Journal of Food Biosciences and Technology,
Islamic Azad University, Science and Research Branch, Vol. 14, No. 1, 71-85, 2024

Phytochemical and Antioxidant Screening of *Lepidium latifolium* L. Extract: Function in Fish Product Preservation

***N. Samadian*[a], *M. Nouri*[a*]**

[a] Department of Food Science and Technology, Roudehen Branch, Islamic Azad University, Roudehen, Iran.

Received: 13 October 2023 Accepted: 4 April 2024

ABSTRACT: The aim of the present study is to investigate physicochemical attribute, antioxidant function, antimicrobial activity and sensory feature in *Lepidium latifolium* L. (*L. latifolium*) extract on fish burgers during the shelf life. Initially, *L. latifolium* was extracted using soxhlet (LES), ultrasound (LEU) and supercritical water (LEW). The major components were assessed using high performance liquid chromatography, where sinigrin and glucosinolates constituents were identified. The results illustrated that satisfactory values were obtained for these values in the treated sample by LEU. In next step, 0.2 % of each extract was added to fish burgers. The control and treated samples were tested to determine pH level, cooking yield, color features, thiobarbituric acid (TBAR), peroxide value (PV), microbial population, sensory evaluation and scanning electron microscope (SEM). The lowest TBAR and PV levels were found to be 0.38 and 0.40 (mg malonaldehyde) as well as 4.22 and 4.28 (meqO_2/kg) for treated fish burgers by LEU and LEW respectively ($p < 0.05$). The most sensory desirability was observed for treated sample by LEU, which also exhibited the uniform structure in SEM images as compared to others. In conclusion, fish burgers obtained by LEU and LEW can be considered as optimal samples to preserve burgers.

Keywords: Antioxidant, Burger, Color, Fish, *Lepidium latifolium* L.

Introduction

Fish contains high-quality protein and ω-3 fatty acids as well as *Salmo trutta caspius* Kessler that is one of the anadromous species that has a special economic value and great popularity (Bagherikakash *et al*., 2022; Duman, 2020; Kaviyani *et al.*, 2020). Ecologically, *S. trutta caspius* was placed in the Nekton group, which was a descendant for brown trout as a native subspecies in western and southern coasts of Caspian Sea that is about 15000 years old (Mattje *et al*., 2019). Physically, *S. trutta caspius* has an elongated bilaterally compressed body with dark spots on surface and a snout (Kaviyani *et al*., 2020). This meat contains large essential amino acids and unsaturated fatty acids from the n-6 especially n-3 series such as eicosapentaenoic acid (20:5) n-3 and docosahexaenoic acid (22:6) n-3 (Campelo *et al*., 2018).

A glance at people's living, the problems in mechanical life, the lack of time for preparing food, producing and supplying ready or semi-ready foodstuffs (such as seafood and fish burger) seem to be a viable solutions (Emir Çoban and Tuna Keleştemur, 2017). One of the benefits in fish products (such as burgers) is to assist the reduction for wastes (Bainy *et al*., 2015).

* Corresponding Author: Marjan.nouri@iau.ac.ir

The natural maintenance can help alleviate concerns over the potential health risks associated with application of artificial preservatives in food products (Kowalczyk *et al*., 2023; Mooliani and Nouri, 2021). Herbal products possess an individual place and have been used over centuries to protect against oxidative damage, specifically in high-fat foods that are prone to spoilage (Nouri and Shafaghi Rad, 2021; Dolea *et al*., 2018). *Lepidium latifolium* L. belongs to the *Brassicaceae* family, which is endemic to southern Europe and Asia (Dzah *et al*., 2020). This plant is a rich source of flavonoid, glucosinolates, protecting against oncogenic mutagenesis and destroying the body cells that are prone to cancerous transformation before they can develop tumors (Belhaj Amor *et al*., 2023; Kaur *et al*., 2013; Xiang *et al*., 2018). *L. latifolium* has many therapeutic function including diuretic, antihypertensive and biological features such as antidepressant and also antiprotozoal effects (Bhat *et al*., 2021).

In previous works, Moringa and Lavandula extracts in tilapia fish burger (Delfino *et al*., 2021), *Zataria multiflora Boiss*. essential oil for catfish burger (Emir Çoban and Tuna Keleştemur, 2017), *Sambucus nigra* extract in salmon burgers (Jonušaite *et al*., 2021), thyme and rosemary extracts for Nile tilapia fillets (Khalafalla *et al*., 2015), olive leaf extract on salmon burger (Khemakhem *et al*., 2019), ginger essential oil and supercritical extract for tilapia fish burger (Mattje *et al*., 2019) were studied. So far, no study has been conducted on the preservative impacts of *L. latifolium* on *S. trutta caspius* burger. Therefore, the aim of present study is to investigate the impacts for extracts obtained by soxhlet, ultrasound and supercritical water on quality features as well as antioxidant, microbiological and sensory attributes of *S. trutta caspius* Kessler fish burger during storage.

Materials and Methods

Fresh *S. trutta caspius* fish with an average weight of 1.5 kg was randomly purchased from a fish market and transported to the laboratory on ice. Other ingredients including sunflower oil, fried flour, breadcrumbs, salt and dry spices were purchased from a local retailer as well as *L. latifolium* plant was obtained from Vezovan City (Isfahan Province, Iran), which was identified by traditional taxonomist and chemical materials were achieved analytically from Merck, Darmstadt, Germany.

After cleaning, *L. latifolium* plant was dried away from sunlight, completely powdered using an industrial mill (SCX600W, China), passed through a laboratory sieve with a suitable mesh (Retsch, Germany) and stored at 5 ℃ until used for further experiments. In this research, three extractions were used as follows:

L. latifolium extraction assisted by soxhlet (LES): *L. latifolium* powder (25 g) was mixed with 500 mL hexane solvent and transferred to a soxhlet apparatus. The mixture was boiled by heating and then kept at atmospheric pressure for 180 min (Kaur *et al*., 2013).

L. latifolium extraction obtained using ultrasound (LEU): Plant powder at 25 g concentration was mixed with 500 mL hexane solvent, which placed in an ultrasound device (Hielscher UP200ST) at 124 W frequency and 25 ℃ for 30 min. After the extraction process, the solution was centrifuged (Domel, Germany) during 10 min at 300 rpm; then, the solvent was removed by a rotary machine (Buchi model B-480, Germany) to obtain extraction (Wang *et al*., 2022).

L. latifolium extraction achieved through supercritical water (LEW): The 25 g *L. latifolium* powder was poured into a cotton bag and placed inside a multipurpose super heater device (System Suprex MPS/225, USA); the final extraction was performed at 60 ℃ a pressure of 20 MPa and for 2 h (Mattje *et al*., 2019).

- ***High performance liquid chromatography* (HPLC) analysis**

Glucosinolate compositions of different extracts (treated samples by LES, LEU and LEW) were analyzed using HPLC, which were performed with the following specifications: HPLC with agilent 1260 Infinity Quaternary LC system equipped by a Zorbax Eclipse Plus C18 column (250 mm × 64.6 mm, 5 µm), 1 mL/min flow rate, sinigrin as an internal standard (0.1-10 mg/L) and glucosinolate detection by comparison with valid standards and also elution profiles of chromatogram identification. The elution flow for glucosinolates were performed by H_2O about 2 min as well as linear gradients of 0 to 60 %, 60 to 100 % and 100 % methanol during 48, 3 and also 3 min, respectively (Lin *et al*., 2021).

- ***Antioxidant assay by 1,1-Diphenyl-2-picrylhydrazyl (DPPH) inhibition***

This function of L. latifolium extract was measured based on ability to inhibit DPPH free radicals. At first, 100 µL extract (6.25, 12.5, 25, 50, 100 and 200 µg/mL) was placed into 96-well plates; then, 100 µL DPPH solution in ethanol (0.2 mM) was added. After incubation for 30 min at room temperature and darkness, the sample absorbance was recorded in the rage of 517 nm wavelength and quercetin was used as a positive control. The radical scavenging activity was calculated as "inhibition percentage":

Eq 1. $$\text{Inhibition percentage (\%)} = [1-(A_i-A_j)/A_c] \times 100\ \%$$

Where A_i, A_j and A_c represented the absorbance of mixture, DPPH solution and also ethanol, respectively (Xiang *et al*., 2018).

- ***TPC (total phenol content) and TFC (total flavonoid content) determination of extracts***

Initially, 0.1 mL extracts were blended with distilled water and then 0.5 mL Folin-Ciocalteu reagent was added. After mixing, the sample was kept at 25 ℃ for 6 min and merged with 0.4 mL of 7.5 % (w/v) sodium carbonate solution. Then, incubation was done at dark and room temperature during 1 h and also absorbance was measured by UV-Visible spectrophotometry at 765 nm wavelength. Finally, TPC was expressed as gallic acid equivalent (GAE) in milligrams per gram (mg GAE/g) of sample (Custódio *et al*., 2015).

The TFC was determined using aluminum chloride and 1 mL extract, 1.25 mL distilled water and also 75 µL nitrite-sodium (5 %) were blended, which placed in dark at 24 °C. After that, 150 µL aluminum trichloride (10 %) was merged to mixture during 5 min; then, 1 mL sodium hydroxide (1 M) was added. Lastly, samples absorbance were recorded at 510 nm wavelength and TFC was reported as mg rutin equivalents in g extract (mg RE/g extract) in this method (Bhat *et al*., 2021).

- ***Preparation of fish burger***

First, the fish of *S. trutta caspius* was washed with water that subjected to decapitation, abdominal emptying, peeling, deboning and also filleting, which were performed manually. Then, fillets were minced using a meat grinder after

washing (Pars Khazar, Iran) with a hole diameter of 4 mm. Minced fish meat (90 %) was mixed with other ingredients including sunflower oil (4 %), fried flour (3 %), water (1.1 %), salt (1 %), dry spices (0.9 %) and plant essence (0.2 %). Then, burger pieces with an approximate weight of 10 grams were prepared using a circular mold and packed separately in polyvinyl chloride-polyethylene bags with zippers. The four treatments were prepared including control fish burger without extract and also fish burgers treated by 0.2 % LES, LEU and LEW.

Fish burgers were transferred to the laboratory for testing and others were frozen at -18 °C to be tested at intervals of 0, 3, 6 and also 8 days.

- ***pH and cooking yield***

Inorder to measure the pH of fish burgers, 10 g sample was mixed with 100 mL distilled water after homogenization and a pH meter (Metrohm 827, Switzerland) was used to read pH according to AOAC (2005). The sample weights were measured using a digital scale (Mettler PM600, Switzerland) with an accuracy of 0.001 before and after cooking to determine cooking yield, which was calculated (Bainy *et al*., 2015):

Eq. 1 Cooking yield (%) =
[cooked weight (g) / raw weight (g)] ×100

- ***Thiobarbituric acid-reactive substance (TBAR) and peroxide value (PV)***

Inorder to determine the amount of TBAR, 5 g fish burger sample was transferred into a laboratory tube containing 5 mL potassium chloride (1 %); then, mixed with 0.25 mL trichloroacetic acid (30 %) and 1 mL TBAR (0.8 %). Next, 5 mL 1-butanol was added to each tube, followed by homogenization; finally, ultraviolet absorption was measured using a spectrophotometer (Shimadzu, model UV 260, Japan) at 533 nm. The results were reported as milligrams of malonaldehyde per kilogram for sample (Fard and Nouri, 2023).

Sample (0.5 g) was mixed with 25 mL acetic acid and chloroform (at a ratio 2:3); next, 1 mL saturated potassium iodide was added, followed by the addition of 30 mL distilled water and 1 mL starch (1 %). The sample was finally titrated with sodium thiosulfate (1 N) until the blue color disappeared and the PVs were calculated (Fard and Nouri, 2023).

- ***Color measurements***

The color assessment for fish burgers was performed on the surfaces of raw material using a colorimeter (Shimadzu, model UV 260, Japan). The results of colorimetric assay were presented based on the L^*, a^* and b^* parameters, where the L^* indicated brightness, the a^* illustrated redness-greenness as well as b^* reflected yellowness-blueness (Delfino *et al*., 2021).

- ***Determination of microbial load (yeast and mold count)***

Fish burgers (10 g) were separated and pounded well in a porcelain mortar; then, 90 mL peptone water (0.1 %) was added at a ratio of 1:10 and homogenized. Afterwards, 1 mL sample inside the mortar was removed and transferred into a sterile plate with a diameter of 8 cm, which 1 mL was poured to a tube containing 9 mL normal saline. Then, 12 to 15 mL agar culture medium (at a temperature of 42 to 44 °C) was added to the plate; finally, mold and yeast enumeration was performed (Dolea *et al*., 2018).

- ***Sensory assessment***

This evaluation was conducted using trained panelists including 15 for both females and males (30 to 40 ages). For sensory analysis, fish burgers were

prepared in sunflower frying oil at 170 °C for 3 min in a deep fryer and then provided to the panel group. Samples were fortuitously presented by three digital counted plates at normal light in laboratory. All panelists served distilled water and unsalted crackers before investigation that samples were pointed by 5 scale including 0: extremely dislike to 5: extremely like (Mattje *et al*., 2019).

- ***Scanning electron microscope (SEM) analysis***

The fish burgers were examined by SEM to observe the microstructure, which samples were stored for 3 day, dried; after that placed in aluminum stubs and coated with gold to determine the microstructure (Pinto *et al*., 2022).

- ***Statistical analysis***

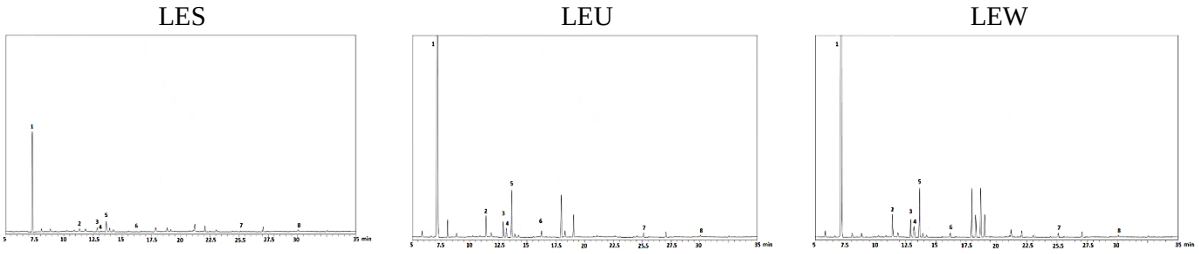
Data were represented as the mean and standard deviation (SD) in triplication and also differences between groups were analyzed using SPSS version 22.0 (SPSS Inc, Chicago, Ill, USA) by one-way analysis of variance (ANOVA). A multivariate experiment was used in a completely randomized design to compare differences between groups. A Duncan multiple range test and variance analysis were used to designate significant differences ($p < 0.05$).

Results and Discussion

- ***Identification of glucosinolate components in extracts***

HPLC chromatogram of glucosinolate substances is identified in LES, LEU and LEW samples according to retention time, which is outlined in Figure 1. The several compounds such as sinigrin, glucoiberin, methylsulfinylpropyl-glucosinolate, glucocochlearin, gluconapin, glucoalyssin, glucotropaeolin and 4-methoxyglucobrassicin were detected at retention times of 7.2, 11.4, 12.9, 13.3, 13.7, 16.2, 25.6 and 30.4 min, respectively. In general, the highest sinigrin and glucosinolates levels were detected in LEU, LEW and also LES extracts. The components of LES and LEW samples were slightly different from each other, which illustrates the efficiency for both treatments in extraction, but the most extraction was related to LEU sample. Glycosinolate compositions in *L. latifolium* play an important role in antimicrobial, antioxidant and cytotoxic effects, which are also precursors of some essential oil substances such as allyl isocyanate (Belhaj Amor *et al*., 2023; Dzah *et al*., 2020). Glucosinolates are hydrolyzed to glucose, hydrogen sulfate and unstable aglycone that are spontaneously converted to a variety of reactive compounds including isothiocyanate, nitrile, epithionitrile, thiocyanate and vinyl oxazolidinethione (Xiang *et al*., 2018). On account of aglycone detection in present study, the formation for different products from this component is related to glucosinolates, pH, metal ions and specifier proteins (Bhat *et al*. 2021). Glycosinolates, polyphenols, carotenoids, colors, anthocyanins and tannins as antimicrobial, antioxidant as well as anticancer compounds are removed effectively by ultrasound from the complex matrix of plant (Wang *et al*., 2022). The ultrasound mechanism is applied to the plant through cavitation action (appropriate intensity) that mechanical influence causes irreversible disruptions in the membrane by sonoporation and allows for successful delivery of materials (Görgüç *et al*., 2022). Glycosinolates (sinigrin, gluconapin, glucoiberin, glucocheirolin, glucoraphanin, glucotropaeolin, gluconasturtiin and glucobrassicin) were isolated using HPLC from different parts

of L. latifolium extract, which sinigrin had the highest level about 90 % (149 to 199 µg/g) (Kaur *et al*., 2013). Sinigrin, glucoiberin, glucoallysin, gluconapin, glucocochlearin, glucotropaeolin and 4-methoxyglucobrassicin have been isolated from L.latifolium L extract (Bhat *et al*., 2021; Dzah *et al*., 2020), which is in line with present research.



Compositons	Peak IDs	Concentration (µg/g)		
	RT(min)	LES	LEU	LEW
[1] 2-proprnyl glucosinolate or Sinigrin	7.2	98.5	305.1	250.4
[2] Glucoiberin	11.4	4	31.3	26.4
[3] Methylsulfinylpropyl-glucosinolate	12.9	7	24.4	21.2
[4] Sec-butylglucosinolate or glucococochlearin	13.3	tr	14.7	10.3
[5] 3-Butenyl glucosinolate or gluconapin	13.7	10	64.9	60.4
[6] 5-methylsulfinylpentyl glucosinolate or glucoalyssin	16.2	tr	4	4.32
[7] Benzyl glucosinilate or glucotropaeolin,	25.6	tr	3	2.8
[8] 4-Methoxy-3-indolylmethyl glucosinolate or 4-methoxyglucobrassicin	30.4	tr	1	0.53

Tr: trace

Fig. 1. HPLC chromatogram related to LES, LEU and LEW (1. sinigrin, 2. glucoiberin, 3. methylsulfinylpropyl-glucosinolate, 4. glucococochlearin, 5. gluconapin, 6. glucoalyssin, 7. glucotropaeolin, and 8. 4-methoxyglucobrassicin).

- ***Comparison of TPC, TFC and antioxidant activity for extracts***

The maximum and minimum for TPC and also TFC were reported in samples of LEU (156.3± 1.4 mg GAE/g and 104.2±1.5 mg RE/g) as well as LES (42.5±0.8 mg GAE/g and 33.2 ± 0.5 mg RE/g), respectively; there was a significant difference between these components in all samples (Figure 2a). Based on the obtained results about antioxidant function (Figure 2b) in distinct concentrations of extract, this factor for LEU and LEW samples were less various from each other. The most inhibition potential for LEU extract and the lowest value were obtained in LES; in general, the most extraction of phytochemical constituents and antioxidant feature were obtained in LEU sample. The inhibition percentages for different extracts containing crude (15.2 to 84.5 %), water (16.6 to 47.6 %), n-butanol (16.0 to 79.4 %), ethyl acetate (19.3 to 83.1 %) and hexane (13.5 to 27.2 %) in various concentrations (6.25, 12.5, 25, 50, 100 and 200 µg/mL) were reported (Xiang *et al*. 2018). The TPC ranged from 0.0 ± 0.0 to 172.4 ± 1.5 mg GAE/g and the highest level was found for ethyl acetate soluble fraction (172.4 ± 1.5 mg GAE/g). The TFC in ethyl acetate (110.8 ± 1.9 mg RE/g) and hexane soluble fractions (0 ±

0.0 mg RE/g) was represented as maximum and also minimum response, respectively (Bhat *et al*., 2021).

According to these results, there was a more correlation between TPC and antioxidant activities for all samples, which is in accordance with previous results (Custódio *et al*. 2015; Görgüç *et al*., 2022).

Compared to other methods, ultrasound-assisted extraction resulted less solvent, shorter time, further efficiency and minimal destruction of heat-sensitive constituents, which was generally considered a green technique (Wang *et al*., 2022). The ultrasound was applied to extract polyphenolic components from propolis and lemon peel, which was an efficient method to extract abundant polyphenols (Görgüç *et al*., 2022). Plant flavonoids are mainly responsible for color, taste, lipid oxidation prevention, enzyme protection and anti-stress physiological features with important role in antioxidant, anti-inflammatory and antimicrobial activities in various studies (Dolea *et al*., 2018; Görgüç *et al*., 2022; Khemakhem *et al*., 2019). The flavonoid extraction using ultrasound from chokeberry (*Aronia melanocarpa L.*) (Görgüç *et al*. 2022) and *Moringa oleifera* L. leaves (Lin *et al*., 2021) with more efficiency was investigated. The results illustrated that TPC, TFC, carotenoid and antioxidant ability of thyme extracts prepared by ultrasound were strongly influenced by extraction conditions (Dolea *et al*., 2018; Dzah *et al*., 2020).

- pH measurement

In Table 1, pH measurements for fish burger including LES showed a decline from 5.99 to 5.77 from day 0 to 8, which was the largest decrease ($p < 0.05$). Overall, pH of samples gradually reduced during storage from day 0 to 8 ($p < 0.05$) and the results represented that *L. latifolium* extract was effective in maintaining this factor. A reduction in acidity can be due to the fact that fish burgers were treated with extracts containing phenolic compounds, preventing microbial growth, protecting fish burgers against internal proteases and avoiding the protein breakdown and also amine production (Khemakhem *et al*., 2019). Researchers have reported an increase in the pH of tilapia fish fillets treated with rosemary and thyme essential oils during refrigeration (Khalafalla *et al*., 2015). The effects of thyme and oregano

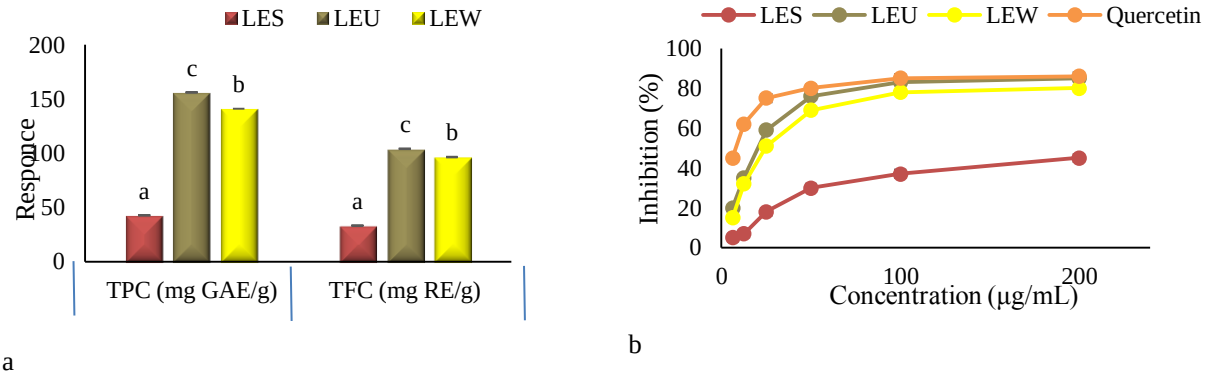


Fig. 2. (a) The TPC (mg GAE/g) and TFC (mg RE/g) levels for extract samples and (b) The antioxidant results (% inhibition) of extracts and quercetino
L. latifolium by soxhlet extraction (LES), *L. latifolium* by ultrasound extraction (LEU), *L. latifolium* by supercritical water extraction (LEW)

essential oils were investigated on the shelf life for salmon burgers, indicating a primary pH of 6.2, which was not significantly different from control, however, pH slightly reduced and then enhanced from day 0 to 14. The initial decline was associated with growth of lactic acid bacteria and the subsequent enhancement in pH was attributed to accumulation for primary spoilage products in fish (Dolea *et al*., 2018). On the other hand, ginger essential oil had no impact on pH in tilapia fish burgers, where this index was reported to be only dependent on storage (Mattje *et al*., 2019). The influences of *Moringa* and *Lavandula* extracts as natural antioxidants founded that storage had no impact on pH of tilapia fish burgers (Delfino *et al*., 2021).

- ***Cooking yield***

The results of cooking yield for fish burgers are outlined in Table 1; in general, treated samples significantly boosted cooking yield compared to control and reciprocal effect between the formulations as well as on 8 days was significant (p <0.05). At the end of storage, the lowest and highest cooking yields were observed in control (78.83 %) and also treated sample consisting of LEU (83.48 %), respectively, which could be related to improvement of water holding capacity or preventing moisture loss during frying (Karimifar *et al*., 2022). The impact of grilling and cooking on texture in tilapia fish burgers indicated that baked samples had a further cooking yield compared to grilled form, which was attributed to the better preservation of moisture (Bainy *et al*., 2015). Also, increasing the level of flaxseed flour was reported to boost the cooking yield for fish burgers (Duman, 2020).

- ***TBAR substances assay***

The TBAR results of fish burgers are demonstrated in Table 1, revealing a significant reciprocal response between the formulations and shelf life (p < 0.05). The level of TBAR displayed a decreasing trend in treated samples by LES, LEU and LEW during 8 days and also an increasing trend in control. The highest TBAR was measured in control (0.83 mg malonaldehyde) and the lowest amount in treated fish burger with LEU (0.33 mg malonaldehyde). On 8th day, the TBAR level in treated sample by LEU was less than permissible limit, which was the most effective in reducing the oxidation rate of fat and spoilage in fish burgers (Dzah *et al*., 2020; Kaur *et al*., 2013; Xiang *et al*., 2018). Researchers have reported that treatment of catfish burgers with the essential oil of *Z. multiflora* reduced TBAR until the 3th day and then elevated it until the 9th day (Emir Çoban and Tuna Keleştemur, 2017). The influences of thyme and oregano essential oils on shelf life for salmon burgers exhibited this index remained constant until the 7th day and then gradually improved in samples (Dolea *et al*., 2018). The application of ginger essential oil prepared by superheat method and Clevenger apparatus in tilapia fish burger exhibited no significant change in TBAR; however, at the end of storage, this factor decreased in all treatments except control (Mattje *et al*., 2019). The *Sambucus nigra* extract had a positive impact on controlling the amount of TBAR in all salmon burger samples during storage, with the lowest increase in TBAR being related to butylated hydroxytoluene (Jonušaite *et al*., 2021). The extracts of *Moringa* and *Lavandula* had no effect on TBAR in tilapia fish burgers during storage (Delfino *et al*., 2021).

Table 1. Results of changes in pH, cooking yield, TBAR and PV for control fish burgers and including *L. latifolium* extract

Tests	pH				Cooking yield (%)			
Shelf life days / Treatments	0	3th	6th	8th	0	3th	6th	8th
C	6.15±0.01^{Aa}	6.10±0.01^{Ba}	6.07±0.05^{CBa}	6.02±0.02^{Ca}	89.45±0.18^{Ad}	86.22±0.23^{Bc}	81.41±0.16^{Cd}	78.93±0.27^{Dc}
LES	5.99±0.03^{Ab}	5.86±0.04^{Bd}	5.79±0.03^{Cc}	5.77±0.04^{Cc}	91.59±0.14^{Aa}	86.27±0.35^{Bc}	83.42±0.13^{Cb}	82.29±0.18^{Db}
LEU	6.11±0.04^{Aa}	6.03±0.03^{Bb}	5.91±0.02^{Cb}	5.94±0.03^{Cb}	89.93±0.16^{Abc}	87.78±0.24^{Ba}	85.50±0.29^{Ca}	83.48±0.31^{Da}
LEW	6.01±0.02^{Ab}	5.93±0.02^{Bc}	5.89±0.04^{Cb}	5.83±0.05^{Cc}	89.88±0.34^{Acd}	87.10±0.13^{Bb}	83.12±0.12^{Cc}	82.07±0.10^{Db}
Tests	**TBAR (mg of malonaldehyde)**				**PV (meqO$_2$/kg)**			
C	0.63±0.04^{Aa}	0.71±0.05^{Aa}	0.73±0.02^{Ba}	0.77±0.06^{ABa}	4.73±0.02^{Da}	4.92±0.04^{Ca}	5.03±0.06^{Ba}	5.13±0.03^{Aa}
LES	0.57±0.02^{Ab}	0.53±0.07^{ABb}	0.48±0.04^{Bb}	0.43±0.03^{Bb}	4.64±0.03^{Ab}	4.53±0.01^{Bb}	4.41±0.07^{Cb}	4.37±0.02^{Cb}
LEU	0.52±0.01^{Ac}	0.48±0.03^{Bb}	0.42±0.02^{Cc}	0.38±0.05^{Cb}	4.49±0.05^{Ac}	4.47±0.02^{Ac}	4.29±0.04^{Bc}	4.22±0.03^{Cc}
LEW	0.55±0.03^{Ac}	0.50±0.04^{Bb}	0.43±0.03^{Cbc}	0.40±0.01^{Cb}	4.57±0.04^{Ac}	4.48±0.05^{Bbc}	4.31±0.03^{Cc}	4.28±0.05^{Cc}

- The results are reported as mean ± standard deviation (Mean ± S.D)
- The data shown in each column with distinct letters (a-d) have significant differences ($p < 0.05$)
- The data in each line with distinct letters (A-D) have significant differences ($p < 0.05$)
- Fish burgers without extract as control (C) and fish burgers with 0.2 % *L. latifolium* by soxhlet extraction (LES), 0.2 % *L. latifolium* by ultrasound extraction (LEU) and 0.2 % *L. latifolium* by supercritical water extraction (LEW)

- *Quantitative analysis of PV*

The results of PV measurement are presented in Table 1, revealing that extract concentration, treatment duration and the formulation versus time interaction had significant effects on this feature ($p < 0.05$). Treated samples by LES, LEU and LEW indicated a reduction in PV of fish burgers during storage; but in control, it represented an increasing trend. The most and least levels were in control (5.16 meqO_2/kg) and also fish burger treated by LEU (4.19 meqO_2 /kg), respectively; which previous studies have also confirmed the antioxidant features of *L. latifolium* (Dzah *et al*., 2020; Kaur *et al*., 2013; Xiang *et al*., 2018). Fish burgers treated with *Z. multiflora* essential oil had lower PVs than untreated burgers during storage (Emir Çoban and Tuna Keleştemur, 2017) and oregano, rosemary and lemon essential oils were reported to prevent the oxidation of fish burgers (Campelo *et al*., 2018). Thyme, rosemary and basil extracts significantly improved PV in all mackerel fish pellets as well as the most was in control and sample with basil (12.98 and 12.77 meqO_2/kg, respectively). Chokeberry leaf extract was employed in burger from dark cutting veal as a natural antioxidant component that was effective in improving the oxidative stability index (Kowalczyk *et al*., 2023).

- *Color determination*

The results for *L**, *a** and *b** as color indices are shown in Figure 3 and also changes in these parameters appeared that treated sample using LES delivered a lower *L** (49.71). In general, the samples turned darker during storage, regarding to *a** color index, the results presented that control and treated fish burger with LES had the highest and also lowest values (11.07 and 3.79, respectively). In terms of *b** color factor, the results revealed that samples became more yellow over storage. In this regard, treated samples containing LEU and LEW with the respective values of 13.88 and also 8.76 proved the most and least *b** parameter. Changes in color factors of *L**, *a** and *b** demonstrated that *L. latifolium* extracts had significant effects in fish burgers ($p < 0.05$); also the interaction influence of time on color indicators was significant ($p < 0.05$). The use of ginger essential oil prepared by superheat method and a Clevenger device demonstrated that formulation and also storage had no significant impact on *L**,

but had an impact on color intensity to became more yellow (Mattje *et al*., 2019). Fish burgers from minced flesh of common barbell supplemented with microalgae illustrated that lower *a** and *b** values were due to more appearance of pale orange in color (Atitallah *et al*., 2019). The *Moringa* and *Lavandula* extracts were found to intensify the yellow discoloration of tilapia fish burgers during storage and treated samples by *Lavandula* had a lower *L** index (Delfino *et al*., 2021).

- ***Microbiological quality (yeast and mold counts)***

Figure 4 illustrates changes in microbial loads, reflecting the significant impacts of *L. latifolium* extracts, treatment duration and formulation on the shelf life for treated samples by LEU and LEW ($p < 0.05$). In total, the amount of microbial load in samples was less than the permissible limit on 8th day (10^7 CFU/g); in current research, mold and yeast counts decreased continuously in all treatments.

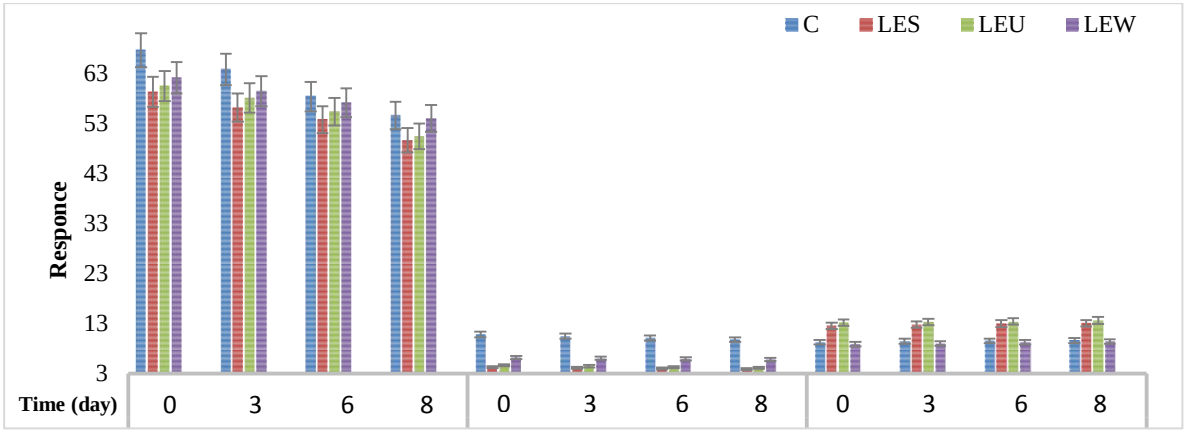


Fig. 3. Color measurements of control fish burgers and containing *L. latifolium* extract
- Fish burgers without extract as control (C), 0.2 % *L. latifolium* by soxhlet extraction (LES), 0.2 % *L. latifolium* by ultrasound extraction (LEU), 0.2 % *L. latifolium* by supercritical water extraction (LEW)

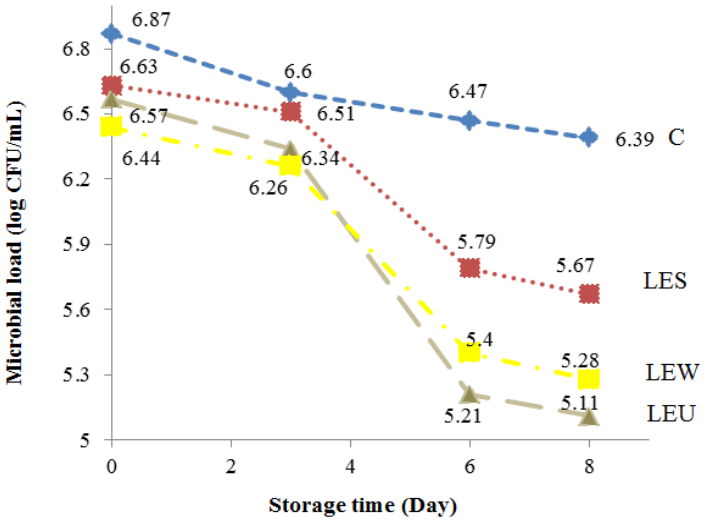


Fig. 4. Variations of microbial load in control fish burgers and enriched with *L. laifolium* extract.

- Fish burgers without extract as control (C), 0.2 % *L. latifolium* by soxhlet extraction (LES), 0.2 % *L. latifolium* by ultrasound extraction (LEU), 0.2 % *L. latifolium* by supercritical water extraction (LEW)

The treated sample by LEU had the greatest inhibitory effect on growth and proliferation of mold and also yeast in fish burgers that antimicrobial properties of *L. latifolium* have been reported for previous studies (Dzah *et al*., 2020; Xiang *et al*., 2018). The essential oil of *Z. multiflora* was noted to reduce the microbial load in all fish burgers (Emir Çoban and Tuna Keleştemur, 2017), while thyme and oregano essential oils could not prevent the microorganism growth in salmon burgers. However, microorganism population seemed to have no effect on microbial spoilage of sample during storage (Dolea *et al*., 2018). The *Moringa* and *Lavandula* extracts were reported to preserve the microbiological safety of tilapia fish burgers after 7 days in the refrigerator, showing a lower load than required by standards of Brazil (Delfino *et al*., 2021). The application of **Ziziphora clinopodioides**/**Rosmarinus** essential oil encapsulated nanoparticles considerably reduced the population of *Escherichia coli O157:H7* and *Staphylococcus aureus* in lamb patties compared to control (Karimifar *et al*., 2022).

- ***Sensory evaluation***

In present study, the treatment effects are evaluated on sensory indicators of appearance, taste, odor, tissue and overall acceptance in fish burgers (Figure 5). In addition, sensory attributes decreased in all treatments over time (8 days), but the reduction trend of treated samples by LES, LEU and LEW was less than control ($p < 0.05$). The evaluators reported that in terms of appearance, the treated fish burger by LEU had a better sensory score (2.84); however, all samples pointed out a decline in the sensory index over time. Regarding the taste index, fish burger treated by LEW demonstrated a better sensory score (2.97) and samples by LEW and also LEU had a significant increase in quality compared to control at the end of the shelf life. The evaluation of odor index indicated that treated sample using LEU obtained the best score (2.95) from the panel and sensory analysis in terms of tissue index exhibited that greatest score belonged to fish burger treated by LEU (2.74) at the 8^{th} day. Regarding the overall acceptance index, it was revealed that the best score was observed in fish burger treated by LEU (2.61) at the end of the storage. The overall results of sensory analysis outlined that treated sample using LES had lower scores than other treatments after 8^{th} day of shelf life, which could be due to the pungent and aromatic characteristics of *L. latifolium*, leading evaluators to perceive a strong aroma and flavor.

Tilapia fish burgers including ginger essential oil prepared by superheat and Clevenger methods were efficiently preserved; however, samples with this essential oil using supercritical CO_2 had a lower tissue score than control (Mattje *et al*., 2019). The influence of *Moringa* and *Lavandula* extracts was examined on tilapia fish burgers, it was shown that there was no significant correlation between shelf life and formulation. Regarding to taste, treated samples by *Moringa* extract and the control displayed an adequate quality, but *Lavandula* indicated a lower this feature, which generally these had a negative impact on the overall acceptance (Delfino *et al*., 2021). The effects of essential oil and extract for *Sambucus nigra* were assessed on salmon burgers that all treatments demonstrated good scores in terms of color, odor, taste as well as overall acceptance (Jonušaite *et al*. 2021).

- ***Microstructure of fish burger samples***

Figure 6 depicts the structure images of

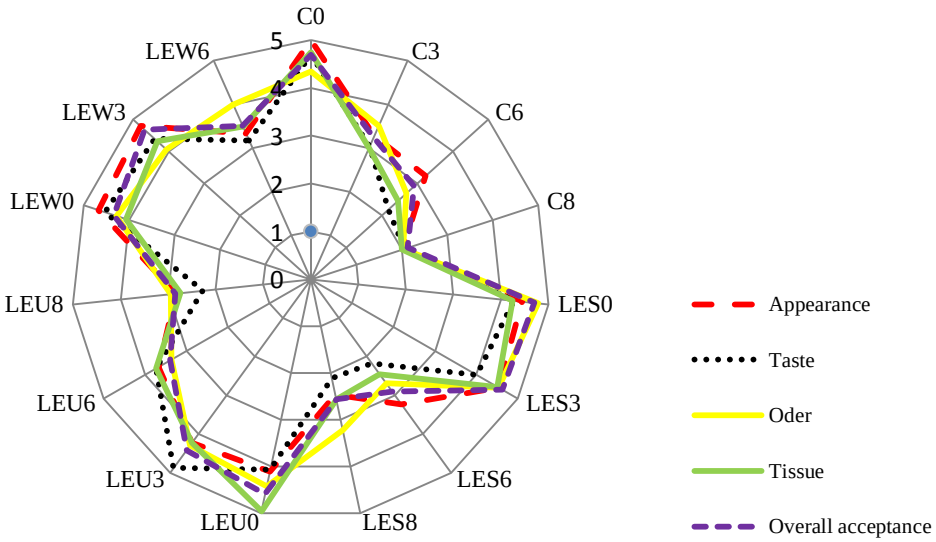


Fig. 5. Sensory evaluation of control fish burgers and enriched with *L. latifolium* extract.
C0, C3, C6, C8: Control (C) on 0, 3, 6 and 8 days of storage/ LES0, LES3, LES6, LES8: treatments by soxhlet extraction on 0, 3, 6 and 8 days of storage/ LEU0, LEU3, LEU6, LEU8: treatments by ultrasound extraction on 0, 3, 6 and 8 days of storage/ LEW0, LEW3, LEW6, LEW8: treatments by supercritical water extraction on 0, 3, 6 and 8 days of storage

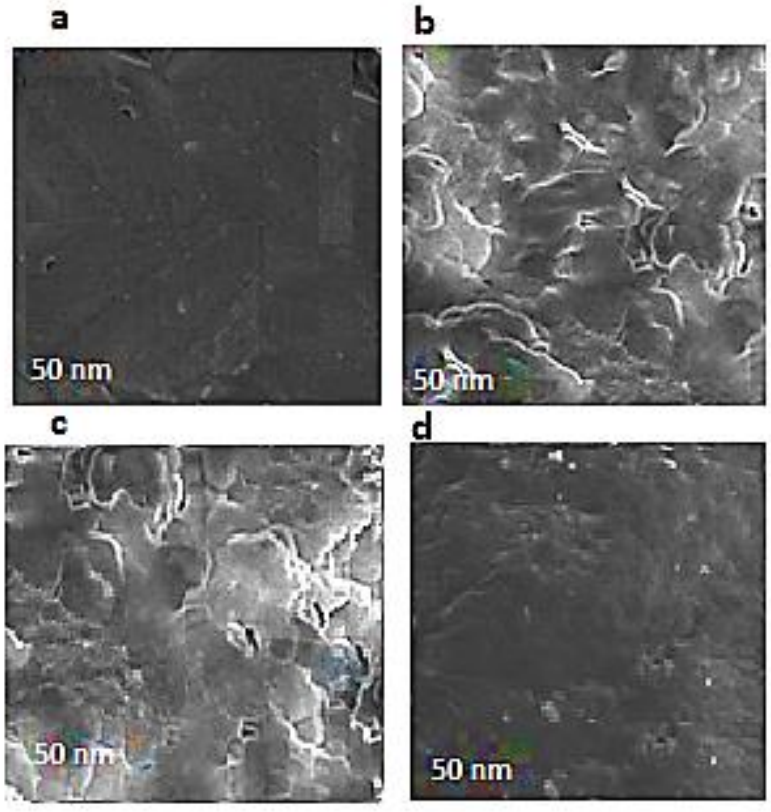


Fig. 6. SEM images (a) control, (b) LES, (c) LEU and (d) LEW in fish burgers on 3th day of shelf life.
- Fish burgers without extract as control (C), 0.2 % *L. latifolium* by soxhlet extraction (LES), 0.2 % *L. latifolium* by ultrasound extraction (LEU), 0.2 % *L. latifolium* by supercritical water extraction (LEW)

Conclusion

Sinigrin and glucosinolates were recognized as the main components through HPLC for *L. latifolium* extract and also TPC, TFC with antioxidant traits were obtained in LEU sample. The results of experiments showed that treated samples had less TBAR level than permissible limit at the end of storage and PV showed a declining trend in treatments during 8 days. The *L** and *b** revealed that fish burgers turned darker and also more yellow during storage, respectively. Treatments decreased the growth of mold and yeast as well as sensory evaluation was acceptable in fish burgers, which was greater in treated sample by LUE with a smooth surface obtained in SEM compared to others. Based on our results, *L. latifolium* extract could increase the quality and microbial safety of burger fish; thus, after confirmation by complementary tests that can be used as a natural preservative in foods.

References

Atitallah, A. B., Barkallah, M., Hentati, F., Dammak, M., Hlima, H. B., Fendri, I., Attia, H., Michaud, P. & Abdelkafi, S. (2019). Physicochemical, textural, antioxidant and sensory characteristics of microalgae-fortified canned fish burgers prepared from minced flesh of common barbel (Barbus barbus). Food Bioscience, 30, 100417. https://doi.org/10.1016/j.fbio.2019.100417

Anon. (2005). Association of Officiating Analytical Chemists (AOAC). Official method of analysis of the AOAC. 18th ed. United States: AOAC.

Bainy, E.M., Bertan, L.C., Corazza, M.L. & Lenzi, M.K. (2015). Effect of grilling and baking on physicochemical and textural properties of tilapia (Oreochromis niloticus) fish burger. Journal of Food Science and Technology, 52, 5111-5119. https://doi.org/10.1007/s13197-014-1604-3

Bagherikakash, S., Hojjatoleslamy, M., Babaei, G. & Molavi, H. (2022). Hydrolysis kinetics and electrophoresis pattern of the impact of the kiwi fruit actinidine on different proteins of Rainbow Trout meat. Journal of Food Biosciences and Technology, 12(4), 57-76. https://doi.org/10.30495/JFBT.2022.20318

Belhaj Amor, G., Ben Farhat, M., Beji-Serairi, R., Selmi, S., Saidani-Tounsi, M. & Abdelly, C. (2023). Impact of cooking treatments on nutritional quality, phytochemical composition and antioxidant properties of Lepidium sativum L. seeds. Journal of Food Measurement and Characterization, 1-9. https://doi.org/10.1007/s11694-023-01851-6

Bhat, R., Faiz, S., Ali, V., Khajuria, M., Mukherjee, D. & Vyas, D. (2021). Effect of temperature and insect herbivory on the regulation of glucosinolate-myrosinase system in Lepidium latifolium. Physiologia Plantarum, 172(1), 53-63. https://doi.org/10.1111/ppl.13289

Campelo, P.H., Sanches, E.A., De Barros Fernandes, R.V., Botrel, D.A. & Borges, S.V. (2018). Stability of lime essential oil microparticles produced with protein-carbohydrate blends. International Food Research Journal, 105, 936-944. https://doi.org/10.1016/j.foodres.2017.12.034

Custódio, L., Patarra, J., Alberício, F., Da Rosa Neng, N., Nogueira, J.M.F. & Romano, A. (2015). Phenolic composition, antioxidant potential and in vitro inhibitory activity of leaves and acorns of Quercus suber on key enzymes relevant for hyperglycemia and Alzheimer's disease. Industrial Crops and Products, 64, 45-51.

https://doi.org/10.1016/j.indcrop.2014.11.001

Delfino, L., Mattje, L., Silva, M., Araujo, M., Tormen, L. & Bainy, E. (2021). Evaluation of Moringa and Lavandula extracts as natural antioxidants in tilapia fish burger. Journal of Culinary Science and Technology, 1-16. https://doi.org/10.1080/15428052.2021.1883494

Dolea, D., Rizo, A., Fuentes, A., Barat, J. & Fernández-Segovia, I. (2018). Effect of thyme and oregano essential oils on the shelf life of salmon and seaweed burgers. International Journal of Food Science and Technology, 24(5), 394-403. https://doi.org/10.1177/1082013218759364

Duman, M. (2020). Nutritional value and sensory acceptability of fish burger prepared with flaxseed flour. Journal of Food Science and Technology, 42. https://doi.org/10.1590/fst.27920

Dzah, C.S., Duan, Y., Zhang, H., Wen, C., Zhang, J., Chen, G. & Ma, H. (2020). The effects of ultrasound assisted extraction on yield, antioxidant, anticancer and antimicrobial activity of polyphenol extracts: A review. Food Bioscience, 35, 100547. https://doi.org/10.1016/j.fbio.2020.100547

Emir Çoban, Ö. & Tuna Keleştemur, G. (2017). Qualitative improvement of catfish burger using Zataria multiflora Boiss. essential oil. Journal of Food Measurement and Characterization, 11, 530-537. https://doi.org/10.1007/s11694-016-9420-2

Fard, M.N. & Nouri, M. (2023). New formulation of fermented sausages towards healthier and quality rectification by adding Ferulago angulata essential oil. Bollettino della Società Italiana di Biologia Sperimentale. https://doi.org/10.4081/jbr.2022.10702

Görgüç, A., Gençdağ, E., Demirci, K., Vayiç, A. & Yılmaz, F.M. (2022). The effect of high-power ultrasound pretreatment on drying efficiency and bioactive compounds of chokeberry (Aronia melanocarpa L.). International Journal of Food Science and Technology. https://doi.org/10.1177/10820132221094787

Jonušaite, K., Venskutonis, P.R., Martínez-Hernández, G.B., Taboada-Rodríguez, A., Nieto, G., López-Gómez, A. & Marín-Iniesta, F. (2021). Antioxidant and antimicrobial effect of plant essential oils and Sambucus nigra extract in salmon burgers. Foods, 10(4), 776. https://doi.org/10.3390/foods10040776

Karimifar, P., Saei-Dehkordi, S.S. & Izadi, Z. (2022). Antibacterial, antioxidative and sensory properties of Ziziphora clinopodioides–Rosmarinus officinalis essential oil nanoencapsulated using sodium alginate in raw lamb burger patties. Food Bioscience, 47, 101698. https://doi.org/10.1016/j.fbio.2022.101698

Kaur, T., Hussain, K., Koul, S., Vishwakarma, R. & Vyas, D. (2013). Evaluation of nutritional and antioxidant status of Lepidium latifolium Linn.: a novel phytofood from Ladakh. PLOS One 8(8), e69112. https://doi.org/10.1371/journal.pone.0069112

Kaviyani, F.E., Naeemi, A.S. & Salehzadeh, A. (2020). Acute toxicity and effects of titanium dioxide nanoparticles (TiO2 NPs) on some metabolic enzymes and hematological indices of the endangered Caspian trout juveniles (Salmo trutta caspius Kessler, 1877). Iranian Journal of Fisheries Sciences, 19(3), 1253-1267. https://doi.org/10.22092/ijfs.2019.119319

Khalafalla, F.A., Ali, F.H. & Hassan, A.R.H. (2015). Quality improvement and shelf-life extension of refrigerated Nile

tilapia (Oreochromis niloticus) fillets using natural herbs. Beni-Suef University Journal of Basic and Applied Sciences, 4(1), 33-40. https://doi.org/10.1016/j.bjbas.2015.02.005

Khemakhem, I., Fuentes, A., Lerma-García, M.J., Ayadi, M.A., Bouaziz, M. & Barat, J.M. (2019). Olive leaf extracts for shelf life extension of salmon burgers. Journal of Food Science and Technology, 25(2), 91-100. https://doi.org/10.1177/1082013218795816

Kowalczyk, M., Domaradzki, P., Materska, M., Florek, M., Kaliniak-Dziura, A., Skałecki, P. & Pabich, M. (2023). Effect of the addition of chokeberry leaf extract on the physicochemical and sensory properties of burgers from dark cutting veal. Food Chemistry, 399, 133978. https://doi.org/10.1016/j.foodchem.2022.133978

Lin, X., Wu, L., Wang, X., Yao, L. & Wang, L. (2021). Ultrasonic-assisted extraction for flavonoid compounds content and antioxidant activities of India Moringa oleifera L. leaves: Simultaneous optimization, HPLC characterization and comparison with other methods. Journal of Applied Research on Medicinal and Aromatic Plants, 20, 100284. https://doi.org/10.1016/j.jarmap.2020.100284

Mattje, L.G.B., Tormen, L., Bombardelli, M.C.M., Corazza, M.L. & Bainy, E.M. (2019). Ginger essential oil and supercritical extract as natural antioxidants in tilapia fish burger. Journal of Food Processing and Preservation, 43(5), e13942. https://doi.org/10.1111/jfpp.13942

Mooliani, H. & Nouri, M. (2021). Optimization of oxidative, physical stability and microbial characteristics of salad dressing emulsions based on avocado and whey protein combined with mint (Mentha spicata L) extract. Journal of Food Measurement and Characterization, 15(6), 5713-5724. https://doi.org/10.1007/s11694-021-01131-1

Nouri, M. & Shafaghi Rad, M. (2021). Encapsulation of Tribulus terrestris and Valeriana officinalis extracts in nanoliposomes and evaluation of its antibacterial and antioxidant properties. Journal of Food Biosciences and Technology, 11(2), 59-68. https://doi.org/1001.1.22287086.2021.11.2.6.0

Pinto, L.A.D.M., Razente, R.A., Benito, C.E., Gubert, L., Stefanello, L.R., Simões, E.P., Júnior, R.C.D.S., Monteschio, J.D.O. & Fernandes, J.I. (2022). Clove essential oil (Syzygium aromaticum L.) as a natural preservative to improve the shelf-life of chicken patties with different degrees of myopathy. Journal of Food Processing and Preservation, 46(11), e17037. https://doi.org/10.1111/jfpp.17037

Wang, S., Song, L., Li, J., Yang, R., Zhai, J., Zhang, H., Liang, Y., Zhou, W., Cui, Z. & Qian, X. (2022). Optimization of ultrasonic-assisted extraction of polysaccharide from fig leaves and its antioxidant activity. Journal of Food Science and Technology, 42. https://doi.org/10.1590/fst.77922

Xiang, Y., Haixia, W., Lijuan, M. & Yanduo, T. (2018). Isolation, purification and identification of antioxidants from Lepidium latifolium extracts. Medicinal Chemistry Research, 27, 37-45. https://doi.org/10.1007/s00044-017-2042-3