Contents lists available at ScienceDirect

Food and Bioproducts Processing

journal homepage: www.elsevier.com/locate/fbp

Maximizing the freeze-dried extract yield by considering the solvent retention index: Extraction kinetics and characterization of *Moringa oleifera* leaves extracts

*M. Kashaninejad, B. Blanco, O. Benito-Román, S. Beltrán, S. Mehdi Niknam, M.T. Sanz**

Department of Biotechnology and Food Science (Chemical Engineering Section), University of Burgos, 09001 Burgos, Spain

ARTICLE INFO

Article history:
Received 17 May 2021
Received in revised form 14 September 2021
Accepted 20 September 2021
Available online 23 September 2021

Keywords:
Moringa leaves
Phenolic compounds
Solvent retention
Kinetic model
Freeze-dried extract

ABSTRACT

A complete chemical characterization of *Moringa oleifera* leaves was carried out showing a high content of extractives. Extraction kinetics of bioactive compounds present in this fraction were performed by conventional and ultrasound assisted extraction (UAE). A 50% (v/v) hydroalcoholic mixture led to the highest total phenolic compounds yield by conventional solvent extraction, 29.5 ± 0.3 mg per gram of moringa leaves. UAE did not bring any improvement when using hydroalcoholic mixtures probably due to the physical properties of the ethanol aqueous mixtures that affect the UAE performance, such as viscosity and vapor pressure of the mixture. The retention index of the different solvents in the raffinate phase was determined revealing the highest retention index for water, 9.5, and a continuous decrease by increasing ethanol concentration. Retention index is a key parameter in a solvent extraction process since it determines the number of stages in an industrial separation process and it is not usually reported in bioactive compounds extraction. Solvent extraction capacity and the retention index determined the final freeze-dried extract yield.

1. Introduction

Moringa oleifera Lam (syn. M. pterygosperma Gaertn.) is a type of medium-sized, evergreen tree belonging to the Moringaceae family. *M. oleifera* is an edible and medicinal perennial plant that presents high nutritional and medicinal value. It is widely cultivated in tropical and sub-tropical regions around the world, due to its ability to grow on both humid and hot dry lands. All parts of this plant, roots, leaves, flowers and inmature pods and seeds can be used for different purposes being defined as a multi-purpose tree. For instance, roots have been described as a good source of tanning agents; while seeds can be used for biodiesel production and also as water purification agent (Amaglo et al., 2010).

Moringa leaves (ML) have been reported as a natural source of antioxidant compounds, including various phenolic compounds, mainly flavonoids that can have positive effects on health. Different epidemiological studies have shown that consumption of foods rich in antioxidants reduce the risk of degenerative diseases, cancer, cardiovascular disease and neurological pathologies (Ames et al., 1993). González-Romero et al. (2020) found that ML presented the highest antioxidant capacity and total polyphenol content compared with 28 different vegetable leaves pre-packaged for consumption as salad, such as various lettuce plants or spinach. Considering the properties of ML, it is certainly important to find the most practical and cost-effective extraction method to obtain extracts from ML rich in antioxidant compounds. Phenolic compounds content and antioxidant capacity of extracts from ML depend on the extraction method, type of solvent, solvent-to-solid ratio, temperature, stirring rate and particle size (Oldoni et al., 2019; Castro-lópez et al., 2017). Extracts of ML have been described as complex mixtures of a large number of phytochem-

* *Corresponding author.*
E-mail address: tersanz@ubu.es (M.T. Sanz).
https://doi.org/10.1016/j.fbp.2021.09.008

icals, mainly phenolic acids and complex flavonoids, like glucosides, rutinosides, malonyl glucosides and acetyl glucosides of quercetin, isorhamnetin and kaempferol (Oldoni et al., 2019). A subsequent drying step of the liquid extracts will help to obtain a dry powder with a high phenolic concentration that could be used as additive for foods.

The simplest method of solid liquid extraction is the single contact batch operation and it takes place in two steps: (1) contact of the solvent and the solid material to transfer the solute to the solvent (2) separation of the solution from the remaining solid. Conventional solid liquid extraction can be intensified by considering the use of ultrasound. Ultrasound assisted extraction (UAE) technology has been described as a clean, green, extraction technique that presents several benefits such as the enhancement of the extraction of heat-sensitive components and the global extraction yield (Chemat et al., 2017)

In a real extraction process, it is impossible to completely separate the liquid phase from the solid fraction. The solution holding capacity of the insoluble matrix depends on the porosity of the matrix, and the density, viscosity and surface tension of the solution, as well as on the method of separation (Berk, 2009). As a result, two phases are obtained after a solid liquid extraction stage, a liquid phase with the solute, the extract phase, and a solid phase composed by the exhausted solid and the adhered liquid solution, the raffinate phase. The effect of solvent type on bioactive compounds extraction has been usually considered only in terms of the composition of the extracts. The liquid solution retained in the solid phase is usually not reported in the extraction studies of biocompounds from different matrix, although this parameter determines economic aspects such as the number of stages of the process and the solvent recovery.

The final aim of this work was to obtain a freeze-dried extract of ML from an optimized solid liquid extraction process by using different ethanol aqueous mixtures by conventional and ultrasound assisted extraction (UAE), considering the extraction kinetic aspects, the extract composition and the solution retention index. Characterization of the freeze-dried extract was carried out in terms of total and individual phenolic compounds, antioxidant capacity, according to ABTS and FRAP assays, and pigments.

2. Materials and methods

2.1. Raw material

ML were purchased from Waka brand (Konso, Ethiopia) in powder form. Moisture content of ML, 5.61 ± 0.02% (w/w), was determined gravimetrically by weighing it before and after drying in an oven at 105 °C until constant weight. Results were expressed as mass of dry moringa leaves, DML. ML were stored in the freezer at −18 °C until extraction studies.

2.2. Determination of chemical composition of moringa leaves

ML were characterized according to the Standard Biomass Analytical Methods provided by the National Renewable Energy Laboratory (Sluiter et al., 2010). After water and ethanol extractives removal, two hydrolysis steps with sulphuric acid were carried out to determine the structural components of the biomass. Carbohydrates were quantified by high-performance liquid chromatography (HPLC) (Agilent 1260, Agilent Technologies, Inc. U.S.A.) equipped with an Aminex-HPX-87H column (Bio-Rad Laboratories Inc. U.S.A.), a variable wavelength detector (VWD) and a refractive index detector (RID) using a mobile phase constituted by 0.005 M sulphuric acid. The column and refractive index detector were maintained at 40 °C. The lipid fraction of ML was measured by Soxhlet extraction using a BUCHI 811 extraction system (Buchi Labortechnik AG, Flawil, Switzerland).

Elemental composition (C, H, N, S) of the ML was determined by an elemental micro-analyzer equipment (Model Flash 2000, Thermo Scientific, Massachusetts, U.S.A.). Protein content of ML was calculated from the nitrogen content by applying a conversion factor of 6.25.

2.3. Extraction procedures

2.3.1. Conventional solvent extraction

Extractions were performed in an orbital shaker (Grant Instruments-OLS 200, Shepreth Cambridgeshire, England) for 1 h at 50 °C. The volume of the extractor was 250 mL and it was filled with 150 mL of the extraction solvent and the corresponding mass of ML. First, the effect of solvent to solid ratio, from 10 to 20 mL/g_{ML} was analysed by using a hydroalcoholic solution with 20% (v/v) ethanol as solvent. Afterwards, the effect of solvent type was studied by using different hydroalcoholic mixtures (100% ethanol, 80% ethanol, 50% ethanol, 20% ethanol and 100% water, v/v) at 50 °C and a constant liquid/solid ratio of 10 mL/g_{ML}. Extraction kinetics were followed by withdrawing samples at regular time intervals and further analysis.

After extraction, the solid and liquid phases were separated by centrifugation and weighed. The liquid phase was considered as the extract phase (EP) and the remaining solid phase was the raffinate phase (RP).

Liquid and solid phases were characterized as followed. An aliquot of the EP, m_{EP}, was oven dried at 105 °C to evaporate the solvent and it was weighed again to determine the mass of soluble solids extracted ($m_{SS,EP}$) and by difference, the mass of solvent evaporated ($m_{S,EP}$). The mass fraction of solvent ($w_{S,EP}$) and total soluble solids ($w_{SS,EP}$) in the extract phase were evaluated as:

$$w_{SS,EP} = \frac{m_{SS,EP}}{m_{EP}} \quad (1)$$

$$w_{S,EP} = 1 - w_{SS,EP} \quad (2)$$

The extraction yield for soluble solids was determined as the ratio of the total amount of soluble solids in the extract phase and the initial mass of DML:

$$Yield = \frac{total\, m_{ss,EP}}{initial\, mass_{DML}} \cdot 100 \quad (3)$$

The raffinate phase (m_{RP}) was dried until constant weight to remove the solvent. Once solvent was evaporated, the samples were weighed again, $m_{RP,\ non\text{-}solvent}$, allowing the calculation of the solvent retained in the raffinate phase, $m_{S,RP}$:

$$m_{S,RP} = m_{RP} - m_{RP,\ non-solvent} \quad (4)$$

The amount of soluble solids in the raffinate phase was evaluated assuming equilibrium stage in a single stage solid liquid extraction defined as one in which the extract phase has the same composition as the solution adhered to the solids (Wisniak et al., 1987):

$$m_{SS,\ RP} = \frac{w_{SS,\ EP}}{w_{S,\ EP}} m_{S,RP} \quad (5)$$

The amount of insoluble solids in the RP phase, $m_{I,RP}$, was evaluated as:

$$m_{I,\ RP} = m_{RP} - m_{S,RP} - m_{SS,RP} \quad (6)$$

With Eqs. 4 to 6, weight fractions of the three components of the raffinate phase, solvent, soluble solids and insoluble solids were evaluated.

The retention index (RI) was evaluated as the solution adhered to the insoluble solids in the raffinate phase after separation of both phases (Oliveira et al., 2019).

$$RI = \frac{m_{SS,RP} + m_{S,RP}}{m_{IS,RP}} \quad (7)$$

2.3.2. *Ultrasound assisted extraction*

Ultrasound assisted extraction (UAE) was performed by using water, 50% and 20% (v/v) ethanol aqueous solutions as solvents at 50 °C. UAE was performed by using a 750 W Sonics Material TM with a 13 mm probe (VibraCell^{TM} 75042, Bioblock Scientific, U.S.A.). ML were processed at a constant ultrasound frequency of 20 kHz and 79 μm of amplitude and a ratio of 10 mL solvent/g_{ML}. ML were introduced in a jacketed vessel (Φ_i = 4.8 cm, V = 199 cm^3) and the probe was submerged in the solution at a constant depth of 2 cm from the bottom of the vessel. Temperature was kept at 50 °C in the vessel by circulating water through the jacket (Illera et al., 2018). Samples were taken periodically to follow the extraction kinetics.

2.4. *Freeze-drying process*

A freeze-dried extract (FDE) was obtained from the liquid extract at 50 °C by using 50% ethanol aqueous mixture (v/v) and a ratio of 10 mL solvent/g_{ML}. Before freeze-drying, ethanol was removed by a rotary evaporator and the remaining extract was submitted to freeze drying. First, samples were frozen with liquid nitrogen (−196 °C), equilibrated at −80 °C for 2 h and then submitted to freeze-drying in a Labconco Freeze Dry System (Labconco Corporation, U.S.A.) at $1.5 \cdot 10^{-4}$ mbar during 48 h. The moisture content of the freeze-dried particles was determined gravimetrically by weighing small amounts of dried particles (around 0.5 g) before and after drying in an oven at 105 °C until constant weight. Solid particles were analyzed in terms of antioxidant activity, individual phenolic compounds and pigments.

2.5. *Characterization of extracts*

2.5.1. *Total polyphenols and flavonoids content determination*

Total phenolic compounds (TPC) were determined by using the Folin-Ciocalteu reagent (VWR International, France) (Singleton et al., 1999). Briefly, 100 μL of the liquid extract were mixed with 2.8 mL of water and subsequently with 100 μL of the Folin-Ciocalteu reagent. After that, 2 mL of sodium carbonate 7.5% (w/v) (LabKem, Barcelona, Spain) were added to the mixture. Absorbance was measured at 750 nm after 60 min of reaction (Spectrophotometer V-750, Jasco, Japan). A calibration curve was prepared with standard solutions of gallic acid (Sigma Aldrich, Chemie GmbH, Germany) and results were expressed as mg of gallic acid equivalent (GAE) per L (mg GAE/L) or mg GAE/g_{DML}. To determine the TPC in the FDE, a solution of 5 mg of FDE/mL in the same solvent used for extraction was used instead of the liquid extract. Results were expressed as mg GAE/g_{FDE}.

The total flavonoids content (TFC) was only determined in the FDE following a similar procedure as described by Chang et al. (Chang et al., 2002). 0.5 mL of a solution of 5 mg of FDE/mL were mixed with 2.8 mL of distilled water and 0.1 mL of $AlCl_3$ (10%, w/v) (VWR International, France). Finally, 0.1 mL of CH3COOK (1 M) and 1.5 mL of ethanol were added. Absorbance at 415 nm was measured after 30 min in darkness (spectrophotometer V-750, Jasco, Japan). A quercetin standard curve (Sigma Aldrich, Chemie GmbH, Germany) in ethanol was determined and results were expressed as mg Quercetin Equivalent (QE) per g of FDE (mg QE/g_{FDE}).

2.5.2. *Antioxidant capacity*

Two assays with different mechanism action were used to determine the antioxidant capacity, the ABTS assay and the FRAP method. Reactives were purchased from Sigma Aldrich (Chemie GmbH, Germany).

The $ABTS^+$ radical was prepared according to Re et al. (1999). 3 mL of a previously prepared $ABTS^+$ solution were added to 100 μL of the liquid extracts. After 1 h being in the darkness, the absorbance was measured with a spectrophotometer (V-750, Jasco, Japan) at 734 nm using ethanol as a blank. A Trolox standard curve was used to express the antioxidant capacity of the samples as mg Trolox Equivalent per liter, (mg TE)/L.

The FRAP method was performed according to Benzie and Strain (Benzie and Strain, 1996). 2850 μL of the working FRAP reagent were added to 150 μL of the ML extract and incubated at 37 °C for 30 min. Absorbance was read at 593 nm. As standard, a solution of $FeSO_4 \cdot 7H_2O$ (0.1 M) was used. Results were expressed in mg $FeSO_4$/L.

To determine the antioxidant capacity of the FDE, different solutions of the FDE were used for the antioxidant tests.

2.5.3. *Identification and quantification of extracted phenolic compounds by HPLC/DAD*

Chromatographic separation was performed on a HPLC/DAD Agilent 1100 (Agilent Technologies, Inc., U.S.A.) with a Kinetex® 5 μm Biphenyl 100 Å, 250 × 4.6 mm column (Phenomenex Inc., CA, U.S.A.). The chromatographic method has been previously described elsewhere (Alonso-Riaño et al., 2020; Kashaninejad et al., 2020). Before injection, extracts were filtered through 0.45 μm pore size. Identification of individual phenolic compounds was carried out by comparing retention times and spectral data with those of authentic standards. Chlorogenic acid, rutin, quercetin, quercetin 3-O-glucoside were purchased from Sigma-Aldrich (Chemie GmbH, Germany). Neochlorogenic acid, kaempferol 3-O-glucoside, vitexin and isovitexin were purchased from Chengdu Biopurify Phytochemicals (Sichuan, China). Apigenin and kaempferol were purchased from Glentham Life Sciences (Corsham, U.K.). Standard solutions were prepared by dissolution of the compounds in methanol to build the corresponding calibration curves.

2.5.4. *Pigments in the freeze-dried extract*

The amount of chlorophylls and carotenoids in the FDE was determined according to Sumanta et al. (2014) and described by Kashaninejad et al. (2020) for pigments in a freeze dried extract from olive leaves. 0.5 *g* of the FDE were dissolved in 10 mL of diethyl ether. The sample was homogenized and then centrifuged for 10.000 rpm for 15 min at 4 °C. 0.5 mL of the supernatant were mixed with 4.5 mL of diethyl ether and the absorbance was determined at different wavelengths to determine the concentration of Chlorophyll-a (Ch-a), Chlorophyll-b

(Ch-b) and carotenoids (Cx + c), μg/mL, according to the equations collected by Sumanta et al. (2014):

$$\text{Ch-a} = 10.05A_{660.6} - 0.97A_{642.2} \quad (8)$$

$$\text{Ch-b} = 16.36A_{642.2} - 2.43A_{660.6} \quad (9)$$

$$\text{Cx} + \text{c} = (1000A_{470} - 1.43\text{Ch-a} - 35.87\text{Ch-b})/205 \quad (10)$$

2.6. Modeling of extraction kinetics and statistical analysis

TPC data along extraction were fitted to the Weibull model:

$$Y\,(mg\,GAE/L) = A \cdot (1 - exp(-kt^n)) \quad (11)$$

The exponent n indicates the shape of the extraction curve. If n > 1, the curve is sigmoidal and if n < 1, the curve is parabolic (Kitanović et al., 2008). A represents the maximum extraction yield when the extraction time tends to infinite.

The estimation of the kinetic parameters was carried out by non-linear regression by using the Marquardt algorithm. The quality of the fitting was evaluated by the relative error, RE

$$RE = \frac{\sum_{i=1}^{n} \frac{abs(Y_{i,exp} - Y_{i,calc})}{Y_{i,exp}}}{n} \cdot 100 \quad (12)$$

where $Y_{i,exp}$ and $Y_{i,calc}$ are the experimental and calculated TPC concentrations and n is the number of experimental data points in each extraction.

The results were presented as the mean ± standard deviation of at least three replicates. To confirm significant differences, the Fisher's least significant difference (LSD) procedure at p-value ≤0.05 was applied. The fitting procedure and analysis were carried out by the Statgraphics Centurion 18-X64 software.

3. Results and discussion

3.1. Biomass composition

Table 1 presents the chemical composition of ML. According to the two-step extractives determination, extractives soluble in water were 37.2 ± 0.5% (w/w) while in ethanol were found to be 11.7 ± 0.4% (w/w). Water and ethanol extractive fractions accounted for soluble proteins (water or ethanol soluble), different carbohydrates (with higher solubility in water) and TPC, among other compounds. High amount of extractives has been also found in other green leaves such as olive leaves (Kashaninejad et al., 2020) and it supports the importance of the study and optimization of different extraction strategies for ML valorization. The structural polysaccharide fraction (glucans and hemicellulose) was 18.7 ± 0.8%.

The lipid fraction was 4.04 ± 0.07% (w/w). It must be highlighted the high total protein fraction of the ML, 31.0 ± 0.2%. This value was of the same order as the values reported by Leone et al. (2015) for different ML from different sources Chad, Sahhrawi camps and Haiti, with values of 31.5 ± 0.1, 27.98 ± 0.12 and 20.80 ± 0.01%, respectively. This makes ML as an alternative good source of protein.

Table 1 – Chemical composition of moringa leaves expressed as % ± standard deviation in a dry weight basis.

Compound	Composition, %
Extractives	48.9 ± 0.9
Water soluble extractives	37.2 ± 0.5
Surcrose	0.40 ± 0.08
Glucose	3.20 ± 0.02
Mannose	1.84 ± 0.02
Galactose	0.66 ± 0.03
Xylose	3.79 ± 0.01
Arabinose	0.69 ± 0.01
Proteins	7.6 ± 0.2
TPC	2.4 ± 0.1
Ethanol soluble extractives	11.7 ± 0.4
Glucose	1.06 ± 0.06
Mannose	0.57 ± 0.03
Galactose	0.21 ± 0.01
Xylose	1.12 ± 0.07
Arabinose	0.64 ± 0.01
Proteins	2.2 ± 0.1
TPC	0.07 ± 0.01
Glucans	9.3 ± 0.4
Hemicellulose	9.4 ± 0.4
Lignin	10.0 ± 0.1
Soluble	7.99 ± 0.03
Insoluble	2.0 ± 0.1
Proteins*	31.0 ± 0.2
Lipids	4.04 ±0.07
Ashes	8.3 ± 0.5
Elemental composicion	
N	4.95 ± 0.03
C	45.3 ± 0.1
H	6.14 ± 0.04

*Total protein content includes the protein content in the water and ethanol extractive fraction.

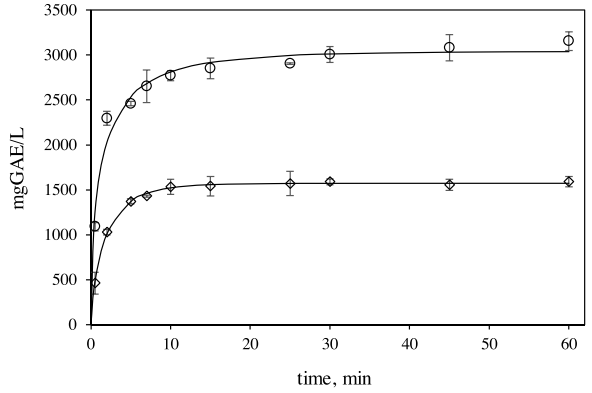

Fig. 1 – Effect of solvent to mass ratio on extraction kinetics by using a 20% (v/v) ethanol aqueous solution as solvent at 50 °C: (○) 10 mL:g_{ML} (◊) 20 mL:g_{ML}. The continuous lines represent the Weibull model.

3.2. Conventional solid liquid extraction of total polyphenol compounds

3.2.1. Effect of solvent to mass ratio

The effect of solvent to ML mass ratio was studied by using a 20% ethanol aqueous solution as solvent (v/v).

Fig. 1 shows the TPC extraction kinetics at 50 °C at two different solvent to ML mass ratios, 10 mL/g_{ML} and 20 mL/g_{ML}. A fast initial extraction period was observed at both solvent to mass ratios followed by a second slow period controlled by diffusion. These type of extraction curves have been also

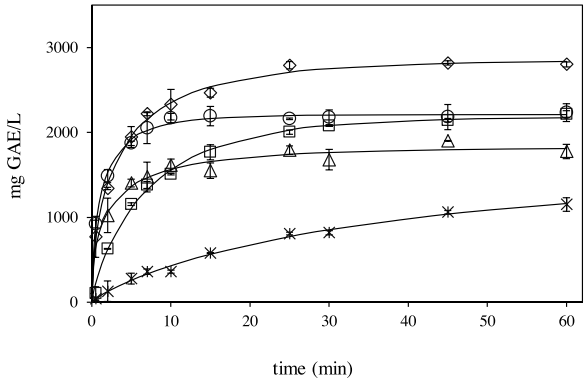

Fig. 2 – Effect of solvent type on extraction kinetics at 50 °C and a ratio of 10 mL/g_{ML}: (△) H_2O, (○) 20% EtOH, (◇) 50% EtOH, (□) 80% EtOH, (×) EtOH. The continuous lines represent the Weibull model.

described in the literature for bioactive compounds extraction from different plant materials (Kitanović et al., 2008). For ML, more than 90% of the TPC extraction was reached after 10 min of extraction time. The final TPC concentrations in the extracts were 2235 ± 104 and 1127 ± 58 mg GAE/L at 10 and 20 mL/g_{ML}, respectively. To compare the results on a dry biomass basis, the maximum extraction yield that could be reached was evaluated assuming that no extract was retained in the raffinate phase after phases separation ($V_{extract\ phase} = V_{solvent}$), yielding values of 23.7 ± 1.1 and 23.9 ± 1.2 mg GAE/g_{DML}, respectively. These two values were not significantly different by applying the Fisher's least significant difference (LSD) procedure at p-value ≤0.05, proving that, there was no limitation by the saturation of the solutes that are soluble in the solvent or mass transfer limitations at any of the ratios essayed. To evaluate the effect of solvent to mass ratio on the extraction rate, the initial extraction rates were determined from the initial linear part of the extraction curves. The values of the initial extraction rate were 807 ± 182 and 381 ± 48 mg GAE/(L·min), or 8.5 ± 1.9 and 8.1 ± 1.0 mg GAE/(g_{DML}·min) on a dry biomass basis, at 10 and 20 mL/g_{ML}, respectively. There were not statistically significant differences among the initial slopes expressed as mg GAE/(g_{DML}·min) at the two solvent to mass ratios used. Therefore, the lowest ratio value of 10 mL/g_{ML} was selected, since a higher solvent to mass ratio resulted in neither higher extraction rates nor final yields, on a biomass basis, but higher solvent consumption and a more diluted extract phase.

3.2.2. *Effect of type of solvent*

The effect of type of solvent was studied by varying the composition of ethanol-water mixtures. Five different mixtures were used as solvents, pure water and ethanol and different ethanol/water mixtures: 80/20, 50/50, 20/80 (v/v).

The results are presented in Fig. 2. The solvent extraction capacity changed by using different ethanol concentration, since it depends mainly on the solubility of the components in the solvent and on the interaction of the components with other constituents of the sample (Oldoni et al., 2019). The fastest extraction kinetic and the highest TPC concentration were achieved for a 50% ethanol aqueous mixture (v/v) with a value of 2802 ± 29 mg GAE/L. For conventional solvent extraction of TPC from green leaves, better extraction efficiency has been usually reported for ethanol-water mixtures compared to pure ethanol or water as solvents (Karthivashan et al., 2013). This fact has been attributed to the double effect of both solvents, since water swells the plant matrix an helps to create a more polar medium that facilitates the extraction of organic compounds, either soluble in ethanol and/or water (Ancut et al., 2018). Rodríguez-Pérez et al. (2015) also found that TPC extraction yield from ML was lower when using pure organic solvents, such as methanol, ethanol and acetone than when using these solvents mixed with water. These authors reported a TPC of 3.82 ± 0.09, 27 ± 2 and 24.3 ± 0.3 mg GAE/g_{DML} for pure ethanol, 50% and 70% (v/v) ethanol aqueous mixtures, respectively, when using 0.5 g of moringa leaves with 25 mL of the corresponding solvent for 1 h. Lin et al. (2021) reported a value of the same order for TPC extraction, 33.3 ± 1.8 mg GAE/g_{DML}, by stirring-assisted extraction at 30 °C for 30 min and 100 mg ML in 4 mL of a 52% ethanol aqueous solvent. To compare the values plotted in Fig. 2 with the values reported in the literature, the maximum theoretical extraction yield achieved was calculated (assuming no extract phase retained in the raffinate phase, $V_{extract\ phase} = V_{solvent}$) for the different solvents were 18.7 ± 0.9, 23.5 ± 1.1, 29.5 ± 0.3, 23.3 ± 0.4 and 12.1 ± 0.8 mg GAE/g_{DML} for pure water, 20%, 50%, 80% (v/v) ethanol aqueous mixtures and pure ethanol, respectively.

The composition of the raffinate and the extract phases, as well as the retention index were evaluated according to Eqs. 1–7 (Table 2). It can be clearly observed that the type of solvent has also a clear impact on the retention of the extract phase by the inert solid. The highest RI was obtained for water and it decreased continuously by increasing the amount of ethanol in the mixture solvent. The lower the RI, the more efficient is the extraction process in terms of losses of the solution. It also determines the cost of the downstream separation process in the raffinate phase for solvent recovery and the number of stages in an industrial extraction process. However, the choice of the right solvent must consider low solvent losses in the raffinate phase, but also the concentration of the targeted bioactive compounds in the extract phase.

In the extraction of soluble solids from murici and pequi seeds by using different types of solvents including ethanol, isopropanol, acetone and hexane, RI reached values between 0.51–1.28 (Araújo et al., 2018). The high values determined in this work for hydroalcoholic mixtures can be attributed to the trend of green leaves to adsorb water in its structure not being possible the comparison with other literature data since RI is usually not reported for extraction of bioactive compounds.

Table 2 also lists the extraction yield of the total soluble solids, as determined by Eq. 3, where the holding capacity of the inert solid was considered. The trend in the yield of total soluble solids agreed with the values reported in the Table 1 for ML characterization where the water extractive fraction was three times the ethanol extractive fraction. By using solvents with high water content, other components such as water-soluble proteins and different sugar monomers were extracted that led to higher values of the extraction yield.

Table 2 also presents the TPC yield reached in the extract phase considering the losses of the solution in the raffinate phase, $TPC_{real\text{-}yield}$. These values are lower than the maximum values considering no solution retention ($TPC_{max\text{-}yield}$). The difference between both values decreased by decreasing the RI. However, in both cases the maximum was observed by 50% (v/v) ethanol aqueous solution. Mass balances considering the initial mass of solvent and ML that split up into the extract and the raffinate phases were above 94% in all cases (Table 2).

3.2.2.1. *Influence of extraction solvent on extract composition and antioxidant capacity.*

The individual phenolic compounds

Table 2 – Composition of the raffinate and extract phases expressed as weight fraction. Retention index (RI) and global soluble solids extraction yield (%). Maximum theoretical TPC yield, assuming no solution retention, and real extraction TPC yield (mg TPC/g_{DML}). Mass balance (MB) for the extraction process.

Solvent	Raffinate phase			Extract phase		RI	Yield (%)	$TPC_{max-yield}$, mg TPC/g_{DML}	$TPC_{real-yield}$ mg TPC/g_{DML}	MB, %
	w_i	w_{ss}	w_s	w_{ss}	w_s					
Water	0.095	0.034	0.870	0.038	0.962	9.5 ± 0.5^{c}	19.1 ± 0.1^{c}	18.7 ± 0.2^{b}	9.0 ± 0.1^{a}	95.3
20% EtOH	0.133	0.030	0.837	0.034	0.966	6.5 ± 0.7^{b}	20.3 ± 0.2^{d}	23.5 ± 0.4^{c}	13.7 ± 0.2^{b}	94.3
50% EtOH	0.205	0.022	0.773	0.028	0.972	3.9 ± 0.3^{a}	18.9 ± 0.2^{c}	29.5 ± 0.5^{d}	20.8 ± 0.4^{d}	96.2
80% EtOH	0.230	0.021	0.749	0.028	0.972	3.4 ± 0.3^{a}	17.8 ± 0.2^{b}	23.3 ± 0.4^{c}	16.9 ± 0.3^{c}	96.4
Ethanol	0.254	0.012	0.734	0.015	0.985	2.9 ± 0.3^{a}	9.0 ± 0.4^{a}	12.1 ± 0.1^{a}	8.5 ± 0.1^{a}	96.4

w_i: mass fraction of insoluble solids, w_{ss}: mass fraction of soluble solids, w_s: mass fraction of solvent.
Values with different letters in each column are significantly different when applying the Fisher's least significant differences (LSD) method at p-value $\leq$0.05.

Table 3 – Concentration of individual phenolic compounds (mg/L) and antioxidant capacity determined in different hydroalcoholic mixture.

Compound/Antioxidant test	Conventional solvent extraction					UAE		
	Water	20% EtOH	50% EtOH	80% EtOH	EtOH	Water	20% EtOH	50% EtOH
Neochlorogenic acid	240 ± 8^{b}	403 ± 2^{c}	424 ± 41^{c}	223 ± 38^{b}	70 ± 2^{a}	263 ± 31^{b}	392 ± 2^{c}	386 ± 15^{c}
Chlorogenic acid	97 ± 2^{b}	160 ± 2^{c}	167 ± 18^{c}	87 ± 21^{b}	33 ± 3^{a}	76 ± 6^{b}	$175 \pm 16^{c,d}$	199 ± 6^{d}
Rutin	4.2 ± 0.8^{a}	$13 \pm 1^{b,c,d}$	$15.9 \pm 0.4^{c,d}$	$15 \pm 3^{b,c,d}$	$11 \pm 2^{b,c}$	9 ± 3^{b}	$15 \pm 0.2^{c,d,}$	17 ± 2^{d}
Apigenin	n.d.	5 ± 3^{a}	20.5 ± 3.0^{b}	n.d.	n.d.	n.d.	n.d.	n.d.
Quercetin 3-glucoside	49 ± 2^{a}	115 ± 1^{b}	212.9 ± 0.8^{c}	215 ± 1^{c}	113 ± 4^{b}	50 ± 6^{a}	129 ± 20^{b}	219 ± 6^{c}
Quercetin	n.d.	n.d.	n.d.	4 ± 1^{a}	9 ± 5^{a}	n.d.	n.d.	n.d.
Kaempferol 3-glucoside	13.8 ± 0.7^{a}	38.9 ± 0.1^{b}	$71 \pm 1^{c,d}$	76 ± 2^{d}	38 ± 10^{b}	16 ± 3^{a}	37 ± 8^{b}	64.5 ± 0.7^{c}
Kaempferol	2.0 ± 0.1^{a}	17.6 ± 0.3^{d}	40.8 ± 0.5^{e}	9.8 ± 0.1^{c}	5 ± 0.4^{b}	1.8 ± 0.4^{a}	16.9 ± 0.5^{d}	10 ± 1^{c}
Vitexin	5.9 ± 0.5^{a}	$13 \pm 3^{c,d}$	23 ± 3^{e}	17 ± 2^{d}	$8.0 \pm 0.6^{a,b}$	$11 \pm 1^{b,c}$	15.3 ± 0.3^{d}	16 ± 0.4^{d}
Isovitexin	3.2 ± 0.1^{a}	$8.7 \pm 0.0.8^{b}$	15 ± 2^{c}	9.2 ± 0.8^{b}	2.1 ± 0.2^{a}	8 ± 2^{b}	10.6 ± 0.1^{b}	9.9 ± 0.9^{b}
ABTS, mg TE/L	2087 ± 29^{b}	$2619 \pm 2^{d,e}$	$2781 \pm 7^{e.f}$	2432 ± 45^{c}	1219 ± 3^{a}	2389 ± 149^{c}	$2516 \pm 81^{c,d}$	2898 ± 124^{f}
FRAP, mg Fe^{2+}/L	1473 ± 7^{b}	2120 ± 53^{d}	2599 ± 89^{f}	2368 ± 57^{e}	998 ± 20^{a}	1701 ± 83^{c}	1994 ± 34^{d}	2731 ± 148^{f}

Values with different letters in each row are significantly different when applying the Fisher's least significant differences (LSD) method at p-value $\leq$ 0.05.
n.d.: non-detected.

that could be identified and quantified by HPLC-DAD have been listed in Table 3 for the different type of solvents. The highest concentration of individual phenolic compounds was determined for the 50% (v/v) ethanol aqueous mixture, that agreed with the maximum observed for TPC. The main phenolic compounds that could be identified and quantified in this work were neochlorogenic and chlorogenic acids and quercetin 3-O glucoside with maximum concentrations of 424 ± 41, 167 ± 18 and 212.9 ± 0.8 mg/L respectively in a 50% (v/v) ethanol aqueous solvent. Pure quercetin was only found at ethanol concentrations higher than 80% (v/v) while apigenin was found for ethanol aqueous mixtures with ethanol content lower than 50% (v/v). Other phenolic compounds were kaempferol, kaempferol 3-O-glucoside, vitexin, isovitexin and rutin.

The comparison of results with other authors is hard since the nature of the ML used in the extraction experiments strongly affects the phenolic compounds profile. However, in the literature it has been consistently reported that the most abundant phenolic acids in moringa leaves are chlorogenic acid and its isomers, whereas the most abundant flavonoids are kaempferol, quercetin, isorhamnetin, and apigenin, generally in the glycoside form, attached to a wide spectrum of sugar substituents (Lin et al., 2018). Rodríguez-Pérez et al. (2016) identified more than 60 compound by HPLC-MS, being kaempferol-3-O-glucoside and quercetin-3-O-glucoside the most abundant compounds. Lin et al. (2021) also identified neochlorogenic and chlorogenic acids as two of the major phenolic compounds in ML together with hyperoside (quercetin 3-D galactoside) and D-(+) catechin, with concentrations of 4.2 ± 0.3, 1.61 ± 0.07, 4.6 ± 0.2, 3.7 ± 0.2 mg/g_{DML}, respectively. These authors also identified kaempferol-3-rutinoside and kaempferol-3-glucoside, with concentrations of 2.2 ± 0.2 mg/g_{DML} and 1.33 ± 0.05 mg/g_{DML}, respectively, as the most abundant flavonoids. Vongsak et al. (2013a) found the best results for cryptochlorogenic and quercetin-3-glucoside extraction when using ethanol aqueous solution (70%, v/v) with concentrations of 13.2 ± 0.6 and 5.3 ± 0.1 mg/g raw material, respectively. When ethanol aqueous (50%, v/v) was used, the concentration of these two compounds was decreased by 25%, demonstrating the importance of the solvent selection in the extraction of phenolic compounds from ML. Bennett et al. (2003) tried different moringa varieties, identifying mainly chlorogenic acid (in the range from 0.38 ± 0.02 to 8.9 ± 0.3 mg/g_{DML}), neochlorogenic acid (up to 4.10 ± 0.08 mg/g_{DML}), kaempferol 3-O-glucoside (up to 1.80 ± 0.02 mg/g_{DML}), quercetin 3-O-glucoside (in the range from 0.08 ± 0.01 to 6.3 ± 0.3 mg/g_{DML}), and other more rare flavonoids, such as quercetin 3-O-(6″malonylglucoside) (up to 10.8 ± 0.4 mg/g_{DML}) and kaempferol 3-O-(6″malonylglucoside) (up to 3.1 ± 0.1 mg/g_{DML}). Nouman et al. (2016) studied the effect of the *M. oleifera* cultivar on the phenolic compounds profile, finding that chlorogenic acid was in the range from 57.1 ± 1.2 to 103 ± 3 μg/g_{DML} and cryptochlorogenic acid

was found in concentrations up to $9.1 \pm 0.5\ \mu g/g_{DML}$ for the cultivars studied. These authors also found kaempferol 3-O-glucoside (from 25.7 ± 2.6 to $39.8 \pm 0.8\ \mu g/g_{DML}$), as well as quercetin 3-O-glucoside (from 3.9 ± 0.8 to $8.8 \pm 0.4\ \mu g/g_{DML}$) and isovitexin (up to $47.8 \pm 1.2\ \mu g/g_{DML}$).

Table 3 shows the results of the antioxidant capacity of the liquid extracts. The maximum in the antioxidant activity agreed with the maximum concentration of TPC in the extracts by using a 50% ethanol aqueous mixture. According to the Pearson product moment correlation, statistically significant non-zero correlations at the 95% confidence level were obtained for TPC and the different antioxidant tests performed in this work ($R^2 = 0.9703$ and 0.9770 for FRAP and ABTS tests, respectively). However, Karthivashan et al. (2013) essayed three different ethanolic aqueous mixtures (ethanol:water, 50:50, 70:30 and 90:10%) as solvent, finding that 90% ethanol was the best solvent for the extraction of bioactive compounds from ML as confirmed by different antioxidant tests, including FRAP and DPPH.

The color variation of the extracts by using different ethanol aqueous mixtures could be visually observed from light orange, by using water as solvent, to a more greenish color by using pure ethanol as solvent. The increase in the green color by increasing the amount of ethanol in the extraction solvent could indicate the presence of chlorophylls in these extracts. This color variation was also observed in the extraction of biocompounds from olive leaves (Kashaninejad et al., 2020).

3.3. *Comparison of conventional solvent extraction with ultrasound assisted extraction*

Fig. 3 shows the extraction kinetics of TPC by using water, and different ethanol aqueous mixtures as solvents (20% and 50% v/v) by UAE. For a better comparison of the results, extraction kinetics by conventional solvent extraction have been also plotted. The use of UAE when using water as solvent resulted in an increase of the initial extraction rate and also an increase in final TPC concentration. This could be attributed to the cavitation created by the ultrasound waves that induced a better penetration of the solvent into the ML and improved the diffusion process. However, when using ethanol aqueous mixtures as solvents the extraction efficiency did not improve compared to conventional solvent extraction.

Rodríguez-Pérez et al. also compared the efficiency of TPC extraction from ML by conventional solid-liquid extraction (maceration) and UAE by using different ethanol aqueous mixtures (Rodríguez-Pérez et al., 2015). These authors concluded that a higher total phenolic content was obtained with UAE than by using maceration. However, these authors performed conventional solvent extraction in a single stage by using a ratio of $50\ mL/g_{ML}$ for 1 h. On the other hand, UAE was carried out by using the same solvent ratio for 15 min but three more consecutive extractions were carried out after removing the solids by centrifugation and treating the pellets (the solids) with fresh solvent under the same conditions in such way that four consecutive extractions were made to complete 1 h of extraction. Although extraction time was the same, results are not comparable. A comparison between conventional maceration and ultrasound assisted extraction in different stages must be carried out in the same extraction conditions (Meullemiestre et al., 2016). In the work of Rodríguez-Pérez et al., conventional solvent extraction was carried out in a single-stage method, while UAE was performed in four succes-

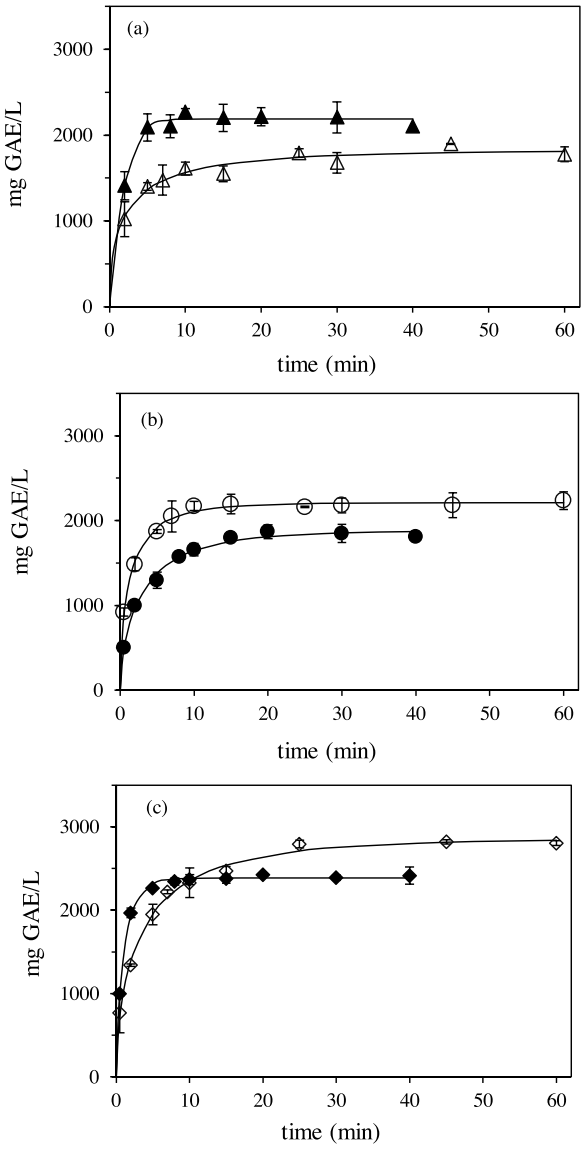

Fig. 3 – Comparison of UAE (solid symbols) and conventional extraction kinetics (empty symbols) at 50 °C and a ratio of $10\ mL/g_{ML}$ for different solvent types: (a) (△, ▲) water; (b) (○, ●) 20% EtOH; (c) (◇, ◆) 50% EtOH. The continuous lines represent the Weibull model.

sive single-stages adding fresh solvent in the different stages leading to an increasing driving force in each step (Rodríguez-Pérez et al., 2015). These authors reported an extraction yield of $24.3 \pm 0.3\ mg\ GAE/g_{DML}$ in one stage conventional extraction while in the 1st extraction run by UAE they reported a value of $12.6 \pm 0.2\ mg\ GAE/g_{DML}$ when using 70% (v:v) ethanol aqueous as solvent and $19 \pm 1\ mg\ GAE/g_{DML}$ after the 4th run. Other values reported by these authors when using a 50% (v/v) ethanol aqueous solution were the following: $27 \pm 2\ mg\ GAE/g_{DML}$ by conventional extraction, while $24 \pm 3\ mg\ GAE/g_{DML}$ after the 1st UAE run ($47 \pm 4\ mg\ GAE/g_{DML}$ after the 4th run). Although comparison of results of TPC by maceration and UAE in the 1st run differ in extraction time, it seems more reliable than after the 4th run of UAE, especially considering that according to the extraction kinetic curves obtained in this work (see Figs. 1–3), 30 min or even shorter times were found to be enough to reach a plateau. In the extraction of phenolic compounds from maritime sawdust waste by different multistage cross-current configurations by conventional and UAE by using acidified water as solvent, Meullemiestre et al. (2016) also concluded

Table 4 – Kinetic parameters of Weibull model for TPC extraction from moringa leaves. Relative error, RE.

Extraction kinetic			Kinetic parameters			
Solvent	Extraction method	S/ML	A	k	n	RE, %
20% EtOH	Conventional	10	2211 ± 22	0.78 ± 0.03	0.59 ± 0.03	2.0
20% EtOH	Conventional	20	1114 ± 6	0.61 ± 0.02	0.75 ± 0.03	1.2
Water	Conventional	10	1825 ± 69	0.61 ± 0.09	0.51 ± 0.11	3.5
50% EtOH	Conventional	10	2857 ± 48	0.45 ± 0.02	0.59 ± 0.03	2.3
80% EtOH	Conventional	10	2183 ± 44	0.19 ± 0.02	0.82 ± 0.05	13.3
EtOH	Conventional	10	1483 ± 221	0.050 ± 0.006	0.84 ± 0.08	5.4
Water	UAE	10	2189 ± 26	0.47 ± 0.09	1.2 ± 0.2	1.6
20% EtOH	UAE	10	1882 ± 26	0.42 ± 0.02	0.71 ± 0.04	2.5
50% EtOH	UAE	10	2385 ± 13	0.96 ± 0.03	0.80 ± 0.04	1.1

S/ML: ratio solvent moringa leaves, mL/g_{ML}.

that the majority of polyphenols were extracted during the first stage of extraction of 14 min. Furthermore, these authors also stated that ultrasound was more effective the first minutes of extraction and then, its effect decreased over time.

The effect of fluid properties on cavitation has been considered to analyse the results obtained in this work. Low values of vapour pressure, viscosity and surface tension of the solvent can improve the formation of high-intensity cavitation (Tao and Sun, 2015). Data for these properties have been collected from literature at 50 °C. An increase in ethanol concentration led to a continuous decrease in surface tension decreasing the cavitation threshold (Vazquez et al., 1995). However, a maximum in viscosity, around 46% (w/w) ethanol concentration, was observed (Belda et al., 2004). An increase in viscosity of the medium increased the resistance of the sample to the movement of the ultrasonic probe and higher intensity is necessary to obtain the mechanical vibration (Chemat et al., 2017). By increasing the ethanol concentration, the vapour pressure of the system continuously increased (Gmehling and Onken, 1981), being a key factor to explain the non-improvement of UAE compared to conventional solvent extraction when using ethanol aqueous mixtures as solvents (at 50 °C, p^{o}_{water} = 92.3 mmHg and $p^{o}_{ethanol}$ = 220.3 mmHg). Solvents with low vapour pressure produces a more intense collapse of cavitation bubble improving extraction (Chemat et al., 2017).

The results obtained by UAE when using ethanol aqueous mixtures could be also due to the low solvent:biomass ratio used in this work. Rodríguez-Pérez et al. (2015) employed a higher solvent:biomass ratio, 50 mL/g_{ML}, when working with UAE. Lower solvent:biomass ratio resulted in lower values of specific energies (kJ/g raw material) and higher resistance to the transference of the ultrasonic wave.

Individual phenolic compounds for the final extracts obtained by UAE are listed in Table 4. Based on the statistical analysis, no difference could be clearly observed in the phenolic profile identified in this work, between conventional and ultrasound assisted extraction.

3.4. *Extraction kinetics modelling*

The Weibull model was used to fit all the experimental extraction kinetics data obtained in this work. Parameters for the model are listed in Table 4. The Weibull model can be considered suitable to describe the extraction curves of TPC from ML with a medium value of the relative error of 3.7% for all the extraction kinetics. The good fitting can be observed in Figs. 1–3 where the Weibull model has been represented. The extraction parameter, A, that can be considered as the maximum concentration of TPC in the extracts, reached a maximum for a 50% ethanol aqueous solution as solvent by conventional solvent extraction. The exponent n, presented values lower than the unity, except for water in UAE (although is close to one). According to Kitanović et al. (2008), in case of extraction of plant materials the n value is usually n < 1 that corresponds to a parabolic curve with a high initial slope followed by an exponential shape (Figs. 1–3).

3.5. *Freeze dried extract (FDE)*

A solid extract was obtained from the liquid ML extract obtained by using a 50% (v/v) ethanol aqueous mixture at 50 °C and 10 mL solvent/g_{ML} ratio. This solvent concentration was used, based on the highest TPC and individual phenolic concentration obtained but also on the value of the retention index that was lower than for 20% (v/v) ethanol aqueous solution (see Table 2).

The final humidity content of the FDE was 3.76 ± 0.05%. The total yield of the FDE respect to the initial ML was 19%, according to the value obtained by this solvent for the total soluble solids yield (see Table 2). Karthivashan et al. (2013) reported lower extraction yield of ML extracts by using different ethanol aqueous mixture as solvent after freeze dried of the liquid extracts with an extraction yield of 8.48% for a 50% ethanol aqueous mixtures. The lower yield obtained by these authors compared to this work, was probably due to a poorer separation of the solid residue and the liquid extract by filtration while in this work was done by centrifugation. Hence, it must be highlighted the importance of the retention index of the selected solvent and an efficient separation process of the liquid and the solid residue to achieve a high final extraction yield in the global process.

A complete characterization of the FDE is presented in Table 5. FDE presented a high content of bioactive compounds as determined by the TPC and total flavonoids compounds, TFC, 82 ± 1 mg GAE/g_{FDE} and 19.6 ± 0.3 mg QE/g_{FDE}, respectively. The reducing power was 127 ± 2 mg Fe^{2+}/g_{FDE}, according to the FRAP method. The ABTS test was carried out to obtain the extract concentration to achieve a 50% inhibition of the radical. The ABTS results were compared with the inhibition response of pure TROLOX in water and ethanol as solvents and are presented in Fig. 4. The TROLOX standard response was linear and a concentration of 0.102 mg TROLOX/mL was needed to achieve a 50% ABTS radical inhibition. On the contrary, the FDE of ML exhibited an asymptotic response with

Table 5 – Characterization of the freeze-dried extract, FDE, from moringa leaves.

Total antioxidants	TPC, mg GAE/g_{FDE}	82 ± 1
	TFC, mg QE/g_{FDE}	19.6 ± 0.3
Antioxidant activity	FRAP, mg Fe^{2+}/g_{FDE}	127 ± 2
	mg FDE/mL$^{50\%\ ABTS\ inhibition}$	0.474
Individual phenolic compounds, mg/g_{FDE}	Neochlorogenic acid	15.0 ± 0.3
	Chlorogenic acid	5.7 ± 0.6
	Rutin	0.5 ± 0.1
	Quercetin 3-O-glucoside	7.3 ± 0.1
	Kaempferol 3-glucoside	2.07 ± 0.01
	Kaempferol	0.48 ± 0.01
	Vitexin	0.95 ± 0.06
	Isovitexin	0.59 ± 0.02
Other components	Ch-a, μg/g_{FDE}	44.8 ± 0.4
	Ch-b, μg/g_{FDE}	20.4 ± 0.1
	Cx + c, μg/g_{FDE}	17.7 ± 0.1

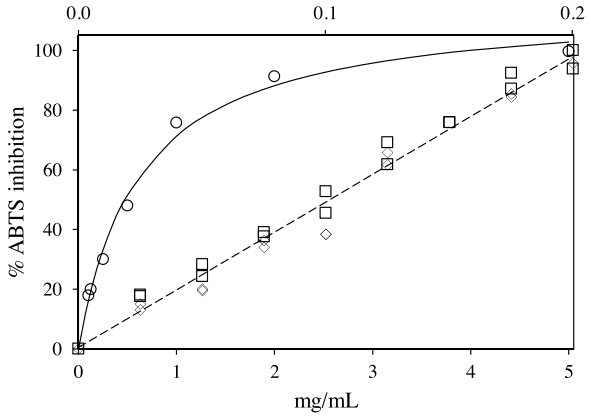

Fig. 4 – ABTS inhibition percentage by FDE from ML (lower x-axis) (○) and comparison with pure TROLOX standard (upper x-axis) dissolved in water (◊) and ethanol (□).

a concentration of 0.474 mg FDE/mL to achieve a 50% ABTS radical inhibition, more than 4.5 times that of TROLOX.

The individual phenolic compounds identified in the FDE have been also listed in Table 5. Similar to the liquid extracts, the main phenolic compounds that could be identified in the FDE were neochlorogenic and chlorogenic acids, quercetin 3-O-glucoside and kaempferol 3-O-glucoside with concentrations of 15.0 ± 0.3, 5.7 ± 0.6, 7.3 ± 0.1 and 2.07 ± 0.01 mg/g_{FDE}, respectively. The presence of pigments such as chlorophylls and carotenoids were responsible of the color of the extract. The amount of Chlorophyll-a, Chlorophyll-b and carotenoids was 44.8 ± 0.4, 20.4 ± 0.1 and 17.7 ± 0.1 μg/g_{FDE}, respectively. Vats and Gupta (2017) reported a higher value for β-carotene refer to ML, 14.1 ± 0.1 mg/g ML, by solvent extraction with a 95% ethanol aqueous mixture as solvent for 24 h in an orbital shaker at 37 °C.

The individual phenolic compounds concentration was followed in the FDE along storage at room temperature and darkness and at 4 °C during 101 days (Fig. 5). Not a significant decrease for the phenolic compounds determined in this work was observed along storage either at room temperature or at cooling conditions. The content of the phenolic compounds varied in the range from 90% to 98% of the initial content, except for vitexin and isovitexin whose content drop to 55–60 of its initial content in the FDE. Vongsak et al. (2013b) also found that after 3 months of storage at 25 °C, antioxidant capacity and bioactive compounds content were not significantly different in the extracts compared with the freshly prepared extracts.

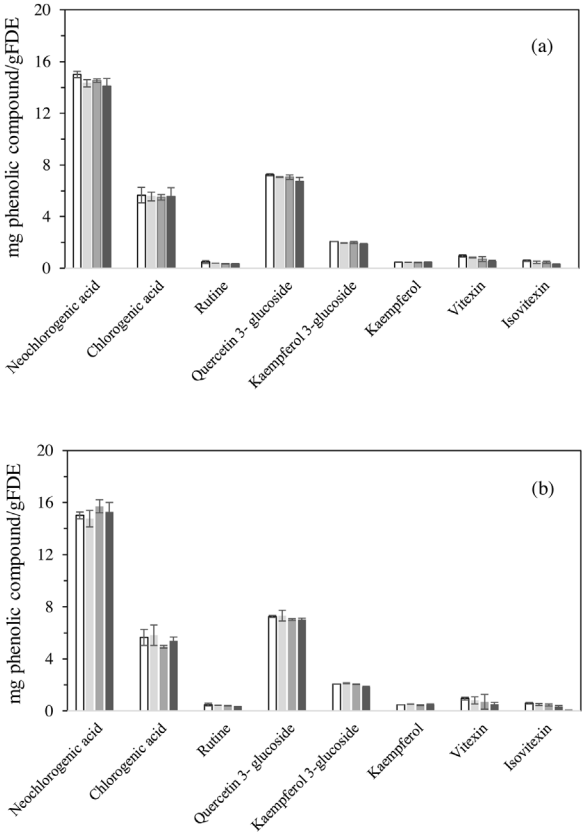

Fig. 5 – Composition of the individual phenolic compounds along storage at room temperature (a) and 4 °C (b): □ fresh prepared FDE, ■ 18 days, ■ 39 days, ■ 101 days.

3.6. Conclusions

Chemical composition of ML showed important amounts of extractives rich in bioactive compounds that makes this plant an attractive raw material as nutraceutical products source.

Extraction of this fraction by using different hydroalcoholic mixtures showed the importance of the type of solvent since it determined the selectivity of the extracted bioactive compounds but also the retention index of the extract phase in the solid residue. It was found that by increasing the amount of water in the ethanol aqueous mixture the retention index also increased. A 50% ethanol aqueous mixture (v/v) was found to be the best extraction solvent with the highest TPC yield and individual phenolic acids concentration. UAE yielded higher extraction yield when using water as solvent, but the presence of ethanol did not bring any improvement.

A freeze-dried extract was obtained with a high content of bioactive compounds such as neochlorogenic and chlorogenic acids and quercetin 3-O-glucoside, among others. Phenolic profile was kept during storage at room temperature and 4 °C for 3 months. Due to the antioxidant capacity and phenolic composition, the ML freeze-dried extract could be used as additive for food, pharmaceutical or cosmetic industries.

Funding

This work was supported by the Junta de Castilla y León (JCyL) and the European Regional Development Fund (ERDF) [grant numbers BU301P18 and BU050P20]; and the Agencia Estatal de Investigación [grant number AEI/10.13039/501100011033].

Conflict of interest

The authors declare no conflict of interest.

Declaration of Competing Interest

The authors report no declarations of interest.

References

Alonso-Riaño, P., Sanz Diez, M.T., Blanco, B., Beltrán, S., Trigueros, E., Benito-Román, O., 2020. Water ultrasound-assisted extraction of polyphenol compounds from Brewer's spent grain: kinetic study, extract characterization, and concentration. Antioxidants 9, 265, http://dx.doi.org/10.3390/antiox9030265.

Amaglo, N.K., Bennett, R.N., Lo Curto, R.B., Rosa, E.A.S., Lo Turco, V., Giuffrida, A., Curto, A.L., Crea, F., Timpo, G.M., 2010. Profiling selected phytochemicals and nutrients in different tissues of the multipurpose tree *Moringa oleifera* L., grown in Ghana. Food Chem. 122, 1047–1054, http://dx.doi.org/10.1016/j.foodchem.2010.03.073.

Ames, B.N., Shigenaga, M.K., Hagen, T.M., 1993. Oxidants, antioxidants, and the degenerative diseases of aging. Proc. Natl. Acad. Sci. U. S. A. 90, 7915–7922, http://dx.doi.org/10.1073/pnas.90.17.7915.

Ancut, S., Färcaå, A.C., Cristian, D., Rusu, B., Tofan, M., 2018. Influence of the extraction solvent on phenolic content, antioxidant, antimicrobial and antimutagenic activities of brewers' spent grain rcas. J. Cereal Sci. 80, 180–187, http://dx.doi.org/10.1016/j.jcs.2018.03.006.

Araújo, A.C.M.A., Resende de Oliveira, É., Menezes, E.G.T., Dias, B.O., Terra, A.W.C., Queiroz, F., 2018. Solvent effect on the extraction of soluble solids from murici and pequi seeds. J. Food Process Eng. 41, http://dx.doi.org/10.1111/jfpe.12813.

Belda, R., Herraez, J.V., Diez, O., 2004. Rheological study and thermodynamic analysis of the binary system (water/ethanol): influence of concentration. Phys. Chem. Liq. 42, 467–479, http://dx.doi.org/10.1080/00319100410001700850.

Bennett, R.N., Mellon, F.A., Foidl, N., Pratt, J.H., Dupont, M.S., Perkins, L., Kroon, P.A., 2003. Profiling glucosinolates and phenolics in vegetative and reproductive tissues of the multi-purpose trees *Moringa oleifera* L. (Horseradish tree) and Moringa stenopetala L. J. Agric. Food Chem. 51, 3546–3553, http://dx.doi.org/10.1021/jf0211480.

Benzie, I.F.F., Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant power": the FRAP assay. Anal. Biochem. 239, 70–76, http://dx.doi.org/10.1006/abio.1996.0292.

Berk, Z., 2009. Food process engineering and technology, chapter 11 "Extraction". In: Food Science and Technology International Series. Elsevier, pp. 259–276.

Castro-lópez, C., Ventura-sobrevilla, J.M., González-hernández, M.D., Rojas, R., Ascacio-valdés, J.A., Aguilar, C.N., Martínez-ávila, G.C.G., 2017. Impact of extraction techniques on antioxidant capacities and phytochemical composition of polyphenol-rich extracts. Food Chem. 237, 1139–1148, http://dx.doi.org/10.1016/j.foodchem.2017.06.032.

Chang, C.C., Yang, M.H., Wen, H.M., Chern, J.C., 2002. Estimation of total flavonoid content in propolis by two complementary colometric methods. J. Food Drug Anal. 10, 178–182.

Chemat, F., Rombaut, N., Sicaire, A.G., Meullemiestre, A., Fabiano-Tixier, A.S., Abert-Vian, M., 2017. Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. Ultrason. Sonochem. 34, 540–560, http://dx.doi.org/10.1016/j.ultsonch.2016.06.035.

Gmehling, J., Onken, U., 1981. Vapor liquid equilibrium data collection. Aqueous Syst. Suppl. 3 Chem. Data Ser. Vol. I Part 1c 53, 1689–1699.

González-Romero, J., Arranz-Arranz, S., Verardo, V., García-Villanova, B., Guerra-Hernández, E.J., 2020. Bioactive compounds and antioxidant capacity of moringa leaves grown in Spain versus 28 leaves commonly consumed in pre-packaged salads. Processes 8, 1–20, http://dx.doi.org/10.3390/pr8101297.

Illera, A.E., Sanz, M.T., Benito-Román, O., Varona, S., Beltrán, S., Melgosa, R., Solaesa, A.G., 2018. Effect of thermosonication batch treatment on enzyme inactivation kinetics and other quality parameters of cloudy apple juice. Innov. Food Sci. Emerg. Technol. 47, http://dx.doi.org/10.1016/j.ifset.2018.02.001.

Karthivashan, G., Tangestani Fard, M., Arulselvan, P., Abas, F., Fakurazi, S., 2013. Identification of bioactive candidate compounds responsible for oxidative challenge from hydro-ethanolic extract of *Moringa oleifera* leaves. J. Food Sci. 78, http://dx.doi.org/10.1111/1750-3841.12233.

Kashaninejad, M., Sanz, M.T., Blanco, B., Beltrán, S., Niknam, S.M., 2020. Freeze dried extract from olive leaves: valorisation, extraction kinetics and extract characterization. Food Bioprod. Process. 124, 196–207, http://dx.doi.org/10.1016/j.fbp.2020.08.015.

Kitanović, S., Milenović, D., Veljković, V.B., 2008. Empirical kinetic models for the resinoid extraction from aerial parts of St. John's wort (*Hypericum perforatum* L.). Biochem. Eng. J. 41, 1–11, http://dx.doi.org/10.1016/j.bej.2008.02.010.

Leone, A., Fiorillo, G., Criscuoli, F., Ravasenghi, S., Santagostini, L., Fico, G., Spadafranca, A., Battezzati, A., Schiraldi, A., Pozzi, F., Di Lello, S., Filippini, S., Bertoli, S., 2015. Nutritional characterization and phenolic profiling of *Moringa oleifera* leaves grown in chad, sahrawi refugee camps, and Haiti. Int. J. Mol. Sci. 16, 18923–18937, http://dx.doi.org/10.3390/ijms160818923.

Lin, M., Zhang, J., Chen, X., 2018. Bioactive flavonoids in *Moringa oleifera* and their health-promoting properties. J. Funct. Foods 47, 469–479, http://dx.doi.org/10.1016/j.jff.2018.06.011.

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. J. Appl. Res. Med. Aromat. Plants 20, http://dx.doi.org/10.1016/j.jarmap.2020.100284.

Meullemiestre, A., Petitcolas, E., Maache-Rezzoug, Z., Chemat, F., Rezzoug, S.A., 2016. Impact of ultrasound on solid-liquid extraction of phenolic compounds from maritime pine sawdust waste. Kinetics, optimization and large scale experiments. Ultrason. Sonochem. 28, 230–239, http://dx.doi.org/10.1016/j.ultsonch.2015.07.022.

Nouman, W., Anwar, F., Gull, T., Newton, A., Rosa, E., Domínguez-Perles, R., 2016. Profiling of polyphenolics, nutrients and antioxidant potential of germplasm's leaves from seven cultivars of *Moringa oleifera* Lam. Ind. Crops Prod. 83, 166–176, http://dx.doi.org/10.1016/j.indcrop.2015.12.032.

Oldoni, T.L.C., Merlin, N., Karling, M., Carpes, S.T., Alencar, S.M. de, Morales, R.G.F., Silva, E.A. da, Pilau, E.J., 2019. Bioguided extraction of phenolic compounds and UHPLC-ESI-Q-TOF-MS/MS characterization of extracts of *Moringa oleifera* leaves collected in Brazil. Food Res. Int. 125, 108647, http://dx.doi.org/10.1016/j.foodres.2019.108647.

Oliveira, R., Carvalho, G.R., Cirillo, M., Queiroz, F., 2019. Effect of ecofriendly bio-based solvents on oil extraction from green coffee bean and its industrial press cake. Brazilian J. Chem. Eng. 36, 1739–1753, http://dx.doi.org/10.1590/0104-6632.20190364s20190102.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26, 1231–1237, http://dx.doi.org/10.1016/S0891-5849(98)00315-3.

Rodríguez-Pérez, C., Quirantes-Piné, R., Fernández-Gutiérrez, A., Segura-Carretero, A., 2015. Optimization of extraction method to obtain a phenolic compounds-rich extract from *Moringa*

oleifera Lam leaves. Ind. Crops Prod. 66, 246–254, http://dx.doi.org/10.1016/j.indcrop.2015.01.002.

Rodríguez-Pérez, C., Gilbert-López, B., Mendiola, J.A., Quirantes-Piné, R., Segura-Carretero, A., Ibáñez, E., 2016. Optimization of microwave-assisted extraction and pressurized liquid extraction of phenolic compounds from *Moringa oleifera* leaves by multiresponse surface methodology. Electrophoresis 37, 1938–1946, http://dx.doi.org/10.1002/elps.201600071.

Singleton, V.L., Orthofer, R., Lamuela-Raventós, R.M., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods Enzymol. 299, 152–178, http://dx.doi.org/10.1016/j.scienta.2016.11.004.

Sluiter, J.B., Ruiz, R.O., Scarlata, C.J., Sluiter, A.D., Templeton, D.W., 2010. Compositional analysis of lignocellulosic feedstocks. 1. Review and description of methods. J. Agric. Food Chem. 58, 9043–9053, http://dx.doi.org/10.1021/jf1008023.

Sumanta, N., Imranul Haque, C., Nishika, J., Suprakash, R., 2014. Spectrophotometric analysis of chlorophylss and carotenoids from commonly grown fern species by using various extracting solvents. Res. J. Chem. Sci. 4, 63–69, http://dx.doi.org/10.1055/s-0033-1340072.

Tao, Y., Sun, D.W., 2015. Enhancement of food processes by ultrasound: a review. Crit. Rev. Food Sci. Nutr. 55, 570–594, http://dx.doi.org/10.1080/10408398.2012.667849.

Vats, S., Gupta, T., 2017. Evaluation of bioactive compounds and antioxidant potential of hydroethanolic extract of *Moringa oleifera* Lam. from Rajasthan, India. Physiol. Mol. Biol. Plants 23, 239–248, http://dx.doi.org/10.1007/s12298-016-0407-6.

Vazquez, G., Alvarez, E., Navaza, J.M., 1995. Surface tension of alcohol + water from 20 to 50 °C. J. Chem. Eng. Data 40, 611–614, http://dx.doi.org/10.1021/je00019a016.

Vongsak, B., Sithisarn, P., Mangmool, S., Thongpraditchote, S., Wongkrajang, Y., Gritsanapan, W., 2013a. Maximizing total phenolics, total flavonoids contents and antioxidant activity of *Moringa oleifera* leaf extract by the appropriate extraction method. Ind. Crops Prod. 44, 566–571, http://dx.doi.org/10.1016/j.indcrop.2012.09.021.

Vongsak, B., Sithisarn, P., Gritsanapan, W., 2013b. Bioactive contents and free radical scavenging activity of *Moringa oleifera* leaf extract under different storage conditions. Ind. Crops Prod. 49, 419–421, http://dx.doi.org/10.1016/j.indcrop.2013.05.018.

Wisniak, J., Hillel, J., Katz, O., 1987. Holdup and extraction characteristics of jojoba meal. J. Am. Oil Chem. Soc. 64, 1352–1354, http://dx.doi.org/10.1007/BF02540796.