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Research article

Low-alcohol light beer enriched with olive leaves extract: Cold mashing technique associated with interrupted fermentation in the brewing process ☆

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GRAPHICAL ABSTRACT

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ABSTRACT

Background: Beer is the most consumed alcoholic beverage globally, and the demand for differentiated beers with peculiar characteristics has intensified among beer consumers, creating a significant market niche. In this study, we developed a low alcohol light craft beer enriched with olive leaf extract (*Olea europaea* L.). The cold mashing technique associated with interrupted fermentation was used in the mashing step. Different concentrations of olive leaf extract (0.5, 1.0 and 2.0%) were added at the maturation stage. The samples were characterized by physicochemical parameters, phenolic and polyphenolic content, bioactive compounds, antioxidant potential, and microbiological quality.
Results: The cold mash technique associated with interrupted fermentation provided a low-alcohol beer (≅1.3%). The bitterness dimension (19.0 to 23.2 IBU) and color (9–17 EBC) parameter were in accordance with the Beer Judge Certification Program (BJCP) for the American Blond Ale-style. The addition of the extract enriched the content of total phenolics (171.09 to 437.4 mg GAE/mL) and polyphenolic (221.4 to 729.0 mg/L). Coumaric, ferulic, and cinnamic phenolic acids were detected in appreciable amounts in the beers. Oleuropein was the major compound in the beverage and plant extract. After adding 2% extract, the ABTS and DPPH radical scavenging activity, as well as the ferric reduction power, increased in beers by 28.4%, 449.1%, and 120.5%, respectively.

☆ Audio abstract available in Supplementary material.

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Conclusions: The extract of *O. europaea* L. promoted the enrichment of low-alcohol beer samples with bioactive compounds and antioxidant potential. The results obtained indicated the potential use of *O. europaea* L. extract as a natural oxidant in other beverages and food products.

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1. Introduction

Beer is one of the most consumed beverages in the world, gaining notoriety in the Brazilian market since it was introduced in the country. According to the Brazilian Beer Industry Association, the country ranks third in annual beer production, having produced more than 14 billion liters in 2021 [1]. Global beer consumption reached 185.6 million kiloliters in 2021, and the Chinese market is the largest consumer of that beverage (360 million hectoliters), followed by the North American market (241 million hectoliters) [2].

Non-alcoholic and low-alcohol beers are also part of this market segment. According to the National Association of the Beer Industry [3], the consumption of non-alcoholic beers increased 30% in 2021, reaching a total consumption of 257 million liters per year. This shows an emerging market and an opening for the production of new varieties of non-alcoholic and low-alcohol beverages. The worldwide non-alcoholic beer market expanded from $18.44 billion in 2022 to $20.16 billion in 2023, reflecting a compound annual growth rate (CAGR) of 9.3% [4].

The rapid growth of the non-alcoholic beer market is associated with several factors, including new beverages and traffic rules and legislation, health concerns, and religious motives. Within this framework, efforts have been made to produce non-alcoholic and low-alcohol beverages that maintain the same palatable sensations as their alcoholic counterparts [5,6].

Non-alcoholic beers can have an alcohol strength by volume (ABV) of up to 0.5%. On the other hand, in many countries such as the United Kingdom and USA, beverages with an alcohol strength of 0.5% to 1.2% by volume are considered low-alcohol beers [7]. In Brazil, beers with up to 2.0% (v/v) alcohol strength are classified as low-alcohol beers [8].

Producing high-quality non-alcoholic or low-alcohol beers with an attractive sensorial profile is still challenging, but the industry has sought new production methods that focus on improving the flavor and aromatic profile of these beers. The cold mashing technique is an alternative for producing low-alcohol beers, which aims to extract the flavor and color of the malt, leaving more body to the beer. This technique also decreases the extraction of fermentable sugars, being favorable for obtaining a low-alcohol beer, and can be used in conjunction with interrupted fermentation to produce alcohol-free beer [7,9].

Cold mashing is a relatively uncommon technique that is employed primarily by a select group of experimental microbreweries to craft unique beer specialties. Using this method, malt and water are blended without additional heating. Following this, the wort (extract) and brewers' grain are separated, bypassing the traditional resting or partial mashing warming steps. After the cold mashing and lautering, the resulting wort is brought to a boil to eliminate undesirable germs, mirroring the conventional brewing process [10].

Beer can be classified in different ways, one of which is associated with the fermentation process that is carried out, including the different types of yeast used and the temperature of the process [11,12]. According to the Beer Judge Certification Program (BJCP), each style must follow a standard and specific characteristics based on the BJCP style guide [13].

Within the group of Ale beers, the American Blonde Ale is an American craft beer that is refreshingly easy to drink due to its light flavor, with malty notes, and a soft touch of hops, and is very suitable for drinking in hot environments. It has a pale yellow to golden color, with white foam of up to medium volume; the bitterness intensity is between low and medium [13,14], with attractive fruit, hop, or malt character notes. Well-balanced and clean, it is a refreshing drink without aggressive flavors. Smooth without harsh bitterness or astringency, and medium to high carbonation. Medium-dry to somewhat sweet finishes, and no diacetyl. According to the BJCP, the American Ale beer style has a bitterness of 15 to 28 IBU, SRM (Standard Reference Method) between 3 and 6, OG (Original Gravity) between 1.038 and 1.054, FG (Final Gravity) between 1.008 and 1.013, and ABV (alcohol by volume) ranges from 3.8 to 5.5% [13].

The high consumption of this beverage raises interest in its nutritional properties and benefits for human health. One of its main characteristics is the high content of phenolic compounds from hops and malt, which are directly associated with the prevention of oxidative stress in those who consume it, and can be ingested in an alcoholic, low-alcohol, and alcohol-free presentation [15].

Phenolic compounds can present several biological activities and are characterized as substances that generally have good antioxidant potential, being efficient in capturing free radicals [16]. The studies by González et al. [17], Ciont et al. [18], and Alrugaibah et al. [19] demonstrated that the extracts obtained from olive leaves are a good source of phenolic compounds, especially flavonoids, which give them great potential to be used to benefit human health.

Olea europaea L. is a plant from the Oleaceae family that is cultivated primarily for its fruit, the olive. The extract from this plant's leaves has medicinal benefits. In the food industry, it has been used for its antioxidant properties due to phenolic compounds that prevent oxidative reactions. The central molecule responsible for these benefits is oleuropein and, to a lesser extent, tyrosol, caffeic acid, and hydroxytyrosol [20].

Oleuropein, a glycoside characterized by its bitter taste and astringency, is abundant in the leaves, trunk, and fruits. This phenolic compound has pharmacological and biological properties such as anti-inflammatory, antioxidant, antimicrobial action, anti-cancer potential, and cardio-protective activity. It interferes with lipid metabolism and reduces weight and oxidative stress [21].

Thus, this study aimed to develop and characterize a low alcohol light craft beer with commercial olive leaf (*O. europaea* L.) extract incorporated. The main focus was to enrich the beverage with bioactive compounds with antioxidant, antimicrobial, and cardio-protective properties. The physicochemical quality parameters and dimension were: color (EBC), final pH, total acidity, alcohol content (ABV), international bitterness units (IBU), apparent extract, real extract, total phenolic and polyphenolic content, phenolic acid, and antioxidant potential were evaluated in the beer samples.

2. Materials and methods

2.1. Inoculum preparation and fermentation process

The commercial yeast *Saccharomyces cerevisiae* (Ale S-04. Fermentis - Lesaffre. France) was used as a fermenting culture in wort fermentation to obtain low-alcohol beers. The lyophilized yeast was rehydrated in a small amount of wort and used as an inoculum at a concentration of 0.575 g/L_{wort}, following the manufacturer's recommendations.

The lyophilized *O. europaea* L. extract containing 20% oleuropein was purchased from a company specialized in vegetable extracts and natural products (ActiveCaldic Company, Paloça, SC, Brazil).

The brewing process followed the standards recommended by the beer style guide of the BJCP [13], seeking to produce a beer of low-alcohol, but with flavor characteristics that are similar to the American Blonde Ale style. A batch of beer was produced on a pilot scale in a microbrewery in the city of Salgado Filho, Paraná, Brazil. In the brewing process, a three-block system consisting of a stove and three pans with a capacity of 50 L was used. Batches of 30 L of beer were produced by using Patogonia Pilsen and Chateau Melano malts (Vêneto Mercantil. Flores da Cunha. Brazil). The malt was broken in a 4RM-PREMIUM electric roller mill (Amantes da Breja, SP, Brazil).

The cold mashing technique was used to prepare the wort, which consists of cold mashing under a controlled temperature (10°C) for 8 h, according to the methodology described by Dalberto et al. [9]. In the mashing stage, the wort was recirculated through the brewer's spent grain by using a recirculating pump that was previously sanitized with peracetic acid (0.2%). After cold mashing, the wort was boiled for 60 min, hopping being carried out after 5 min of boiling (0.25 g/L, bittering hop) and at the end of the process (0.5 g/L, aroma hop). Nugget and Cascade hops (Barth Haas - LNF Latino América, Brazil) were used as bittering and aroma hops, respectively.

The hopped wort was cooled to 20°C, inoculated with yeast (0.575 g/L_{wort}), and fermented at a temperature of 15°C until the fermented broth reached a 1.005 g/cm^3 density. Fermentation was carried out in a fermentation bucket with an airlock placed inside a refrigerator with a temperature (15°C) that was controlled by thermostat.

O. europaea L. extract at concentrations of 0.5%, 1.0%, and 2.0% (w/v) were added to the beers in the maturation stage, which occurred under atmospheric pressure for 14 d at 3°C. After maturation, the beers were transferred to barrels with a capacity of 10 L and subjected to forced carbonation with CO_2 cylinders attached to the barrels. Carbonation occurred under constant pressure (1 kg/cm^2 of CO_2) controlled by a pressure valve model FR-420B/FSA (Famabras, SP, Brazil) for 5 d at 3°C. The carbonated beer was bottled in 600 mL amber glass bottles that were previously sanitized with 0.2% peracetic acid. The bottling was conducted using a counter-pressure filling machine for PEGAS model bottles (All Brew, Erechim, RS, Brazil).

The carbonated bottles were then subjected to slow pasteurization (62°C, 20 min) and were kept under refrigeration at 4°C until analysis. Fig. 1 shows the flowchart of the brewing process which was followed in this study.

2.2. Physicochemical quality and microbiological analyses

Beer samples were decarbonized before physicochemical analysis by sonication in an ultrasonic bath (ME18163A01, Cristófoli, Brazil) for 15 min, and filtered on Whatman® grade 1 qualitative filter to remove any suspended particles.

The physicochemical analyses that were carried out on the control beer samples (B0: without extract addition) and beers enriched with *O. europaea* L. extract (B05: 0.5%, B1: 1%, and B2: 2% w/v) were: volatile acidity (neutralization volumetry), final pH (ASBC Beer-2 method), alcohol content (gas chromatography, ASBC Beer-4 method), apparent extract (ASBC Beer-3 method), real extract (ASBC Beer-5 method) and original extract (ASBC Beer-6 method), density (ASBC Beer-2 method), calories (ASBC Beer-33 method) [22]. Foam stability was analyzed following the protocol described by Kanauchi et al. [23]. The color and bitterness dimensions were also determined in the samples, respectively (EBC - European Beer Color, ASBC Beer-10 method; and IBU - international bitter units, ASBC Beer-10 method).

The microbiological quality of the beer samples was evaluated by searching for coliforms at 35°C, following the requirements of the Brazilian law [24].

2.3. Analysis of antioxidant activity, total phenolics, polyphenolics and phenolic compounds (HPLCs)

The antioxidant potential of *O. europaea* L. extract and beer samples was evaluated by their ability to scavenge ABTS [25] and DPPH [26] radicals, as well as by the ferric ion reducing antioxidant power (FRAP) [27], following the methods that are described in the scientific literature. Total phenolic content was determined by the Folin-Ciocalteau method [28], and the polyphenol content was evaluated following the protocol described by the European Brewery Convention [29]. Phenolic acids and flavonoids were analyzed through high-performance liquid chromatography (HPLC-DAD) following the previously described protocol [30].

3. Results and discussion

3.1. Physicochemical and microbiological parameters of quality

Table 1 shows the physicochemical quality parameters that were analyzed in beer samples without the addition of extract (B0) and with the addition of different concentrations of extract (B05, B1, and B2).

The alcohol content of the beer samples that were produced in this work ranged from 1.3 to 1.4% (v/v); therefore, they were classified as low-alcohol beers in accordance with Brazilian legislation (Normative Instruction n°65 of December 2019 of the Ministério da Agricultura, Pecuária e Abastecimento).

This study used the non-enzymatic cold mashing technique associated with the interruption of fermentation (interrupted fermentation) before the yeast completely assimilated the sugars as a tool to obtain a beverage with low-alcohol content. The process was ineffective for obtaining a non-alcoholic beer (up to 0.5% v/v of ethanol). However, it was possible to obtain a beer with a reduced alcohol content according to the Brazilian law.

A study described by Dalberto et al. [9] that used the cold mashing technique made it possible to obtain beers with an alcohol strength ranging from 0.97 to 2.35% (v/v). The cold mashing technique contributed to the reduction of the wort boiling time and reduced the required mass of hops, which is advantageous from a cost-benefit point of view. Additionally, beers that are produced through the cold mashing technique align with the trend toward creating beers with reduced alcohol content and fewer calories [10]. The beers obtained had a low caloric value (12 to 15 Kcal/100 mL) and adding olive leaf extract did not promote changes in energy content. The beers obtained are classified as light beers since they present a caloric value of less than 35 kcal/100 ml according to Brazilian legislation [8]. It is worth

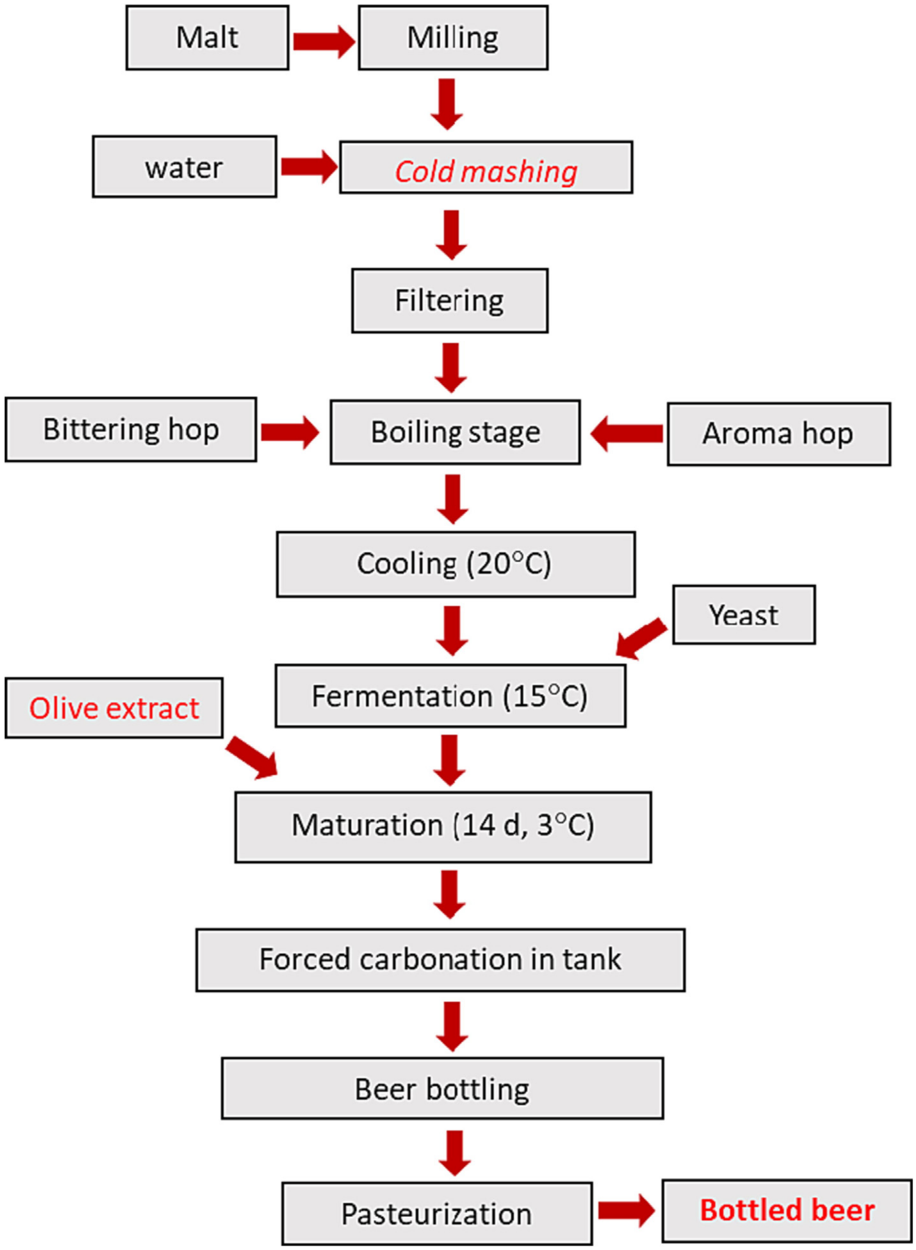

Fig. 1. Flowchart of the brewing process.

Table 1
Beer samples' physicochemical profile and microbiological quality.

Physicochemical parameters	Beer samples			
	B0	B05	B1	B2
Alcohol content (% v/v)	1.4[a]	1.3[a]	1.3[a]	1.3[a]
Calories (Kcal/100 mL)	15.0[a]	12.0[a]	14.0[a]	15.0[a]
Apparent extract (°P)	1.2[a]	1.2[a]	1.3[a]	1.3[a]
Real extract (°P)	1.8[a]	1.6[a]	1.8[a]	1.9[a]
Original extract (°P)	3.7[a]	2.8[b]	3.6[a]	3.7c[a]
Bitterness (IBU)	22.5[a]	23.25[a]	21.0[a]	19.0[a]
Original gravity - OG (g/cm^3)	1.013[a]	1.013[a]	1.013[a]	1.013[a]
Final gravity – FG (g/cm^3)	1.005[a]	1.005[a]	1.005[a]	1.005[a]
Color (EBC)	9.0[c]	9.0[c]	15.0[b]	17.0[a]
Volatile acidity (mEq/L)	12.0[a]	12.0[a]	12.0[a]	12.0[a]
pH	5.0[a]	5.0[a]	4.9[a]	4.9[a]
Foam reduction (%)	37.5[b]	33.3[b]	33.3[b]	67.0[a]
Coliforms at 35°C (MPN/mL)[*]	nd[#]	nd[#]	nd[#]	nd[#]

[*] Most Probable Number/mL. B0: Control beer - no added extract. B05: 0.5% extract. B1: 1.0% extract. B2: 2.0% extract. [#]Not detected. Different letters on the same line indicate a significant difference at the 95% confidence level ($p < 0.05$).

noting that low-carb and low-alcohol beers are presented as healthier consumption options and have gained market share in recent years.

An analytical parameter that is widely used by brewers in the production of beers is the value of beer extract. Beer extract can be comprehended as real extract, apparent extract, and original extract. The real extract represents all the solids that are present in the beer and is related to the beverage's body, indicating the amount of sugars that are present. The apparent extract is a critical parameter in the fermentation of low-malt beer. This variable indicates the degree of fermentation because it corresponds to the total residual concentration of the three main assimilable sugars in the wort (glucose, maltose, and maltotriose). The brewing process concludes upon achieving the desired apparent extract concentration [31]. The original extract is the amount of substances (wort extract) of the wort that give rise to the beer and are expressed in percentage (%) by weight [32].

All the beer samples obtained showed low values of real, apparent, and original extract, with no statistically significant differences between samples for apparent and real extract at a 95% significance level ($p < 0.05$). According to Brazilian legislation, soft beer is the one that has an original extract equal to or greater than 5 and less than 10.5 percent, by weight. The beers produced in this study showed values of original extract between 2.8°P (B05) and 3.7°P (B0 and B2). This shows that the cold mashing technique promoted a reduced extraction of sugars, dextrins, and proteins from the malt, which made it possible to obtain a soft beverage with low caloric value and low alcohol content.

The bitterness of the beers ranged from 19.0 IBU (B2) to 23.25 IBU (B05) and is in accordance with the BJCP [13] reference values for the American Blond Ale style. Bitterness is largely derived from the content of iso-α-acids that are generated during the boiling of the hop-added wort [33].

The original gravity (OG) of the beer samples, measured before wort fermentation, was 1.013 g/cm^3. After fermentation, the density of the beers dropped to 1.005 g/cm^3 (final gravity, FG). The FG value we found in the beers produced was below the one that is recommended by the BJCP for the American Blond Ale style, which is justified by the reduced content of extracts in the beverage. The reduced content of extract in the beverage is due to a lower extraction of soluble solids from the malt by the cold mashing technique, hence a lower content of fermentable sugars in the wort.

Regarding the color of the beers, we noticed that adding plant extract intensified this parameter, with a variation of 9.0 EBC in samples B0 (control) and B05 (0.5% extract) to 15.0 EBC in the B1 samples (1% extract) and 17.0 in B2 (2% extract). In this context, the samples with the highest extract content (B1 and B2) did not fit into the American Blonde Ale style, whose value limit is 11.82 EBC [13].

Another interesting aspect is that adding the extract to the beer did not influence the values of pH (4.9–5.0) and volatile acidity (12.0 mEq/L).

The organic acids that yeast produces during fermentation are responsible for the acidity of beers and impact the sensory acceptance of the beverage as they influence its flavor and aroma. Acetic acid constitutes the primary component of volatile acids that are found in beer. Typically falling within the 57–145 mg/L range, its threshold varies between 71 and 200 mg/L across beer varieties. The presence of acetic acid imparts an unfavorable taste to beer and if its concentration surpasses the taste threshold, it can lead to a considerable drop in quality [34].

Foam stability is a crucial element of beer quality and is valued by consumers and brewers. Adding 0.5% and 1% extract in beer does not contribute to a change in foam stability. On the other hand, when 2% extract (B2) was added, the foam percentage was reduced by 67%. Higher concentrations of the extract could decrease the surface tension of the system, promoting instability in the foam. A particular study conducted by Guglielmotti et al. [35] showed that including *O. europaea* L. leaves in beer samples significantly elevated polyphenol content. This increase in polyphenols was associated with an enhanced colloidal instability of the beer.

The surface tension, viscosity, and density of the beer play pivotal roles in the formation, motion, and surface stability or lifespan of the bubbles [36]. Aliyari et al. [37] discovered a noteworthy correlation between the foaming characteristics of protein dispersions and surface hydrophobicity. Their research revealed a distinct inverse relationship between the level of surface hydrophobicity and the foaming capacity of protein–phenolic complexes, particularly as the concentration of phenolic compounds increased.

Contrary to what was observed in our study, Francesco et al. [38] reported that beer samples enriched with phenolic extracts showed better stability in terms of turbidity, color formation, and foam quality. In relation to the foam quality, the authors mention that the presence of tannins in the phenolic extracts that were added in the beers studied are responsible for foam stability. According to these authors, some tannins present in the phenolic extracts demonstrate a protective effect against foam collapse. Mazengia et al. [39] reported that adding *Moringa stenopetala* (LEMS) leaf extract contributed to foam stability and attributed

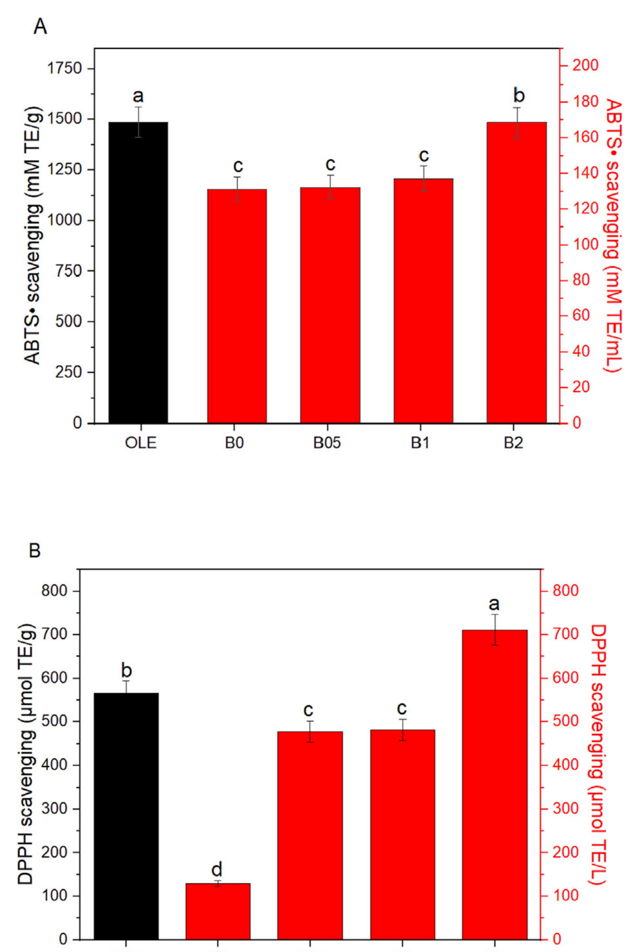

Fig. 2. Antioxidant potential. (A) ABTS$^•$ and (B) DPPH radical scavenging and (C) FRAP - ferric ion reducing antioxidant power of *Olea europaea* L. (OLE) *Olea europaea* extract; (B0) Beer 0% of extract; (B05) Beer with 0.5% of extract; (B1) Beer with 1% of extract; (B2) Beer with 2% of extract.

this phenomenon to the presence of foam-promoting agents (polypeptides and iso-α-acids) and a deficit of foam-negative materials (lipid-binding proteins).

Coliforms are commonly used to indicate the sanitary quality of beer. As it can be seen in Table 1, no coliforms at 35°C were detected in the samples, which indicates their microbiological quality.

3.2. Antioxidant potential, phenolic and polyphenolic compounds

Fig. 2 shows the ABTS• and DPPH radical scavenging potential and ferric ion reducing antioxidant power (FRAP) of *O. europaea* L. extract samples and extract-enriched beers.

The olive leaf extract showed appreciable antioxidant activity (Fig. 2), with a high capacity for eliminating ABTS• (1482.2 mM Trolox equivalent/g) and DPPH (566.3 μM Trolox equivalent/g) radicals, as well as the capacity to reduce ferric ion (1812.0 mM Fe (II) equivalent). Similarly, regarding what was found in our study, Ribas et al. [40] reported that leaf extracts of different olive tree cultivars, especially the Manzanilla variety (radical scavenging: 93.56% DPPH and 78.15% ABTS), have a high potential for eliminating ABTS• and DPPH radicals. These authors also reported that olive leaf extracts were rich in phenolics, ranging from 13.27 to 22.81 mg GAE/g. This study found a higher content of total phenolics (135.4 mg GAE/g) (Table 2), as well as a high content of polyphenols (1016.8 mg/100 mL).

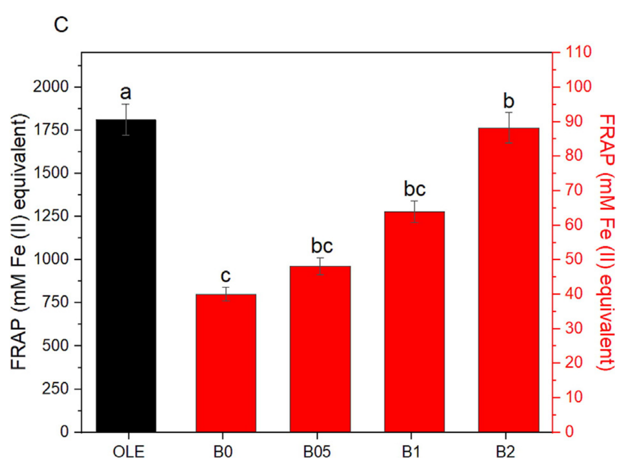

Fig. 2 (*continued*)

Lins et al. [41] found similar values for total phenolics (131.7 mg GAE/g, see Table 2) and oleuropein content (25.5 mg/g, see Table 3) in olive leaf extract that was obtained by solid–liquid extraction by using methanol/water (80:20, v/v) as a solvent.

Olive leaves have a rich variety of phenolic compounds, including simple phenols, flavonoids (flavones, flavanones, flavonols, and 3-flavonoids), and secoiridoids [40]. Phenolic compounds and polyphenols are responsible for the antioxidant capacity of different plant extracts [42]. We can observe that the natural antioxidant compounds of the olive leaves extract were transferred to the beer (Table 2), which was reflected in the antioxidant potential of the samples, mainly when the highest concentration of extract (2%) was used in the formulation (formulation B2) (Fig. 2). The main objective of adding olive leaf extract to beer was to increase the antioxidant capacity of beer and enrich it with bioactive compounds, which would contribute to beer quality and increase the stability of the product to oxidative changes. Furthermore, it can have beneficial effects on consumer health.

The chromatographic analysis (HPLC-DAD) of the olive leaf extract (Table 3, Fig. 3) revealed the presence of phenolic compounds, including chlorogenic acid, epicatechin, caffeic acid, and coumaric acid. The identification and quantification of these compounds were obtained by comparing their retention times and spectra with those of traditional standards. Additionally, the characteristic absorption profile at 280 nm confirmed the presence of oleuropein, a predominant phenolic compound in olive leaves. The extract that we obtained from olive leaves was notably rich in oleuropein, which is consistent with previous studies that highlight the high concentration of this compound in olive plant parts [43,44].

Three different extract concentrations were added to different batches of beer to investigate the impact that olive leaf extract has on beer. Our analysis focused on changes in the concentration

Table 2
Phenolic and polyphenols content in *Olea europaea* L. extract and beer samples.

Evaluated parameter	OLE mg GAE/g	B0 mg GAE/mL	B05	B1	B2
Total phenolics	135.4	171.09^{d}	234.53^{c}	317.6^{b}	437.4^{a}
Total polyphenols (mg/100 mL)	1016.8^{e}	221.4^{d}	341.9^{c}	618.3^{b}	729.0^{a}

OLE: *Olea europaea* L. extract. B0: Control beer - no added extract. B05: 0.5% extract. B1: 1.0% extract. B2: 2.0% extract. GAE: Galic Acid Equivalent. Different letters on the same line indicate a significant difference at the 95% confidence level ($p < 0.05$).

Table 3
Phenolic compounds detected by HPLC-DAD.

Phenolic compound	RT (min)	Wavelength (nm)	Concentration (μg /500 mL)				Concentration (mg/g)
			B0	B05	B1	B2	Extract
Coumaric acid	22.7	309	359.2	346.0	412.0	478.1	0.14
Ferulic acid	25.0	322	400.2	369.4	431.0	462.0	<LD
Cinnamic acid	39.0	276	72.3	72.3	109.6	124.5	<LD
Chlorogenic acid	-	-	<LD	<LD	<LD	<LD	0.38
Caffeic acid	-	-	<LD	<LD	<LD	<LD	0.03
Epicatechin	-	-	<LD	<LD	<LD	<LD	1.21
			Concentration (% Area)				
Oleuropein	33.7	280	<LD	5.7	11.2	17.4	26.1

<LD: Value below detection limit; B0: Control beer - no added extract; B05: 0.5% extract; B1: 1.0% extract; B2: 2.0% extract.

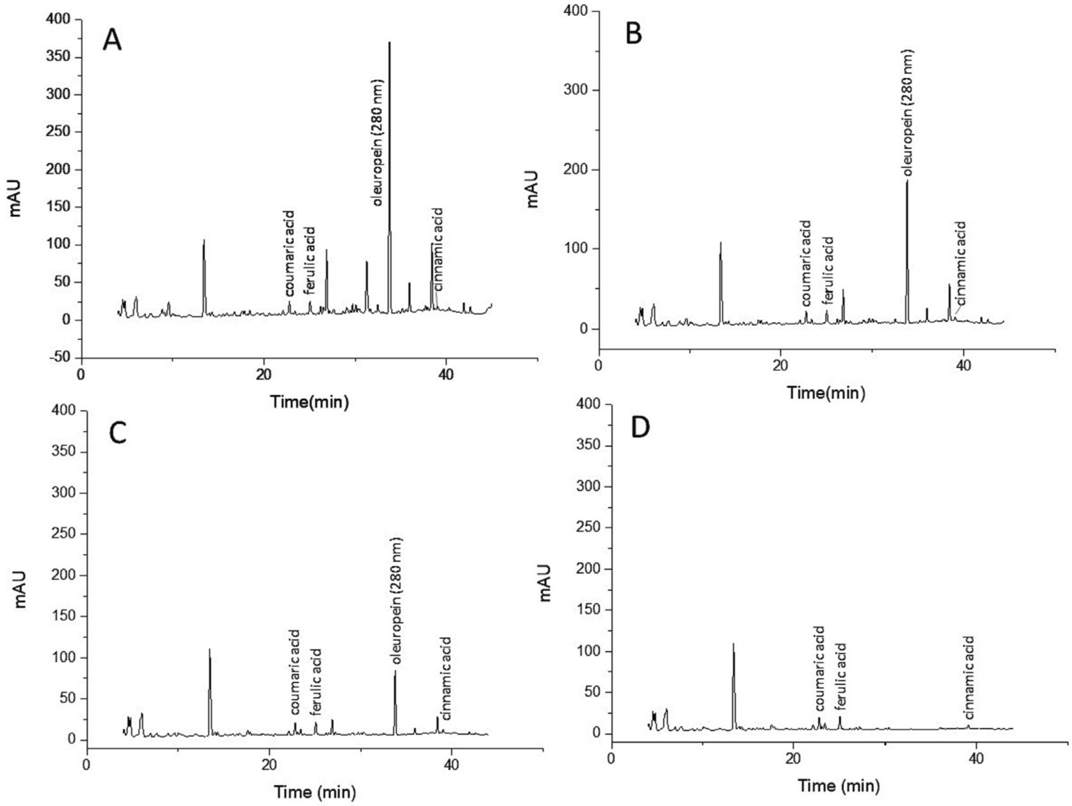

Fig. 3. Chromatograms of beer samples obtained from 280 nm. (A) Beer with 2% of extract; (B) Beer with 1% of extract; (C) Beer with 0.5% of extract; (D) Beer with 0% of extract.

of the identified phenolic compounds, with particular attention to oleuropein, the major component. Thus, by adding olive leaf extract to beer, we observed significant variations in the concentration of certain phenolic compounds similar to those that were described by Guglielmotti et al. [35]. The concentration of coumaric and cinnamic phenolic acids increased in beers after adding the extract. Notably, the concentration of oleuropein showed substantial changes, indicating that adding the extract mainly influenced the concentration of this compound in the beer. This finding suggests that oleuropein strongly contributed to the variations observed in total phenolic concentration in the beer samples.

In addition to oleuropein glycoside, other phenolic compounds such as 3-hydroxytyrosol (which is derived from oleuropein hydrolysis), luteolin-7-glucoside, apigenin-7-glucoside, and verbascoside which are found in olive leaves, and contribute to increased antioxidant capacity and shelf-life [35]. The increased concentration of phenolic compounds in beer can contribute to the bitterness, aroma, and flavor of the drink, therefore potentially influencing the acceptance of the product. Further descriptive sensory analysis and hedonic tests are needed to confirm the perceptible effects on the organoleptic properties of the beer.

4. Conclusions

The non-enzymatic cold mashing technique associated with interrupted fermentation proved to be effective in obtaining a light beer with low alcohol content. A soft, low-calorie beer with relatively reduced bitterness was obtained. *Olea europaea* L. extract was rich in phenolic compounds and polyphenols, especially oleuropein. Adding *Olea europaea* L. extract enriched the beer with bioactive compounds and potentiated its antioxidant activity, mainly when higher extract concentrations were used. Concentrations of 0.5% and 1% extract did not influence the quality of the beer foam, but when 2% extract was added, the stability of the beverage foam was reduced. The addition of the extract contributed to the enhancement of the EBC color of the beer, and the beer produced could be considered innovative and could arouse consumers' interest in unique craft beers and low-alcohol beverages.

Author contributions

– Study conception and design: E Cappelin, DH Hendges, MAA Cunha.
– Data collection: E Cappelin, MLK Marchioro, D Meneguzzi.
– Analysis and interpretation of results: MLM Daltoé, TLC Oldoni, MAA Cunha.
– Draft manuscript preparation: E Cappelin, MAA Cunha.
– Revision of the results and approval of the final version of the manuscript: MAA Cunha.

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Conflict of interest

There are no conflicts to declare.

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Supplementary material

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Data availability

Data will be made available on request.

References

[1] CERVBRASIL. Associação Brasileira da Indústria da Cerveja 2023. [cited 2023 Feb 3]. Avaliable from: http://www.cervbrasil.org.br/novo_site/dados-do-setor/.
[2] Dziedziński M, Stachowiak B, Kobus-Cisowska J, et al. Supplementation of beer with *Pinus sylvestris* L. shoots extracts and its effect on fermentation, phenolic content, antioxidant activity and sensory profiles. Electron J Biotechnol 2023;63:10–7. https://doi.org/10.1016/j.ejbt.2023.01.001.
[3] Sindicato Nacional da Indústria da Cerveja. Vendas de cerveja crescem 7,7% em 2021 2023. [cited 2023 Feb 3]. Avaliable from: https://www.sindicerv.com.br/noticias/vendas-de-cerveja-crescem-77-em-2021/.
[4] Research and Markets. Non-alcoholic beer global market report 2023. [cited 2023 Nov 3]. Avaliable from: https://www.researchandmarkets.com/report/low-alcohol-beer.
[5] Nehra M. Non alcoholic beers: Review and methods. Madridge J Food Technol 2022;7(1):200–6. https://doi.org/10.18689/mjft-1000130.
[6] Salantă LC, Coldea TE, Ignat MV, et al. Non-alcoholic and craft beer production and challenges. Processes 2020;8(11):1382. https://doi.org/10.3390/pr8111382.
[7] Muller C, Neves LE, Gomes L, et al. Processes for alcohol-free beer production: A review. Food Sci Technol 2020;40(2):273–81. https://doi.org/10.1590/fst.32318.
[8] BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa No 65, de 10 de Dezembro de 2019. [cited 2023 Feb 3]. Available from: https://pesquisa.in.gov.br/imprensa/jsp/visualiza/index.jsp?data=11/12/2019&jornal=515&pagina=31&totalArquivos=217.
[9] Dalberto G, da Rosa MR, Niemes JP, et al. Cold mash in brewing process: Optimization of innovative method for low-alcohol beer production. ACS Food Sci Technol 2021;1(3):374–81. https://doi.org/10.1021/acsfoodscitech.0c00099.
[10] Schöttke N, Rögener F. Cold mashing - Analysis and optimization of extraction processes at low temperatures in the brewing process. E3S Web Conf 2021;247:01036. https://doi.org/10.1051/e3sconf/202124701036.
[11] Iorizzo M, Coppola F, Letizia F, et al. Role of yeasts in the brewing process: Tradition and innovation. Processes 2021;9(5):839. https://doi.org/10.3390/pr9050839.
[12] Palomino-Vasco M, Rodríguez-Cáceres MI, Mora-Díez N. Discrimination based on commercial/craft origin and on lager/ale fermentation of undiluted Spanish beer samples: Front-face excitation-emission matrices and chemometrics. J Food Compos Anal 2023;115:. https://doi.org/10.1016/j.jfca.2022.104946104946.
[13] Strong G, England K. Beer Judge Certification Program. 2021 Style Guidelines. Beer Style Guidelines. [cited 2023 Feb 3]. Available from: https://www.bjcp.org/bjcp-style-guidelines/.
[14] da Costa PMC, de Almeida ILML, Bianchini A, et al. Blond Ale craft beer production with addition of pineapple pulp. J Exp Agric Int 2019;38(2):1–5. https://doi.org/10.9734/jeai/2019/v38i230294.
[15] Ambra R, Pastore G, Lucchetti S. The role of bioactive phenolic compounds on the impact of beer on health. Molecules 2021;26(2):486. https://doi.org/10.3390/molecules26020486. PMid: 33477637.
[16] Bertan FAB, da Silva Pereira Ronning E, Marchioro MLK, et al. Valorization of pineapple processing residues through acetification to produce specialty vinegars enriched with red-Jambo extract of *Syzygium malaccense* leaf. Sci Rep 2022;12:19384. https://doi.org/10.1038/s41598-022-23968-2. PMid: 36371484.
[17] González E, Gómez-Caravaca AM, Giménez B, et al. Evolution of the phenolic compounds profile of olive leaf extract encapsulated by spray-drying during *in vitro* gastrointestinal digestion. Food Chem 2019;279:40–8. https://doi.org/10.1016/j.foodchem.2018.11.127. PMid: 30611506.
[18] Ciont C, Difonzo G, Pasqualone A, et al. Phenolic profile of micro- and nano-encapsulated olive leaf extract in biscuits during *in vitro* gastrointestinal digestion. Food Chem 2023;428:. https://doi.org/10.1016/j.foodchem.2023.136778. PMid: 37421669136778.
[19] Alrugaibah M, Yagiz Y, Gu L. Novel natural deep eutectic solvents as efficient green reagents to extract phenolic compounds from olive leaves and predictive modelling by artificial neural networking. Food Bioprod Process 2023;138:198–208. https://doi.org/10.1016/j.fbp.2023.02.006.
[20] Acar-Tek N, Ağagündüz D. Olive leaf (*Olea europaea* L. folium): Potential effects on glycemia and lipidemia. Ann Nutr Metab 2020;76(1):10–5. https://doi.org/10.1159/000505508. PMid: 31901903.
[21] Menezes RCR, Peres KK, Costa-Valle MT, et al. Oral administration of oleuropein and olive leaf extract has cardioprotective effects in rodents: A systematic review. Rev Port Cardiol 2022;41(2):167–75. https://doi.org/10.1016/j.repc.2021.05.011. PMid: 36062705.
[22] ASBC. Beer methods. Am Soc Brew Chem 2023. [cited 2023 Feb 3]. Available from: https://www.asbcnet.org/Methods/BeerMethods/Pages/default.aspx.
[23] Kanauchi M, Kultgen E, Bamforth C. Low-molecular-weight materials from heavily roasted barley and malt with strong foam-stabilising potential. J Inst Brew 2019;125(1):39–46. https://doi.org/10.1002/jib.538.
[24] BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa No 161, de 1 de Julho de 2022. [cited 2023 Feb 3]. Available from: https://www.in.gov.br/en/web/dou/-/instrucao-normativa-in-n-161-de-1-de-julho-de-2022-413366880.
[25] de Oliveira CT, Maia BHLNS, Ferriani AP, et al. Chemical characterization, antioxidant capacity and antimicrobial potential of essential oil from the leaves of *Baccharis oreophila* Malme. Chem Biodivers 2019;16(2):e1800372. https://doi.org/10.1002/cbdv.201800372. PMid: 30673172.
[26] Oldoni TLC, Santos S, Mitterer-Daltoé ML, et al. *Moringa oleifera* leaves from Brazil: Influence of seasonality, regrowth age and region in biochemical markers and antioxidant potential. Arab J Chem 2022;15(11):104206. https://doi.org/10.1016/j.arabjc.2022.104206.
[27] Antonelo FA, Rodrigues MS, Cruz LC, et al. Bioactive compounds derived from Brazilian Myrtaceae species: Chemical composition and antioxidant, antimicrobial and cytotoxic activities. Biocatal Agric Biotechnol 2023;48:102629. https://doi.org/10.1016/j.bcab.2023.102629.
[28] Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteau reagent. Methods Enzymol 1999;299:152–78.
[29] European Brewery Convention. Analytica-Microbiologica-EBC. 2 sd. Nürnberg: Fachverlag Hans Carl; 2005.
[30] Iurckevicz G, Dahmer D, Santos VAQ, et al. Encapsulated microparticles of (1→6)-β-D-glucan containing extract of *Baccharis dracunculifolia*: Production and characterization. Molecules 2019;24(11):2099. https://doi.org/10.3390/molecules24112099. PMid: 31163607.
[31] Kobayashi M, Nagahisa K, Shimizu H, et al. Simultaneous control of apparent extract and volatile compounds concentrations in low-malt beer fermentation. Appl Microbiol Biotechnol 2006;73:549–58. https://doi.org/10.1007/s00253-006-0516-1. PMid: 16865344.
[32] Alves MM, Rosa MS, dos Santos PPA, et al. Artisanal beer production and evaluation adding rice flakes and soursop pulp (*Annona muricata* L.). Food. Sci Technol 2020;40(2):545–9. https://doi.org/10.1590/fst.36119.
[33] Klimczak K, Cioch-Skoneczny M. Changes in beer bitterness level during the beer production process. Eur Food Res Technol 2023;249:13–22. https://doi.org/10.1007/s00217-022-04154-0.
[34] Zhang Y, Jia S, Zhang W. Predicting acetic acid content in the final beer using neural networks and support vector machine. J Inst Brew 2012;118(4):361–7. https://doi.org/10.1002/jib.50.
[35] Guglielmotti M, Passaghe P, Buiatti S. Use of olive (*Olea europaea* L.) leaves as beer ingredient, and their influence on beer chemical composition and antioxidant activity. J Food Sci 2020;85(8):2278–85. https://doi.org/10.1111/1750-3841.15318. PMid: 32652593.
[36] Zenit R, Rodríguez-Rodríguez J. The fluid mechanics of bubbly drinks. Phys Today 2018;71(11):44–50. https://doi.org/10.1063/PT.3.4069.
[37] Aliyari MA, Motahar SFS, Salami M, et al. Structural, functional, and anti-cancer properties of conjugates of quinoa protein isolate and olive leaf polyphenolic extract: Application in production of bread. Food Struct 2022;33:. https://doi.org/10.1016/j.foostr.2022.100292100292.
[38] de Francesco G, Bravi E, Sanarica E, et al. Effect of addition of different phenolic-rich extracts on beer flavour stability. Foods 2020;9(11):1638. https://doi.org/10.3390/foods9111638. PMid: 33182668.
[39] Mazengia G, Dessalegn E, Dessalegn T. Effect of *Moringa stenopetala* leaf extracts on the physicochemical characteristics and sensory properties of lagered beer. Food Sci Nutr 2022;10(2):507–14. https://doi.org/10.1002/fsn3.2672. PMid: 35154687.

[40] Ribas JCR, Lazzari A, Gonzalez LBF, et al. Bioactive compounds and antioxidant activity of leaves from olive trees grown in Paraná. Brazil Pesqui Agropecuária Bras 2023;58:e03025. https://doi.org/10.1590/s1678-3921.pab2023.v58.03025.

[41] Lins PG, Pugine SMP, Scatolini AM, et al. *In vitro* antioxidant activity of olive leaf extract (*Olea europaea* L.) and its protective effect on oxidative damage in human erythrocytes. Heliyon 2018;4(9):e00805. https://doi.org/10.1016/j.heliyon.2018.e00805. PMid: 30255162.

[42] Savi A, Calegari MA, Calegari GC, et al. Bioactive compounds from *Syzygium malaccense* leaves: Optimization of the extraction process, biological and chemical characterization. Acta Sci Technol 2020;42(1):e46773. https://doi.org/10.4025/actascitechnol.v42i1.46773.

[43] Chigurupati S, Alharbi FS, Almahmoud S, et al. Molecular docking of phenolic compounds and screening of antioxidant and antidiabetic potential of *Olea europaea* L. ethanolic leaves extract. Arab J Chem 2021;14(11):103422. https://doi.org/10.1016/j.arabjc.2021.103422.

[44] Romero-Márquez JM, Navarro-Hortal MD, Jiménez-Trigo V, et al. An oleuropein rich-olive (*Olea europaea* L.) leaf extract reduces β-amyloid and tau proteotoxicity through regulation of oxidative- and heat shock-stress responses in *Caenorhabditis elegans*. Food Chem Toxicol 2022;162:112914. https://doi.org/10.1016/j.fct.2022.112914. PMid: 35276233.