerates with *Hypericum perforatum* and *Matricaria recutita*

The contents of fat-soluble vitamin A (retinol), β-carotene, and individual isomers of vitamin E (tocopherols) in *Hypericum perforatum* and *Matricaria recutita* macerates are shown in Table 3.

Table 3

Content of fat-soluble vitamins in the corn oil macerates

Vitamin	Corn oil macerate with	
	H. perforatum	***M. recutita***
Vitamin A (retinyl acetate), mg/kg	0.78	n.a
β-carotene, mg/kg	0.49	0.26
Vitamin E (tocopheryl acetate), mg/kg:		
δ-tocopherol	7.33	8.09
γ-tocopherol	15.10	15.10
α-tocopherol	514.00	509.00

Note: n.a., not applicable.

Vitamin A in the form of retinol was detected only in the *H. perforatum* macerate (0.78 mg/kg), whereas in the *M. recutita* macerate its concentration was below the detection limit of the method. The β-carotene content was 0.49 mg/kg in *H. perforatum* (klamathweed) and 0.26 mg/kg in *M. recutita* (chamomile), indicating a limited provitamin A potential in both macerates.

In the tocopherol profile of both samples, α-tocopherol predominated (over 500 mg/kg), while the γ- and δ-isomers were present in much smaller amounts (15.1 and 7.33 – 8.09 mg/kg, respectively).

These results suggest that the presence of β-carotene and high levels of α-tocopherol in the investigated macerates may play an important role in slowing oxidative processes and supporting quality preservation during storage. At the same time, the relatively low concentrations of γ- and δ-tocopherols indicate a potential need for additional antioxidant supplementation to prolong product shelf life (Niki, 2014).

These findings align with existing evidence that α-tocopherol is the primary antioxidant component in most refined vegetable oils and provides strong radical scavenging activity. However, the absence of detectable retinol in *M. recutita* macerate and the relatively low β-carotene levels in both samples point to a limited provitamin contribution, which may not be sufficient for long-term stabilisation under ambient storage. The predominance of α-tocopherol suggests that these macerates already possess a significant inherent antioxidant potential, but the lack of diversity in the tocopherol profile could reduce resilience against prolonged oxidative stress. Therefore, fortification with additional antioxidants or the use of controlled storage conditions may be required to ensure oxidative stability over an extended shelf life.

Changes in the acid value of the corn oil macerates with *Hypericum perforatum* and *Matricaria recutita* under the influence of fat-soluble vitamin supplementation

In this study, the effect of fat-soluble vitamin supplementation on the acid value of klamathweed (*Hypericum perforatum*) and chamomile (*Matricaria recutita*) macerates was investigated. Vitamin E was applied at concentrations of 500, 1000, and 1500 mg/kg, and vitamin A at 170, 340, and 690 mg/kg. The samples were stored under two temperature regimes – 20±2°C (ambient) and 4±1°C (refrigerated). Acid value was measured weekly over a period of six weeks (Figure 3, 4).

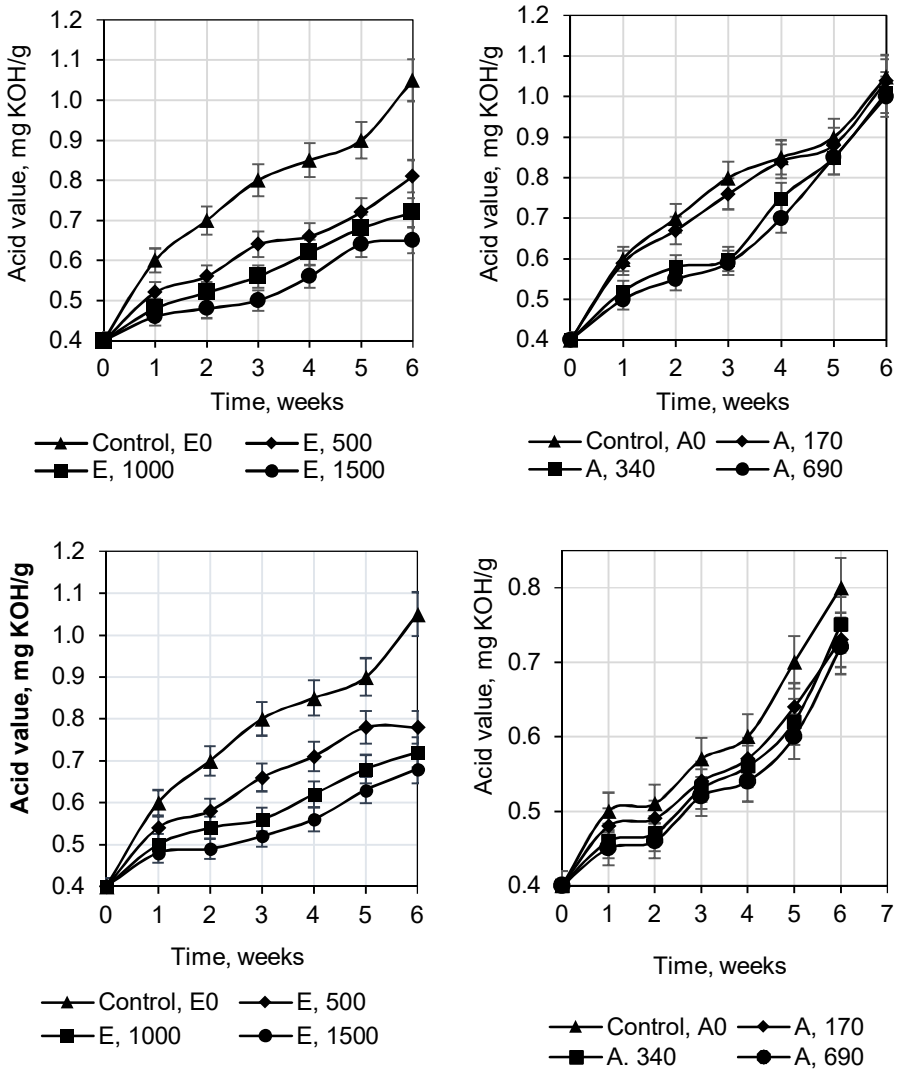

Figure 3. Changes in acid value of *Hypericum perforatum* macerates during 6-week storage with fat-soluble vitamins.
Vitamin E, mg/kg: ▲ – 0 (control); ◆ – 500; ■ – 100; ● - 1500 at 20oC (a) and 4 °C (c);
Vitamin A, mg/kg: ▲ – 0 (control); ◆ – 170; ■ – 340; ● - 690 at 20°C (b) and 4 °C (d).

The selected concentration ranges were justified by previously published studies (Kunik et al., 2016) and safety considerations: the lower levels correspond to doses recommended for slowing oxidative processes in vegetable oils without affecting organoleptic properties, while the upper levels are close to the maximum permitted concentrations used in food and cosmetic fat-based products to extend shelf life (Syed, 2016). This approach allowed the evaluation of the effectiveness of vitamins as antioxidants under realistic production conditions.

Figure 3 shows the changes in the acid value of *Hypericum perforatum* macerates during six weeks of storage at 20±2°C and 4±1°C with the addition of fat-soluble vitamins E and A at different concentrations. In the control samples (without supplementation) stored at ambient temperature, acid value increased most intensively, exceeding 1.0 mg KOH/g by week six, with an average rate of 0.0964 mg KOH/g per week. Lowering the storage temperature to 4 °C significantly slowed both hydrolytic and oxidative processes across all sample groups: even in the controls, final acid values were 25–35% lower compared with those at 20°C.

The addition of vitamin E at concentrations of 500–1500 mg/kg produced a pronounced antioxidant effect, reducing the rate of acid value increase, with the greatest protection observed at 1500 mg/kg. The effect was evident at both ambient and refrigerated storage, though at 4 °C the increase in acid value remained minimal regardless of dose. Vitamin A at 170–690 mg/kg also reduced the accumulation of free fatty acids, but its protective action was less pronounced than that of vitamin E, particularly at 20°C. This may be attributed to the lower stability and weaker antioxidant capacity of vitamin A compared with tocopherols. Under refrigeration (4 °C), the differences between doses of vitamin A were marginal, and final acid values generally did not exceed 0.6 mg KOH/g.

Thus, the combination of refrigeration and supplementation with vitamin E at 1500 mg/kg proved to be the most effective strategy for slowing oxidative degradation of Hypericum perforatum macerates.

In *Matricaria recutita* macerates, acid value also increased over the six-week storage period (Figure 4) at both 20°C and 4°C, but the rate of accumulation was higher than in Hypericum perforatum. This indicates a somewhat lower inherent resistance of chamomile macerates to oxidative processes (Li et al., 2019). At ambient temperature, control samples exceeded 1.0 mg KOH/g by the end of the trial, whereas the corresponding increase in H. perforatum was less pronounced.

The addition of tocopheryl acetate at 500–1500 mg/kg significantly slowed the rise in acid value, with the strongest effect again observed at the maximum concentration. Retinyl acetate also reduced the rate of oxidative change, but its action was consistently weaker than that of tocopheryl acetate, in line with the results for *H. perforatum* (Figure 3). Lowering the storage temperature to 4°C further improved the stability of M. recutita macerates in all series; however, even under refrigeration their acid values rose faster than in the corresponding H. perforatum samples. This confirms that the chemical composition of the raw plant material – in particular, the content and profile of endogenous antioxidants – plays a decisive role in the oxidative stability of macerates (Abdalla et al., 2024).

The results clearly demonstrate that both the type of antioxidant and the storage temperature had a decisive influence on the acid value dynamics of the studied macerates. Tocopheryl acetate consistently provided the strongest protective effect, in agreement with previous findings on its chain-breaking activity in vegetable oils (Shahidi and Zhong, 2010). Its effectiveness was dose-dependent, with the highest concentration (1500 mg/kg) maintaining acid values well below the critical threshold during six weeks of storage. Retinyl acetate, although beneficial, showed weaker activity, which may be related to its lower oxidative stability and different radical scavenging mechanisms.

The greater increase in acid value in *M. recutita* compared with *H. perforatum* supports the idea that intrinsic plant-derived antioxidants, such as β-carotene and retinol (found in H. perforatum), play a significant role in lipid stability. Nevertheless, refrigeration alone already slowed acid value growth by 25–35%, emphasising temperature as a key determinant of oxidative and hydrolytic stability.

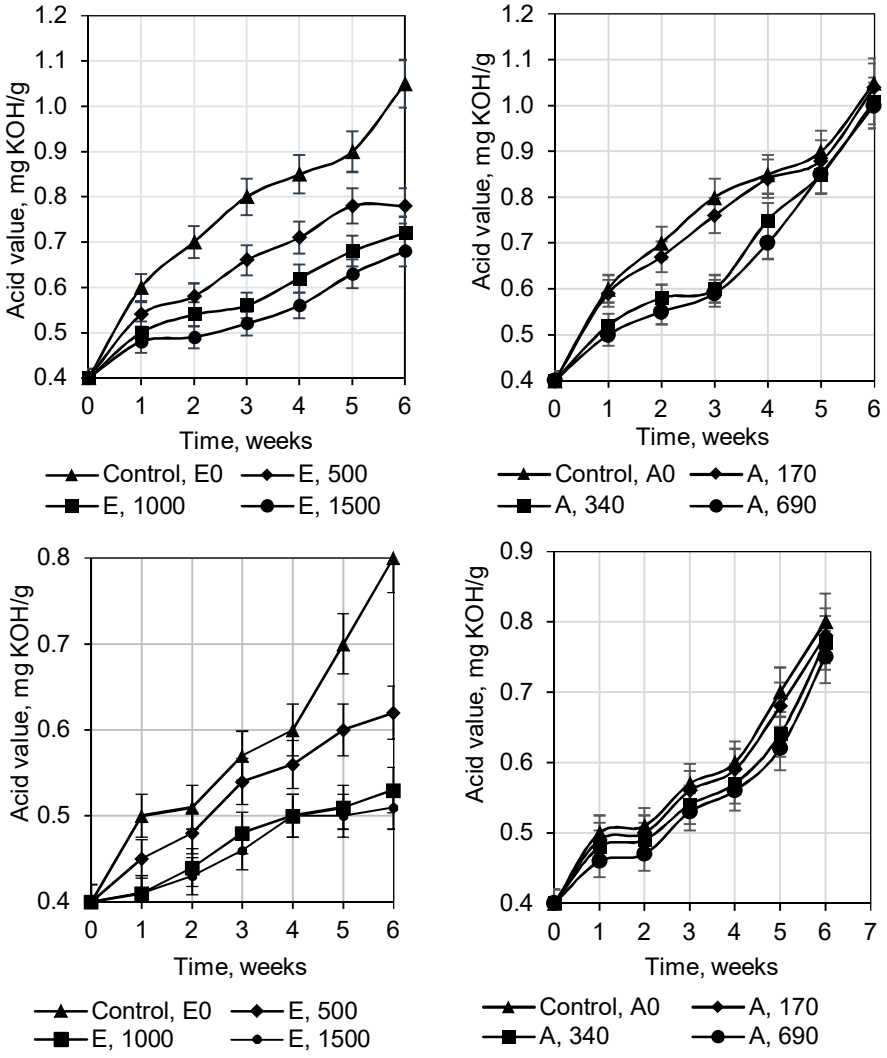

Figure 4. Changes in acid value of *Matricaria recutita* macerates during 6-week storage with fat-soluble vitamins.
Vitamin E, mg/kg: ▲ – 0 (control); ◆ – 500; ■ – 100; ● - 1500 at 20°C (a) and 4°C (c); vitamin A, mg/kg: ▲ – 0 (control); ◆ – 170; ■ – 340; ● - 690 at 20°C (b) and 4°C (d).

From a practical perspective, these results highlight that combining cold storage with tocopheryl acetate supplementation at 1500 mg/kg provides a robust strategy for prolonging the shelf life of herbal macerates. Such an approach is directly applicable to cosmetic emulsions and other formulations where oxidative stability is a prerequisite for product quality and consumer safety.

Conclusions

Macerates of *Hypericum perforatum* and *Matricaria recutita* in refined corn oil contained high levels of linoleic acid (~60–62%), rendering them highly prone to oxidation. Initial radical scavenging activity (RSA) was higher in corn oil macerate with *H. perforatum* (~40%) than with *M. recutita* (~36%), reflecting its greater β-carotene and vitamin A content. After six weeks at 20 °C, acid values in controls exceeded 1.0 mg KOH/g, while refrigeration at 4 °C reduced this increase by 25–35%. Tocopheryl acetate (500–1500 mg/kg) significantly delayed acid value increase, with the strongest effect at 1500 mg/kg, particularly at 4 °C. Retinyl acetate (170–690 mg/kg) also conferred protection but was consistently less effective, especially at 20 °C. Overall, *H. perforatum* macerates were more oxidation-resistant than *M. recutita*, and the combination of refrigeration with 1500 mg/kg tocopheryl acetate provided the most effective stabilization strategy.

Acknowledgment. This publication is connected to a research project funded by the German National Academy of Sciences Leopoldina under a Leopoldina Ukraine Distinguished Fellowship.

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Cite:

UFJ Style

Kunyk O., Leal W., Pasichniy V., Marynin A., Stabnikova O. (2025), Effect of tocopheryl and retinyl acetates on oxidative stability of *Hypericum perforatum* L. and *Matricaria recutita* L. macerates in corn oil during storage, *Ukrainian Food Journal*, 14(3), pp. 420–432, https://doi.org/10.24263/2304-974X-2025-14-3-5

APA Style

Kunyk, O., Leal, W., Pasichniy, V., Marynin, A., & Stabnikova, O. (2025). Effect of tocopheryl and retinyl acetates on oxidative stability of *Hypericum perforatum* L. and *Matricaria recutita* L. macerates in corn oil during storage. *Ukrainian Food Journal*, *14*(3), 420–432. https://doi.org/10.24263/2304-974X-2025-14-3-5