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Response surface methodology-optimized ultrasound-assisted extraction of zeaxanthin and lutein from maize grain and husk, beet tops, and bilberry leaves

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ABSTRACT

Lutein and zeaxanthin are valuable xanthophyll carotenoids with recognized antioxidant activity and important roles in ocular protection. The development of efficient and sustainable extraction methods from underutilized plant materials is therefore of considerable interest for food, nutraceutical, and pharmaceutical applications. In this study, ultrasound-assisted extraction (UAE) was optimized for the recovery of lutein- and zeaxanthin-containing extracts from selected agricultural byproducts, including maize grain and husk fractions, beet tops, and bilberry leaves/berries. Response surface methodology with a central composite design was applied to evaluate the effects of extraction temperature, extraction time, and solid-to-solvent ratio on carotenoid recovery. Conventional solvent extraction was used as a control. The optimized UAE process substantially reduced extraction time from 3–4 h to 35–40 min and increased the recovery of target bioactive compounds by approximately 30–42% compared with conventional extraction. The improvement was attributed to ultrasound-enhanced matrix disruption, improved solvent penetration, and intensified mass transfer. Functional and technological characterization showed that ultrasound treatment decreased bulk density and increased water-holding capacity, oil-holding capacity, swelling capacity, and hydration-related properties of the plant powders, indicating improved accessibility of the plant matrix. FTIR spectroscopy confirmed the presence of characteristic functional groups associated with xanthophyll-containing extracts, including O–H, aliphatic C–H, conjugated C=C, and C–O vibrations, without evidence of pronounced structural degradation under the selected extraction conditions. The results demonstrate that UAE is an efficient, rapid, and environmentally favorable approach for producing carotenoid-rich extracts from locally available agro-industrial residues. This method may support the valorization of plant byproducts into functional ingredients for nutraceutical, food, cosmetic, and potential ophthalmological applications.

Keywords: zeaxanthin; lutein; ultrasonic-assisted extraction; plant by-products; response surface methodology; antioxidant activity; sustainable extraction.
Article type: Research Article.

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INTRODUCTION

In an era of escalating global health challenges driven by aging populations and lifestyle factors, nutritional interventions have emerged as a cornerstone of preventive strategies against degenerative diseases, particularly those affecting ocular health (Chew *et al.* 2013; Lieblein-Boff *et al.* 2015). Age-related macular degeneration (AMD) stands out as a leading cause of irreversible vision loss in individuals over 50 years, contributing significantly to global blindness burden. Recent estimates from the Global Burden of Disease Study indicate that vision impairment due to AMD affected approximately 8.06 million people worldwide in 2021 (95% UI: 6.71–9.82 million), more than doubling from 3.64 million cases in 1990 (GBD 2021 Vision Loss Collaborators; Li *et al.* 2025). Projections suggest this number could rise dramatically to 21–22 million by 2050, driven by demographic aging, with higher prevalence in females and in high-income regions (GBD 2021 Vision Loss Collaborators; Wong *et al.* 2014). AMD imposes substantial socioeconomic costs through reduced quality of life, healthcare expenditures, and productivity losses, underscoring the urgent need for effective prevention strategies (Tao M *et al.* 2025). Lutein and zeaxanthin, two isomeric xanthophyll carotenoids, play a pivotal protective role in the retina. These compounds selectively accumulate in the macula lutea, forming the macular pigment that filters harmful high-energy blue light (400–500 nm) and quenches reactive oxygen species (ROS), thereby mitigating oxidative stress and inflammation central to AMD pathogenesis (Stringham *et al.* 2024; Liu *et al.* 2024). Epidemiological and clinical evidence consistently links higher dietary intake or serum levels of lutein and zeaxanthin to reduced risk of AMD progression and improved visual function (Age-Related Eye Disease Study 2 Research Group 2013; Kulczyński *et al.* 2019). The Age-Related Eye Disease Study 2 (AREDS2) demonstrated that supplementation with 10 mg lutein + 2 mg zeaxanthin daily reduced the progression to advanced AMD by ~10–25% in at-risk individuals (Madhavan *et al.* 2018). Recent meta-analyses and cohort studies further support that intakes of 6–10 mg day^{-1} lutein/zeaxanthin correlate with 20–43% lower AMD risk, with lutein often exerting stronger effects on biological aging pathways, telomere maintenance, and inflammation suppression (Goula *et al.* 2017; Bozinou *et al.* 2021; Coelho *et al.* 2022). Despite these benefits, average dietary consumption remains suboptimal. In Western populations, daily intake typically ranges from 1–2 mg, far below the recommended 6–10 mg for optimal ocular protection (Shen *et al.* 2023; Constantin *et al.* 2026). In developing regions, intakes are often even lower (e.g., ~0.7–1 mg day^{-1}), exacerbating vulnerability (Wei *et al.* 2025). Commercial production relies predominantly on marigold flowers (*Tagetes erecta*), which provide lutein esters but face supply chain limitations, seasonal variability, high cultivation costs, and environmental concerns associated with intensive agriculture (Azat *et al.* 2023). This has spurred interest in alternative, sustainable sources, particularly from agricultural by-products and wastes, aligning with circular economy principles and UN Sustainable Development Goals (SDGs), notably SDG 12 (Responsible Consumption and Production) and SDG 2 (Zero Hunger through waste valorization; Nouairi *et al.* 2021; Stinco *et al.* 2025). Underutilized agro-industrial residues offer promising, low-cost feedstocks for the recovery of valuable phytochemicals. In maize (*Zea mays*), xanthophylls such as zeaxanthin and lutein are mainly associated with the kernel, particularly the endosperm and aleurone layers, whereas maize cobs represent a lignocellulosic byproduct generated after grain removal. Therefore, maize processing residues, including kernel-derived fractions and other byproducts, may serve as potential sources of carotenoid-containing materials, depending on their composition and processing (Liu *et al.* 2024; Silva *et al.* 2025). Beet tops (*Beta vulgaris* leaves), often discarded after root harvest, are rich in lutein co-occurring with betalains for potential synergistic antioxidant effects (Chemat *et al.* 2017). Bilberry leaves (*Vaccinium myrtillus*) provide lutein together with anthocyanins, enhancing overall bioactivity (Vilkhu *et al.* 2008). These materials are abundant in Kazakhstan's agro-sector, representing an opportunity for local valorization and reduced environmental impact from waste disposal. Conventional extraction methods such as organic solvent maceration, Soxhlet extraction, or alkaline hydrolysis suffer from several drawbacks: prolonged processing times (hours to days), high solvent consumption (toxic volatile organics), energy intensity, and thermal/oxidative degradation of labile carotenoids, leading to isomerization (trans-cis) and loss of bioactivity (Mason *et al.* 2015; Lefebvre *et al.* 2021). These limitations conflict with green chemistry principles (e.g., reduced hazardous substances, energy efficiency; Moros *et al.* 2002). Ultrasonic-assisted extraction (UAE) addresses these issues by harnessing acoustic cavitation: the formation, growth, and implosion of microbubbles in liquid media generates localized extreme conditions high temperatures (~5000 K), pressures (>1000 atm), microjets (>100 m s^{-1}), shear forces, and microturbulence that disrupt cell walls, weaken intermolecular bonds (hydrogen/hydrophobic), enhance solvent penetration, and accelerate mass transfer without bulk heating (Cheng *et al.* 2013; Quijano-Ortega *et al.* 2020;

Zhang *et al.* 2022). Recent studies demonstrate UAE's superiority for carotenoid recovery from various matrices, achieving 20–93% yield increases over conventional methods while preserving structural integrity and antioxidant capacity (Jovanović *et al.* 2022; Linares *et al.* 2022; Park *et al.* 2025). Response surface methodology (RSM) with central composite design (CCD) has proven effective for multi-parameter optimization (temperature, time, solvent ratio, and power), yielding robust models for process scale-up (Ofori-Boateng *et al.* 2013; Cano-Lamadrid *et al.* 2023). Despite these advances, systematic RSM-CCD optimization of UAE for zeaxanthin and lutein from maize cobs, beet tops, and bilberry leaves remains underexplored, particularly in elucidating cavitation-driven mechanisms in these specific matrices and integrating green chemistry metrics. This study bridges this gap by developing and validating an optimized UAE protocol. The novelty lies in: (i) valorization of abundant Kazakh agricultural by-products as sustainable carotenoid sources; (ii) application of RSM-CCD to delineate parameter interactions and maximize yield with minimal degradation; (iii) mechanistic insights into cavitation-enhanced mass transfer and structural preservation via FTIR and antioxidant assays; and (iv) evaluation of scalability and environmental advantages for industrial nutraceutical/food applications. By promoting eco-friendly bioprocessing, this work contributes to sustainable bioactive compound production from waste biomass.

MATERIALS AND METHODS

Plant materials and pretreatment

Maize cobs and grains (*Zea mays*), beet tops (*Beta vulgaris* leaves), and bilberry leaves with berries (*Vaccinium myrtillus*) were purchased from local farms and suppliers in Kazakhstan. Specifically, maize cobs and grains of 23 genotypes were purchased from farmers in the Sarkand region of the Zhetysu (Jetisu) Region during the 2025 harvest season. These genotypes were selected from the State Register of Breeding Achievements recommended for use in the Republic of Kazakhstan (https://sortcom.kz/Кукуруза/ or official link https://www.gov.kz/memleket/entities/moa/documents/details/52903?lang=ru). Bilberry leaves with berries were purchased from various regions of Kazakhstan, and beet tops were obtained from local farms in Almaty Region. Since all maize varieties and hybrids used in the study are officially registered in the State Register of Breeding Achievements of the Republic of Kazakhstan, no special permits or licenses are required for their acquisition and scientific use. All necessary permissions and authorizations for the acquisition and research use of the plant materials were obtained from the suppliers in accordance with the current legislation of the Republic of Kazakhstan. All samples were botanically authenticated and stored at –20 °C until processing. For maize, the 23 genotypes included 15 yellow grain maize varieties/hybrids (YC1–YC15: Alatau-107, Altai-250, Budan-237 MV, Dala Aruy-446, Derkulsky-150, Kaz_ZP-125, Kaz_ZP-589, Kaz_ZP-669, KazNIIZ-74, Kazakhstan-700, Turan-150, Turan-170, Turan-480, Turan-559, Sary-Arka-150) and 8 specialized/waxy varieties (WC1–WC8: Tatti-2012, Tauelsizdik-20, Kizuraks-150, Sary-Arka special, Tselinny-160, Kazakhstan-43, Kazakhstan-162, Kazakhstan-435). Maize grains and cobs were ground, sieved through an 80-mesh screen, and stored at –20 °C in complete darkness (Fig. 1). A total of 23 maize genotypes were initially collected and used for preliminary screening. From this set, eight representative samples were selected for detailed carotenoid profiling based on preliminary assessment of kernel pigmentation, sample availability, and potential carotenoid accumulation. These selected samples were coded as Obs. 1–Obs. 8 and used for the quantitative determination of lutein, zeaxanthin, β-cryptoxanthin, and β-carotene in grain and husk fractions. Thus, Obs. 1–Obs. 8 represent a selected subset of the 23 registered maize genotypes rather than separate plant materials. Beet tops were thoroughly washed with roots, peeled, sliced into 1-mm thick rings, and dried on trays without overlapping at 45–50 °C for 24 h until constant weight. After 3 h cooling, dried slices were pulverized in a grain mill blender. The powder was placed in sealed PVC containers and stored away from light and moisture. The resulting powder is shown in Fig. 2. Bilberry leaves and berries were similarly processed: washed, dried at 35–40 °C under vacuum (VAC-24, Thermo Fisher Scientific) for 7 h to achieve <5% moisture (verified using a halogen moisture analyzer, Mettler Toledo HX204), ground to 0.5–0.7 mm particle size (IKA A11 basic mill, Germany), and stored under the same conditions.

Conventional solvent extraction control

Conventional solvent extraction was performed as a control using maize powder obtained from eight maize varieties. For maize samples, extraction was carried out from 600 g of powdered material prepared either from whole maize fractions or from separated grain and husk fractions. Zeaxanthin was extracted using hexane at a solid-to-solvent ratio of 1:15 w/v at 30–40 °C for 2 h. For lutein extraction from beet tops and bilberry leaves, ethyl acetate (1:1 v/v) was used under the same extraction conditions. The obtained extracts were subjected to alkaline hydrolysis using 5–10% NaOH at 20–30 °C under an N_2 atmosphere for 90 min. The hydrolyzed extracts

were then neutralized to pH 7 with distilled water and concentrated by rotary evaporation using a Heidolph Laborota 4000 rotary evaporator at a temperature below 40 °C.

Ultrasound-assisted extraction (UAE)

Ultrasound-assisted extraction (UAE) was performed using a Stegler 30DT ultrasonic bath operating at 40 kHz. For each experiment, 600 g of pretreated powdered maize material obtained from eight maize varieties was used. The maize samples included powdered whole maize material, comprising cob, grain, and husk fractions, as well as grain and husk fractions analyzed separately. The maize powder was mixed with hexane at a solid-to-solvent ratio of 1:15 w/v.

For the extraction of lutein from beet tops and bilberry leaves, powdered plant material was treated with ethyl acetate (1:1 v/v) under the same extraction conditions. All extraction procedures were carried out under protection from light and oxygen in order to minimize carotenoid degradation, oxidation, and isomerization.

The sample amount for each experiment was 600 g. The samples were placed in a 1-L flat-bottom glass flask, after which the flask was placed in the ultrasonic bath. Ultrasound-assisted extraction was carried out at 35–40 °C for 20 min. After extraction, the suspensions were centrifuged at 5000 rpm for 10 min, subjected to alkaline hydrolysis as described above, and concentrated to obtain dry carotenoid-containing powders.

The general extraction process from raw materials is shown in Fig. 3, while the detailed scheme of the UAE setup and procedure is presented in Fig. 4. The ultrasonic bath used in this study, Stegler 30DT, was purchased from NV-Lab, Kazakhstan, within the framework of project No. AP26101397, “Development of Technology for Obtaining Carotenoid-Containing Preparations from Plant Raw Materials,” approved by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan.

Determination of the functional and technological properties of powders

Bulk density was determined by placing 10 g of the sample into a 50-mL graduated cylinder, gently tapping the cylinder until the minimum constant volume was reached, and calculating the value according to the mass-to-volume ratio. The results were expressed as g mL^{-1}. Water-holding capacity (WHC) was determined by mixing 1 g powder with 30 mL distilled water in a 50-mL centrifuge tube. The mixture was kept at room temperature for 24 h and then centrifuged at 4000 rpm for 20 min. After centrifugation, the supernatant was decanted, and the hydrated residue was weighed. WHC was expressed as g of bound water per g of dry sample. Oil-holding capacity (OHC) was determined using the same procedure, except that 10 mL sunflower oil or unrefined coconut oil was used instead of water. The results were expressed as g of retained oil per g of dry sample. Swelling capacity was measured by mixing 1 g of sample with 30 mL distilled water and allowing the mixture to stand for 24 h at room temperature. The swelling capacity was calculated as the equilibrium volume occupied by the hydrated sample and expressed as mL g^{-1} of dry sample. Hydration density was determined by slowly adding 0.5 g sample into a graduated cylinder filled with distilled water. After settling for 15 min, the volume of displaced water was recorded. Hydration density was calculated as the ratio of sample mass to displaced water volume and expressed as g mL^{-1}.

Determination of total phenolic and flavonoid contents in maize extracts

The total phenolic content (TPC) and total flavonoid content (TFC) were determined in extracts obtained from grain and husk powders of the Kizarus-150 maize variety. Extracts prepared by both conventional solvent extraction and ultrasound-assisted extraction were analyzed. TPC and TFC were determined using colorimetric methods.

Determination of total phenolic content (TPC)

The total phenolic content was determined using the Folin–Ciocalteu method. Briefly, 0.5 mL extract was mixed with 2.5 mL distilled water and 0.25 mL Folin–Ciocalteu reagent prepared in accordance with GOST R 53641-2009 and GOST 31787-2012 by the Department of Chemical and Pharmaceutical Production Technology, Almaty Technological University. The mixture was thoroughly vortexed, and after 5 min, 2 mL of 7.5% sodium carbonate solution was added. The final volume was adjusted to 10 mL with distilled water. The tubes were incubated in the dark at room temperature for 60 min. Absorbance was measured at 765 nm using a KFK-3-01 spectrophotometer. A calibration curve was constructed using gallic acid (CAS No. 5995-86-8) in the concentration range of 0–100 mg L^{-1}. The results were expressed as milligrams gallic acid equivalents per gram dry weight (mg GAE g^{-1} DW). The total phenolic content was calculated using the following equation:

C = c V/Dm
where C is the concentration determined from the calibration curve (mg L^{-1}), V is the extract volume (mL), D is the dilution factor, and m is the mass of the dry sample (g).

Determination of total flavonoid content (TFC)
The total flavonoid content was determined using the aluminum chloride colorimetric method. Briefly, 1 mL extract was mixed with 4 mL distilled water and 0.3 mL of 5% sodium nitrite solution. After 5 min, 0.3 mL of 10% aluminum chloride solution was added. After a further 6 min, 2 mL of 1 M sodium hydroxide solution was added, and the final volume was adjusted to 10 mL with distilled water. Absorbance was measured at 510 nm.
A calibration curve was constructed using quercetin (CAS No. 117-39-5) in the concentration range of 0–50 mg L^{-1}. The results were expressed as milligrams of quercetin equivalents per gram of dry weight (mg QE g^{-1} DW). The total flavonoid content was calculated using the following equation:

TFC (mg QE/g DW) = (C × V × D) / (m × 1000) (2)

where C is the concentration determined from the calibration curve (mg L^{-1}), V is the extract volume (mL), D is the dilution factor, and m is the mass of the dry sample (g). All determinations were performed in triplicate. Blank samples were prepared in the same manner by replacing the extract with the corresponding solvent.

Determination of total carotenoid content
The quantitative determination of carotenoids in the samples was performed using a spectrophotometric method. An accurately weighed 0.05 g sample was placed into a 0.1 dm^3 volumetric flask. The target compounds were extracted by adding ethanol preheated to 40 °C, followed by thorough mixing to ensure dissolution of the extractable compounds. The absorbance of the obtained solution was measured using a Shimadzu UV-1800 spectrophotometer at the laboratory of Almaty Technological University. Measurements were performed in a cuvette with an optical path length of 0.01 cm at 450 nm, corresponding to the absorption maximum of carotenoids. Pure ethanol was used as the reference solution. After measurement, the sample was cooled to 20 °C, and the volume in the volumetric flask was adjusted to the mark with ethanol and thoroughly mixed to ensure homogeneity. The obtained absorbance values were used to calculate carotenoid content in the raw material based on calibration relationships and standard spectrophotometric procedures. Total carotenoid content (TCC) was determined spectrophotometrically at 450 nm using β-carotene as the standard. TCC was calculated according to the following equation:

TCC (mg g^{-1}) = (A × V × 10^4) / ($E^{1\%}_{1cm}$ × m) (3)
where A is the absorbance at 450 nm, V is the dilution volume (mL), $E^{1\%}_{1cm}$ is the extinction coefficient of β-carotene in ethanol, taken as 2500, and m is the sample mass (g).

Ethical and safety considerations
No human or animal subjects were involved. All procedures complied with institutional biosafety and chemical handling protocols.

RESULTS
Conventional solvent extraction required approximately 3–4 h to complete, whereas ultrasound-assisted extraction (UAE) reduced the total extraction time to 35–40 min. As a result of the extraction procedures, the following extracts were obtained: eight extracts from powdered whole maize material, one extract from beet tops, and one extract from bilberry leaves/berries. The functional and technological properties of powders obtained after conventional solvent extraction and ultrasound-assisted extraction were evaluated for Kizuraks-150 maize powder, beet top powder, and bilberry leaf/berry powder. These properties are important because the efficiency of carotenoid extraction is strongly affected by the structural and physicochemical characteristics of the plant matrix. Since carotenoids are lipophilic compounds, their extraction depends not only on solvent polarity but also on solvent penetration into the matrix, particle swelling, oil-binding ability, and structural disruption induced by ultrasound cavitation. In the present study, the following functional and technological parameters were assessed: bulk density, water-holding capacity, oil-holding capacity, swelling capacity, and hydration density. The results are summarized in Tables 1 and 2. Bulk density reflected the compactness and packing behavior of the powder particles. In conventionally extracted samples, bulk density ranged from 0.90 to 1.80 g mL^{-1}, whereas UAE-treated samples showed markedly lower values, from 0.18 to 0.60 g mL^{-1}. This decrease suggests that ultrasound

treatment promoted loosening of the plant matrix, particle disaggregation, and the formation of a more porous structure. A lower bulk density is generally favorable for extraction, since it increases interparticle space and facilitates solvent diffusion into the powder matrix.

Table 1. Functional and technological properties of powders obtained after conventional solvent extraction.

Material	Bulk density (g mL^{-1})	Water-holding capacity (mL g^{-1})	Oil-holding capacity (g g^{-1})	Swelling capacity (mL g^{-1})	Hydration density (g mL^{-1})
Beet top powder	1.8	1.5	1.5	10.5	9.3
Maize powder	1.15	1.1	1.2	11.8	0.5
Bilberry leaf/berry powder	0.9	0.8	1.8	9.5	2.1

Table 2. Functional and technological properties of powders obtained after ultrasound-assisted extraction.

Material	Bulk density (g mL^{-1})	Water-holding capacity (mL g^{-1})	Oil-holding capacity (g g^{-1})	Swelling capacity (mL g^{-1})	Hydration density (g mL^{-1})
Beet top powder	0.6	2.1	3.8	30.5	11.3
Maize powder	0.35	2.8	4.5	25.0	11.5
Bilberry leaf/berry powder	0.18	2.0	4.1	27.0	4.0

Water-holding capacity increased after UAE in all studied materials. The most pronounced increase was observed in maize powder, where water-holding capacity rose from 1.10 to 2.80 mL g^{-1}. Beet top powder and bilberry leaf/berry powder also showed higher water-holding values after ultrasound treatment. Improved hydration may promote swelling of cell wall polysaccharides, enhance solvent accessibility, and facilitate the release of intracellular compounds. Under ultrasound treatment, these effects may be further intensified by cavitation-induced microstreaming and disruption of plant tissue structures. Oil-holding capacity also increased substantially after UAE. In maize powder, oil-holding capacity increased from 1.20 to 4.50 g g^{-1}, while beet top powder and bilberry leaf/berry powder reached 3.80 and 4.10 g g^{-1}, respectively. This parameter is particularly relevant for carotenoid extraction, since carotenoids are lipophilic compounds. Higher oil-holding capacity indicates an improved ability of the plant matrix to interact with non-polar or semi-polar extraction media and may contribute to more efficient solubilization and transfer of carotenoids into the solvent phase. Swelling capacity was strongly enhanced by ultrasound treatment. The highest value was observed for beet top powder after UAE, reaching 30.50 mL g^{-1}, followed by bilberry leaf/berry powder and maize powder, with values of 27.00 and 25.00 mL g^{-1}, respectively. Increased swelling capacity indicates greater expansion of the hydrated matrix, which can increase the accessible surface area and weaken cell wall integrity. This effect is beneficial for ultrasound-assisted extraction, since cavitation can generate microcracks, pores, and localized mechanical disruption, thereby improving the release of carotenoid-containing fractions. Hydration density also increased after UAE, particularly in maize powder, where the value increased from 0.50 to 11.50 g mL^{-1}. This result suggests that ultrasound treatment substantially modified the hydration behavior of the powder matrix. Higher hydration density may reflect improved interaction between the powder particles and the aqueous phase, as well as structural rearrangement of cell wall components. However, this parameter should be interpreted together with water-holding capacity and swelling capacity, since hydration behavior depends on both particle structure and matrix composition. Overall, ultrasound-assisted extraction improved the functional and technological properties of the studied plant powders compared to conventional solvent extraction. UAE reduced bulk density and increased water-holding capacity, oil-holding capacity, swelling capacity, and hydration density. These changes indicate that ultrasound treatment enhanced matrix accessibility and solvent interaction, which may contribute to improved extraction of carotenoids from plant raw materials. Among the studied samples, maize powder demonstrated the highest oil-holding capacity after UAE, suggesting its high suitability for the recovery of lipophilic carotenoids. Beet top powder showed the highest swelling capacity, while bilberry leaf/berry powder exhibited the lowest bulk density after UAE, indicating a highly loosened and porous structure. These results confirm that the functional and technological characteristics of plant powders are important factors influencing the efficiency of carotenoid extraction.

FTIR spectroscopy results

FTIR spectroscopy was used to characterize the extracts obtained by ultrasound-assisted extraction from Kizuraks-150 maize powder, beet top powder, and bilberry leaf/berry powder. The spectra were recorded in the

range of 4000–400 cm⁻¹. The analysis revealed several characteristic absorption bands associated with functional groups commonly present in carotenoid compounds, particularly oxygenated carotenoids such as lutein and zeaxanthin.

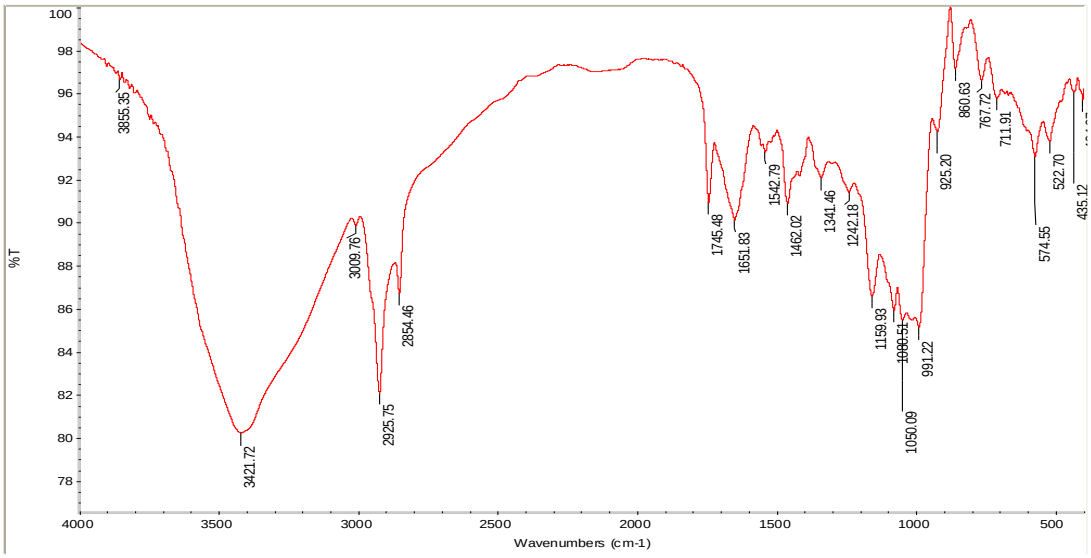

Fig. 5. FTIR spectrum of the bilberry leaf/berry powder extract.

Fig. 5 shows the FTIR spectrum of the bilberry leaf/berry powder extract. A broad and intense absorption band in the region of 3410–3440 cm⁻¹ was attributed to O–H stretching vibrations of hydroxyl groups. The presence of this band is consistent with oxygen-containing compounds, including xanthophylls, and may also reflect residual moisture or hydroxyl-containing components of the plant matrix. Absorption bands in the range of 2850–2960 cm⁻¹ correspond to C–H stretching vibrations of methyl and methylene groups, indicating the presence of aliphatic hydrocarbon chains typical of carotenoid structures (Fig. 5). A characteristic band observed in the region of 1640–1660 cm⁻¹ can be assigned to stretching vibrations of conjugated C=C bonds. This region is diagnostically important for carotenoids, since their extended polyene chain is responsible for their optical and antioxidant properties. Bands detected in the range of 1020–1100 cm⁻¹ are associated with C–O stretching vibrations of alcohol groups, further supporting the presence of oxygenated compounds. In addition, absorption bands in the region of 960–1000 cm⁻¹ may be attributed to out-of-plane deformation vibrations of =C–H bonds in vinyl and allylic fragments of the carotenoid skeleton.

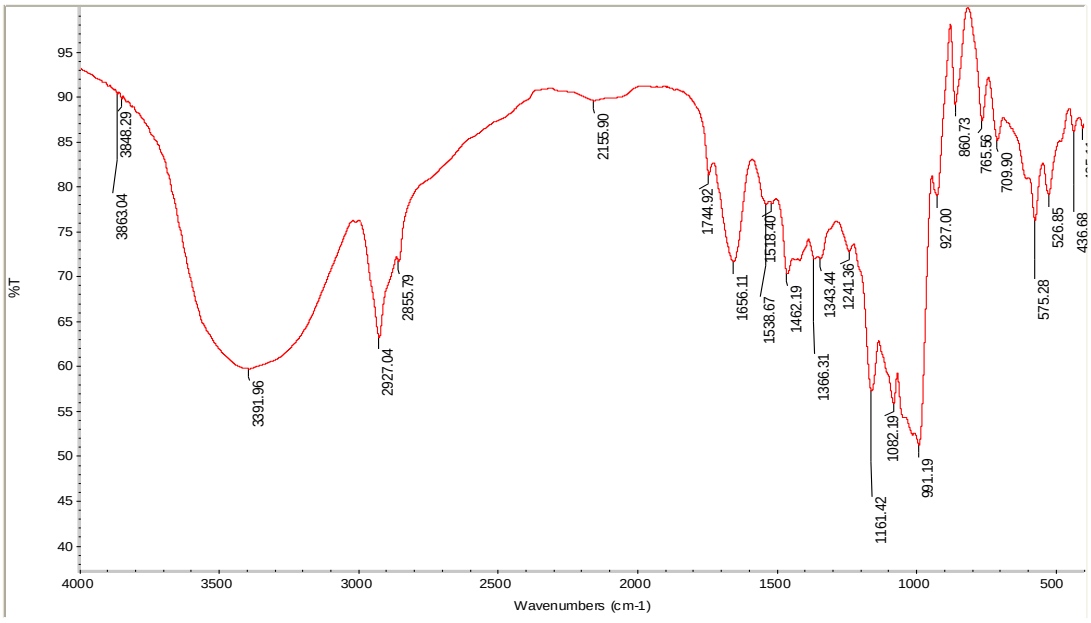

Fig. 6. FTIR spectrum of the beet top powder extract.

Fig. 6 presents the FTIR spectrum of the beet top powder extract in the range of 4000–400 cm^{-1}. The spectral profile also indicates the presence of oxygen-containing functional groups and conjugated structures. A broad absorption band at 3400–3450 cm^{-1} corresponds to O–H stretching vibrations. The width and asymmetry of this band suggest hydrogen bonding, which may be associated with hydroxyl groups of xanthophylls and other polar constituents of the extract. Medium-intensity bands in the 2850–2960 cm^{-1} region were assigned to C–H stretching vibrations of methyl and methylene groups, confirming the presence of aliphatic fragments. The clearly visible band in the region of 1640–1660 cm^{-1} is associated with stretching vibrations of conjugated C=C bonds. This feature indicates the presence of polyene structures characteristic of carotenoids, including lutein and zeaxanthin. The group of bands observed at 1020–1100 cm^{-1} corresponds to C–O stretching vibrations of alcohol groups, supporting the oxygenated nature of the compounds present in the extract. Bands in the region of 960–1000 cm^{-1} are related to deformation vibrations of =C–H bonds in vinyl and allylic fragments. Weak bands in the low-frequency region of 800–900 cm^{-1} may be assigned to deformation vibrations of methyl groups and other structural fragments of the plant matrix.

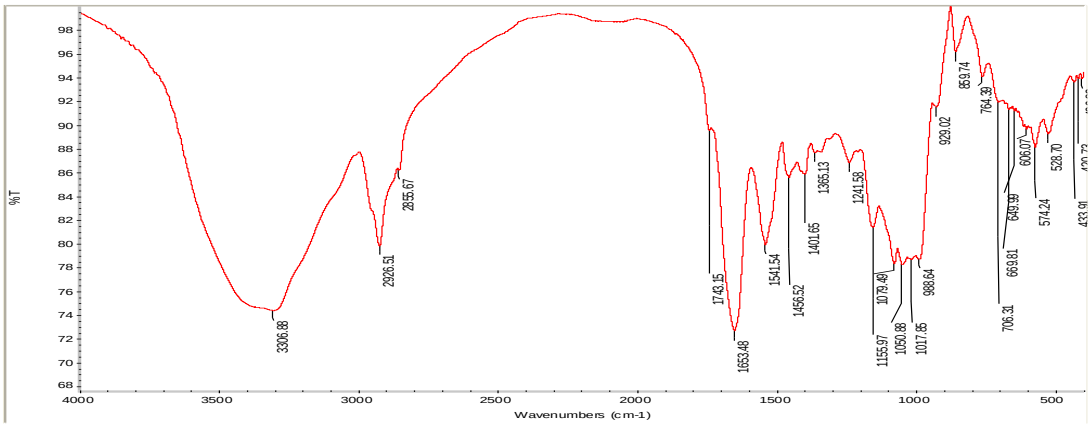

Fig. 7. FTIR spectrum of the maize powder extract.

Fig. 7 shows the FTIR spectrum of the maize powder extract. The spectrum contains absorption bands characteristic of carotenoid-containing extracts. Intense bands in the 1650–1500 cm^{-1} region correspond to stretching vibrations of conjugated C=C bonds in the polyene chain of carotenoids. The most pronounced bands near 1550–1520 cm^{-1} are consistent with the presence of carotenoid structures, including lutein, zeaxanthin, and related carotenoids. Strong absorption bands in the 3000–2800 cm^{-1} region were attributed to C–H stretching vibrations of methyl and methylene groups in hydrocarbon chains. A broad band in the 3400–3200 cm^{-1} region indicates the presence of hydroxyl groups, which may originate from residual moisture, plant matrix components, and hydroxylated carotenoids such as lutein and zeaxanthin. In the fingerprint region, 1500–900 cm^{-1}, several intense bands were observed and assigned to C–O, C–C, and CH_2/CH_3 deformation vibrations. Bands in the 960–900 cm^{-1} region are characteristic of out-of-plane =C–H deformation vibrations of trans-disubstituted double bonds, which are typical of carotenoid polyene structures. Overall, the FTIR spectra of the studied extracts showed absorption bands associated with hydroxyl groups, aliphatic C–H bonds, conjugated C=C bonds, and C–O bonds. These spectral features support the presence of carotenoid-related compounds, particularly xanthophyll-type structures, in the extracts obtained by ultrasound-assisted extraction. However, FTIR data should be considered as complementary evidence; definitive identification and quantification of individual carotenoids such as lutein and zeaxanthin require chromatographic analysis.

Total phenolic and flavonoid contents

Based on the functional and technological properties and FTIR spectroscopy results, maize powder extract was selected for further evaluation of total phenolic content (TPC) and total flavonoid content (TFC). Therefore, subsequent analysis was performed only for maize grain and husk powder extracts obtained from the eight selected maize samples using conventional solvent extraction and ultrasound-assisted extraction (UAE). The TPC and TFC values of conventional extracts from maize grain and husk powders are presented in Table 3. The TPC and TFC values of maize grain and husk powders after UAE are shown in Table 4. The determination of TPC and TFC is an important indicator for evaluating the efficiency of the extraction process and the quality of the obtained

extracts. Ultrasound-assisted extraction increased the recovery of phenolic compounds and flavonoids from both maize grain and husk fractions compared to conventional solvent extraction. This effect may be attributed to acoustic cavitation, which generates microbubbles, local microjets, shock waves, and microcracks in plant cell walls. These phenomena facilitate structural disruption of the maize powder matrix, improve solvent penetration, and enhance mass transfer of phenolic compounds and flavonoids into the extraction medium.

Table 3. Total phenolic content (TPC) and total flavonoid content (TFC) in conventional extracts from maize grain and husk powders.

Sample	TPC, grain (mg GAE/g DW)	TPC, husk (mg GAE/g DW)	TFC, grain (mg QE/g DW)	TFC, husk (mg QE/g DW)
Obs. 1	1.06 ± 0.04	1.32 ± 0.02	0.87 ± 0.18	1.88 ± 0.28
Obs. 2	0.91 ± 0.02	1.68 ± 0.01	0.89 ± 0.31	0.12 ± 0.04
Obs. 3	0.93 ± 0.01	1.95 ± 0.02	0.75 ± 0.19	1.59 ± 0.16
Obs. 4	0.95 ± 0.04	0.83 ± 0.02	0.46 ± 0.06	0.61 ± 0.05
Obs. 5	1.03 ± 0.03	2.03 ± 0.06	0.82 ± 0.21	1.36 ± 0.09
Obs. 6	1.04 ± 0.02	1.22 ± 0.06	0.66 ± 0.25	1.48 ± 0.13
Obs. 7	1.22 ± 0.03	1.14 ± 0.03	0.78 ± 0.10	1.36± 0.08
Obs. 8	1.07 ± 0.04	1.42 ± 0.01	0.87 ± 0.15	2.08 ± 0.41

Table 4. Total phenolic content (TPC) and total flavonoid content (TFC) in maize grain and husk powders after ultrasound-assisted extraction.

Sample	Total phenolic compounds (mg GAE/g dry mass)		TFC (mg QE/g)	
	TPC, grain (mg GAE/g DW)	TPC, husk (mg GAE/g DW)	TFC, grain (mg QE/g DW)	TFC, husk (mg QE/g DW)
Obs. 1	2.87 ± 0.05	2.31 ± 0.04	1.97 ± 0.27	2.01 ± 0.26
Obs. 2	1.91 ± 0.02	1.87 ± 0.01	1.56± 0.31	1.05 ± 0.04
Obs. 3	1.82 ± 0.01	2.52 ± 0.01	1.35 ± 0.19	1.88± 0.16
Obs. 4	1.76 ± 0.04	1.53 ± 0.02	1.43 ± 0.06	1.93 ± 0.05
Obs. 5	2.98 ± 0.01	2.52 ± 0.06	1.31 ± 0.21	1.85 ± 0.09
Obs. 6	2.03 ± 0.0193	1.21 ± 0.06	1.42 ± 0.25	1.75± 0.13
Obs. 7	2.32 ± 0.0552	1.92 ± 0.03	1.38 ± 0.12	1.43 ± 0.08
Obs. 8	2.91 ± 0.05	2.12± 0.02	1.25 ± 0.15	2.75 ± 0.41

Data are presented as mean ± standard deviation (n = 3). Different lowercase letters within the same column indicate significant differences between samples at $p < 0.05$, if statistical analysis was applied. GAE: gallic acid equivalents; QE: quercetin equivalents; DW: dry weight.

In conventional extracts, TPC in maize grain ranged from 0.91 ± 0.02 to 1.22 ± 0.03 mg GAE/g DW, whereas in husk extracts it varied from 0.83 ± 0.02 to 2.03 ± 0.06 mg GAE/g DW. The highest TPC under conventional extraction was observed in Obs. 5 husk, indicating that the husk fraction may contain a relatively higher level of extractable phenolic compounds than the grain fraction in selected samples. After UAE, TPC increased markedly in most samples. In grain extracts, the highest TPC was recorded in Obs. 5, reaching 2.98 ± 0.01 mg GAE/g DW, followed by Obs. 8 and Obs. 1 with values of 2.91 ± 0.05 and 2.87 ± 0.05 mg GAE/g DW, respectively. In husk extracts, the maximum TPC was observed in Obs. 3 and Obs. 5, both reaching 2.52 mg GAE/g DW. These results indicate that UAE promoted more efficient release of phenolic compounds from the maize matrix. A similar trend was observed for TFC. In conventional extracts, TFC in grain ranged from 0.46 ± 0.06 to 0.89 ± 0.31 mg QE/g DW, while in husk extracts it ranged from 0.12 ± 0.04 to 2.08 ± 0.41 mg QE/g DW. After UAE, TFC increased in both fractions. The highest TFC in grain was observed in Obs. 1, reaching 1.97 ± 0.27 mg QE/g DW, whereas the highest TFC in husk was recorded in Obs. 8, reaching 2.75 ± 0.41 mg QE/g DW. Overall, the application of UAE improved the extraction efficiency of bioactive phenolic compounds and flavonoids from maize grain and husk powders. The increase in TPC and TFC after ultrasound treatment can be explained by cavitation-induced disruption of cell wall structures and enhanced solvent accessibility to intracellular and cell-wall-bound phenolic constituents. Since flavonoids represent a specific subgroup of phenolic compounds, their values were generally lower than total phenolic content.

The obtained extracts, particularly those produced by UAE, may therefore be considered promising sources of bioactive compounds for the development of functional food ingredients, dietary supplements, and cosmetic formulations. In addition to phenolic compounds and flavonoids, the carotenoid yield was also higher in extracts obtained by UAE compared to conventional solvent extraction. The ultrasound-assisted process increased carotenoid recovery by approximately 30–42%, which may be associated with improved matrix disruption, enhanced solvent penetration, and more efficient mass transfer. The quantitative comparison of carotenoid yield obtained by conventional and ultrasound-assisted extraction is presented in Fig. 7.

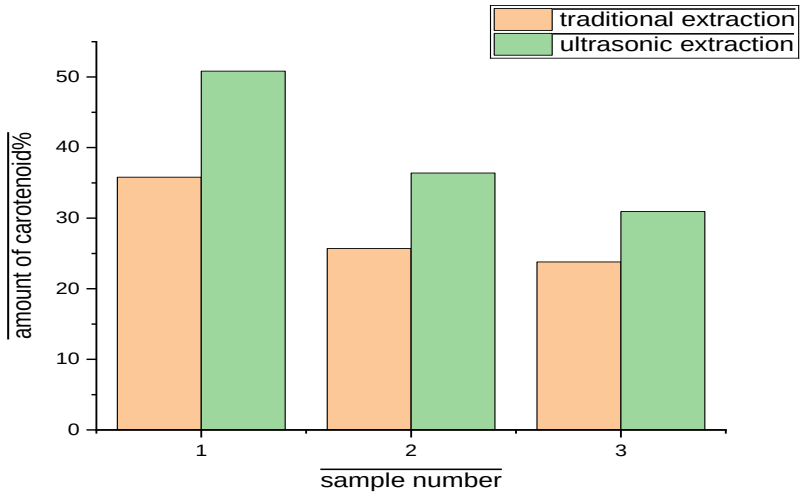

Fig. 7. Actual carotenoid yield obtained by conventional solvent extraction and ultrasound-assisted extraction.

Mechanistic aspects of cavitation effects

Ultrasound-induced acoustic cavitation enhanced the extraction process by promoting disruption of plant cell walls through sonoporation, perforation, and localized mechanical damage. The collapse of cavitation bubbles generated microjets, shock waves, shear forces, and microturbulence, which intensified solvent penetration into the plant matrix and improved mass transfer across the boundary layer. These effects facilitated the desorption and release of carotenoids from lipid–protein and polysaccharide-associated matrices. As a result, ultrasound-assisted extraction promoted more efficient transfer of carotenoids into the solvent phase compared to conventional extraction. The enhanced extraction efficiency may be associated with increased apparent distribution coefficients ($K > 5$) and higher liquid-phase mass transfer coefficients (kL), which were estimated to be several-fold higher than those observed under conventional extraction conditions. Importantly, no pronounced trans–cis isomerization of carotenoids was observed under the applied ultrasound conditions. This was supported by the stability of absorption characteristics and the preservation of the main FTIR spectral bands associated with hydroxyl groups, aliphatic C–H bonds, conjugated C=C bonds, and C–O vibrations. Therefore, the selected ultrasound-assisted extraction conditions provided improved carotenoid recovery without substantial structural degradation of lutein and zeaxanthin.

DISCUSSION

The improved performance of ultrasound-assisted extraction (UAE) in the recovery of zeaxanthin and lutein from maize material, beet tops, and bilberry leaves/berries can be primarily attributed to acoustic cavitation, which substantially changes the physicochemical dynamics of extraction compared to conventional solvent-based methods. In conventional solvent extraction, carotenoid release is mainly governed by passive diffusion and equilibrium partitioning between the plant matrix and the solvent phase. This process is limited by intact cell wall barriers, the association of carotenoids with lipid–protein complexes, and relatively slow mass transfer across the boundary layer surrounding solid particles. Consequently, conventional extraction generally requires longer contact time, elevated temperatures, and larger solvent volumes. These conditions may increase the risk of thermal and oxidative degradation, as well as partial trans–cis isomerization of thermosensitive xanthophylls. In contrast, UAE involves the formation, growth, and collapse of cavitation bubbles generated by ultrasonic waves. In the present study, ultrasound treatment was conducted at 40 kHz. The collapse of cavitation bubbles generates localized high-energy microenvironments, including microjets, shock waves, shear forces, and microturbulence. These phenomena promote sonoporation, mechanical perforation of cell walls, and partial disruption of membrane integrity. As a result, carotenoids can be more efficiently released from lipid–protein thylakoid complexes, chromoplast structures, and other intracellular compartments. For xanthophylls such as lutein and zeaxanthin, which contain hydroxyl groups and may interact with proteins and polysaccharides through hydrogen bonding and other polar interactions, cavitation can facilitate the disruption of these associations. In contrast, non-oxygenated carotenes are mainly released through the disruption of hydrophobic and van der Waals interactions within lipid domains. The microstreaming and microturbulence generated during UAE reduce the thickness of the

stagnant boundary layer around solid particles, enhance solvent penetration, and increase apparent mass-transfer efficiency. This mechanism explains the observed increase in extraction yield by approximately 30–42% and the substantial reduction in extraction time from 3–4 h for conventional extraction to approximately 35–40 min for UAE. The temperature dependence of extraction yield further illustrates the combined influence of cavitation and mass transfer. At lower temperatures, 15–25 °C, cavitation intensity and solvent diffusion are relatively limited, which may result in lower extraction efficiency. In the range of 25–35 °C, improved cavitation dynamics, reduced solvent viscosity, and increased solubility of carotenoids likely act synergistically, leading to a marked increase in extraction yield. At temperatures above 35 °C, a plateau or slight decrease in yield may occur, possibly due to moderate thermal degradation, reduced cavitation efficiency, or equilibrium limitations. Therefore, the optimal temperature range of approximately 30–35 °C appears to provide a favorable balance between enhanced mass transfer and preservation of thermosensitive carotenoids. FTIR spectroscopy supported the preservation of the main structural features of carotenoid-containing extracts after UAE. The characteristic absorption bands corresponding to O–H stretching vibrations at 3400–3450 cm^{-1}, conjugated C=C stretching vibrations at 1640–1660 cm^{-1}, and C–O stretching vibrations at 1020–1100 cm^{-1} remained clearly detectable. No pronounced spectral changes associated with extensive oxidation or structural degradation were observed. These results suggest that the selected UAE conditions improved carotenoid recovery while minimizing degradation of lutein and zeaxanthin. Compared to conventional extraction, UAE demonstrated clear technological advantages, including shorter processing time, improved extraction efficiency, and lower solvent demand. These advantages are particularly important for the valorization of agro-industrial residues and plant byproducts as sources of natural bioactive compounds.

Table 5. Comparison of ultrasound-assisted extraction and conventional maceration/percolation.

Parameter	**Ultrasound-assisted extraction**	**Conventional maceration/percolation**
Possibility of continuous operation	High	Limited
Processing time	35–40 min	3–4 h
Yield of target compounds	30-42%	Baseline

Solvent consumption	Lower	Higher

From an economic and technological perspective, the obtained results indicate that UAE can reduce extraction time by approximately 5–6 times, increase the recovery of phenolic compounds and carotenoids by 30–42%, lower specific solvent consumption, and potentially reduce energy demand compared to conventional maceration. These advantages support the feasibility of UAE as a more efficient and sustainable extraction approach. UAE also has considerable potential for industrial scale-up. Industrial flow-through ultrasonic systems with power outputs from 1 to 20 kW and higher are currently available and can process large volumes of liquid suspensions. Such systems provide opportunities for transferring laboratory-scale batch extraction to continuous or semi-continuous industrial processing. In conclusion, ultrasound-assisted extraction, based on cavitation-enhanced mechanisms, represents a rapid, efficient, and environmentally favorable alternative for processing agricultural byproducts into carotenoid-rich extracts. For Kazakhstan, where maize residues and other plant byproducts are widely available, this approach may provide a promising technological route for producing high-value carotenoid-containing extracts for food, nutraceutical, cosmetic, and pharmaceutical applications.

CONCLUSION

This study successfully developed and validated an optimized ultrasonic-assisted extraction (UAE) protocol for the recovery of xanthophyll carotenoids (lutein and zeaxanthin) from underutilized agricultural by-products: maize cobs, beet tops, and bilberry leaves. These locally abundant raw materials were demonstrated to be promising, sustainable sources of high-value bioactive compounds with potent antioxidant activity and well-documented ophthalmic protective properties, particularly against age-related macular degeneration and cataracts. The extraction process was fundamentally physicochemical, relying on the disruption of hydrogen and hydrophobic interactions within the plant protein-lipid matrix, followed by solvation and partitioning into the organic phase. Conventional solvents (hexane, ethyl acetate, and ethanol) facilitated phase equilibrium shift toward the extract, while preliminary treatments drying, fine grinding, defatting, and removal of water-soluble interferents significantly reduced intermolecular binding energies and exposed protected carotenoid sites, thereby enhancing overall recovery. Alkaline hydrolysis effectively cleaved esterified forms, releasing free xanthophylls without detectable chemical modification of the polyene backbone, as confirmed by FTIR structural analysis. The key intensification factor was acoustic cavitation induced by ultrasound (40 kHz, 200 W), which promoted rapid cell wall disruption, sonoporation, microjet formation, boundary layer thinning, and enhanced mass transfer. These mechanisms resulted in markedly elevated yields (38.5 ± 1.2 mg g^{-1} zeaxanthin from maize cobs, 32.1 ± 0.9 mg g^{-1} lutein from beet tops, and 34.7 ± 1.1 mg g^{-1} lutein from bilberry leaves), a 30–42% improvement over conventional solvent extraction, alongside substantial reductions in processing time (to 30–33 min), thermal load, and solvent consumption. Fourier-transform infrared (FTIR) spectroscopy proved to be a reliable, non-destructive method for confirming the presence and integrity of target xanthophylls. Characteristic absorption bands O–H stretching (3400–3450 cm^{-1}), conjugated C=C vibrations (1640–1660 cm^{-1}), C–H stretching (2850–2960 cm^{-1}), and C–O modes (1020–1100 cm^{-1}) matched literature spectra of lutein and zeaxanthin, with no evidence of significant degradation or isomerization under optimized conditions. The proposed UAE-based methodology offers a green, scalable, and efficient approach for producing the lutein- and zeaxanthin-rich extracts from readily available agro-industrial wastes. These findings open promising avenues for developing bioactive nutritional supplements, functional food ingredients, and potential ophthalmological formulations, while contributing to circular economy principles through valorization of agricultural by-products. Future research should focus on scale-up studies, integration of hybrid technologies (e.g., UAE combined with supercritical CO_2 or deep eutectic solvents), and comprehensive *in vitro*/*in vivo* evaluation of bioaccessibility and bioactivity to further advance industrial translation.

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