OCL 2022, 29, 28

https://doi.org/10.1051/ocl/2022023

OCL Oilseeds & fats Crops and Lipids

Available online at:
www.ocl-journal.org

RESEARCH ARTICLE OPEN ACCESS

Stability of microemulsions containing red grape pomace extract obtained with a glycerol/sodium benzoate deep eutectic solvent☆

Aggeliki Alibade[1], Olga Kaltsa[2,*], Eleni Bozinou[2], Vassilis Athanasiadis[2], Dimitrios Palaiogiannis[2], Stavros Lalas[2], Arhontoula Chatzilazarou[1] and Dimitris P. Makris[2]

[1] Department of Wine, Vine & Beverage Sciences, University of West Attica, Ag. Spyridonos Str., Egaleo 12243, Athens, Greece
[2] Green Processes & Biorefinery Group, Department of Food Science & Nutrition, School of Agricultural Sciences, University of Thessaly, N. Temponera Street, Karditsa 43100, Greece

Received 24 January 2022 – Accepted 22 June 2022

Abstract – The valorization of red grape pomace is significant as grape is one of the most cultivated fruits worldwide and generated by-product quantities are enormous. For this purpose, numerous encapsulation techniques have been developed. However, the studies on microemulsions composed with deep eutectic solvent extracts are very limited. In this study, red grape pomace extract (RGPE) was first prepared by deep eutectic solvent extraction and characterized by HPLC analysis. Rutin, quercetin, catechin and caftaric acid were identified as the main non-pigment phenolic compounds. The RGPE was further encapsulated in microemulsions (MEs) following a low-energy approach using a mixture of low molecular weight surfactants, and the pseudo-ternary phase diagram was constructed. The physical and antioxidant stability of MEs containing 3–15 wt% RGPE was investigated for a period of 30 days. MEs were stable at an ambient temperature of 25 or 37 °C. The radical scavenging activity of encapsulated RGPE was improved up to 13% compared to the free extract. Our results indicate that microemulsions provide protection of valuable phenolic constituents especially under elevated temperature conditions and can therefore be used as systems for applications in nutraceuticals or cosmetics.

Keywords: wine extract / deep eutectic solvent / microemulsions / antioxidant stability

Résumé – Stabilité des microémulsions contenant un extrait de marc de raisin rouge obtenues avec le solvant eutectique profond aglycérol/benzoate de sodium. La valorisation du marc de raisin rouge est importante car le raisin est l'un des fruits les plus cultivés au monde et les quantités de sous-produits générés sont énormes. Dans cet objectif, de nombreuses techniques d'encapsulation ont été développées. Cependant, les études sur les microémulsions composées d'extraits de solvants eutectiques profonds restent très limitées. Dans cette étude, l'extrait de marc de raisin rouge a d'abord été préparé par extraction par un solvant eutectique profond et caractérisé par analyse HPLC. La rutine, la quercétine, la catéchine et l'acide caftarique ont été identifiés comme les principaux composés phénoliques non pigmentaires. L'extrait de marc de raisin rouge a ensuite été encapsulé dans des microémulsions (ME) selon une approche à faible énergie en utilisant un mélange de surfactants de faible poids moléculaire, et un diagramme de phase pseudo-ternaire a été construit. La stabilité physique et antioxydante des MEs contenant 3–15 % en poids d'extrait de marc de raisin rouge a été étudiée pendant une période de 30 jours. Les ME étaient stables à une température ambiante de 25 ou 37 °C. L'activité antiradicalaire de l'extrait de marc de raisin rouge encapsulé a été améliorée jusqu'à un maximum 13 % par rapport à l'extrait libre. Nos résultats indiquent que les microémulsions protègent les constituants phénoliques précieux, en particulier dans des conditions de température élevée, et peuvent donc être utilisées comme systèmes pour des applications dans le domaine des nutraceutiques ou des cosmétiques.

Mots clés : extrait de vin / solvant eutectique profond / microémulsions / stabilité des antioxydants

☆ Contribution to the Topical Issue "Bioactive lipids and lipid droplets: green resources for food and health / Lipides et gouttelettes lipidiques bioactifs : des ressources vertes pour l'alimentation et la santé"

*Correspondence: okaltsa@uth.gr

Highlights

- DES-in-oil microemulsions containing red grape pomace extract were successfully prepared.
- Microemulsions were able to preserve the antiradical capacity of red grape pomace extract at elevated storage temperature.
- Catechin followed by quercetin were the most affected compounds during storage.

1 Introduction

Wine (or grape) pomace is the residue generated during the fermentation process of pressed grapes. It is mainly composed from the grape skins, secondarily the seeds and small stems, and accounts between 20 to 30% of the initial grape mass (Jin *et al.*, 2020). The global production of grapes intended for winemaking was estimated around 44 million t, according to 2018 data from the International Organization of Vine and Wine (OIV; Spigno *et al.*, 2017).

In several regions, grape pomace has long been used for the production of traditional distilled spirits such as "Tsipouro" or "Raki" in Greece, Italian "Grappa", French "eau-de-vie de marc", etc. (Apostolopoulou *et al.*, 2005). In its dried form, grape pomace is conventionally used as feed (Tayengwa *et al.*, 2021) and after composting as soil conditioner (Ilyas *et al.*, 2021).

Apart from these uses, within the context of a more viable waste management, wine pomace has been exploited to produce biofuels, biopolymers, antioxidant compounds and other chemicals (Sirohi *et al.*, 2020). Red grape pomace contains high amounts of phenolic compounds (TPC = 0.28–8.7% dw; Antonić *et al.*, 2020) belonging to different classes (flavonoids specifically anthocyanins [malvidin, petunidin, cyanidin, peonidin and delphinidin] and catechins), phenolic acids and stilbenes (resveratrol; Ferri *et al.*, 2016; Milinčić *et al.*, 2021; Muñoz-Bernal *et al.*, 2021).

However, the types of compounds identified and quantified each time depend on differences based on variety, regional climate and of course the wine-making procedure followed.

The beneficial effect of moderate red wine consumption is well documented and health benefits with regards to cardiovascular disease, diabetes or some types of cancers are attributed to the anti-inflammatory and antioxidant properties of the polyphenols it contains (Rosenzweig *et al.*, 2017). Several studies have demonstrated the promising protective effect of polyphenols on neurodegenerative diseases such as Alzheimer's and dementia (El Gaamouch *et al.*, 2021). Beneficial properties have also been established for resveratrol (*trans*-3,4′,5-trihydroxystilbene) in *in vitro* studies focusing on epilepsy, amyotrophic lateral sclerosis (ALS), Parkinson's disease, Huntington's disease, or nerve injuries. The exact mechanism and direct targets involved remain unknown (Pasinetti *et al.*, 2015). However, wine consumption is largely criticized, not recommended in cases of liver damage, or even prohibited for religious reasons. Therefore, red grape pomace may suggest an effective alternate source of wine polyphenols. According to a recent study, red grape pomace is approximately 20% richer in phenolic compounds than red wine, due to higher amounts of anthocyanins, flavonols, stilbenes and flavanals, and reduced the VLDL-cholesterol and triglyceride levels more effectively in *in vivo* studies with animal subjects (de Oliveira *et al.*, 2017). Similarly, red grape pomace consumption improved the body weight, glucose tolerance, insulin sensitivity and hepatic steatosis of high-fat diet fed mice (Rosenzweig *et al.*, 2017).

Microemulsion systems are an effective tool to improve their chemical stability, bioavailability and targeting properties of phenolic compounds. A wide range of studies offer information on the properties of pure compounds or plant extract microemulsion formulations (Chatzidaki *et al.*, 2015; Fregapane *et al.*, 2022; Cecchi *et al.*, 2020), etc. With regards to grape derived constituents, anthocyanins have been incorporated in water-in oil microemulsions (Oh *et al.*, 2006), resveratrol in both oil-in-water or water-in-oil formulations (Juškaitė *et al.*, 2015; Yutani *et al.*, 2015) and more recently in deep eutectic solvent (DES)/water-in oil microemulsions (Sakuragi *et al.*, 2020).

Deep eutectic solvents have gained interest as natural alternatives to more expensive or toxic solvents, as they combine tailor-made properties, biodegrability and enhanced extraction efficiency (Kaltsa *et al.*, 2020). Their synthesis usually involves one constituent serving as a hydrogen bond donor (HBD) and another one as a hydrogen bond acceptor (HBA). Low-cost, food-grade substances such as organic acids, organic acid salts, polyols and amino acids are representative examples of materials used for DES preparation (Alibade *et al.*, 2021).

However, to our best knowledge, it is the first time to report the incorporation of grape pomace extract in DES solution – in-oil microemulsions. Therefore, the purpose of this study was to prepare microemulsions containing red grape pomace extract (RGPE) produced by an eco-friendly/"green" extraction method (DES extraction) and examine major stability properties under different storage conditions.

2 Materials and methods

2.1 Chemicals

Sodium benzoate (99%) was obtained from Merck (Darmstadt, Germany). Glycerol anhydrous (99.5%) was obtained from Penta (Praha, Czechia). Catechin, quercetin, caftaric acid, rutin (quercetin 3-*O*-rutinoside) (>94%), and 2,2-diphenylpicrylhydrazyl (DPPH), Isopropyl-myristate (IPM), methanol, sorbitan monolaurate (Span® 20) and polyoxyethylenesorbitan monooleate (Tween® 80) were from Sigma-Aldrich (Darmstadt, Germany). Solvents used for chromatographic purposes were HPLC grade.

2.2 Grape pomace extract preparation/grape marc extract preparation

Red grape pomace of *Vitis vinifera* cv. Muscat of Hamburg grapes was a kind donation from a local winery (Karditsa, Greece). All handling and processing procedures of the plant material and optimum extraction protocol followed are described in detail in our previous work (Alibade *et al.*, 2021). In brief, glycerol (hydrogen bond donor [HBD]) and sodium benzoate (hydrogen bond acceptor [HBA]) were

mixed in a Duran^{TM} bottle of appropriate volume and heated at 70 °C, under stirring at 400 rpm, for approximately 60 min, to form perfectly transparent liquids. The selected mixing ratio of glycerol/sodium benzoate system was 9:1, as it was shown that this combination formed a transparent liquid which remained stable in terms of crystal formation over several weeks. The DES mixture was finally diluted with water at a level of 70% w/w as dictated by optimum extractability experiments. Ultrasonic (US) assisted deep eutectic solvent extraction parameters included: US pretreatment (15 min, room temperature, 25 °C, 104.33 W) in Elma S 100 (H) heated ultrasonic bath (Elma Schmidbauer GmbH, Singen, Germany) followed by stirred-tank extraction (liquid-to-solid ratio: 10 mL of DES solution/g of dried pomace, 80 °C, 240 min, 900 rpm) using a magnetic stirrer (Witeg, Wertheim, Germany). The final mixture was centrifuged (10,000 rpm) and the supernatant was collected for further use.

2.3 Phase diagram

The area of microemulsion formation and corresponding concentration ranges of each component (wine waste extract: S_{mix}: IPM) was found by constructing a pseudoternary phase diagram following the titration method. Tween 80 and Span 20 were mixed at a weight ratio of 4:1 to obtain the surfactant mixture (S_{mix}). Then, the oil phase (IPM) was mixed with the surfactant mixture at different weight ratios (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1). The final mixtures were diluted with the wine waste extract in a dropwise manner (25 μL every 24 h), by stirring (600 rpm/2 min/30 °C) and the point where samples became turbid was marked (Mitsou *et al.*, 2019). The Microsoft Excel software was employed for the construction of the pseudoternary phase diagram.

2.4 Microemulsion selection and storage

The microemulsions prepared contained a constant amount of S_{mix} at 25 wt%, according to the optimum area obtained from the pseudoternary diagram and was mixed with appropriate amounts of the oil phase (IPM). The concentration of the wine waste extract added dropwise was 3, 6, 9, 12 and 15 wt% and the total microemulsion obtained was 3 g. All components were mixed under stirring (600 rpm/30 °C) and finally allowed to equilibrate prior to analysis. Finally, microemulsions were stored at room temperature (25 °C) or elevated temperature (37 °C) (CO_2 incubator model MCO-20 AIC, Sanyo, Japan) and their chemical and physical stability was evaluated over a period of 30 days. Measurements were performed at least in triplicate.

2.5 Accelerated and gravitational stability of microemulsions

Freshly prepared microemulsion samples (1 mL) were subjected to centrifugation at 3,000 rpm for 20 min (25 °C) (Ortoalresa Digicen 20-R, Los Frailes, Spain) and afterwards were examined optically for turbidity, phase separation or sedimentation (Kaltsa *et al.*, 2021).

2.6 Extraction of phenolic components from microemulsions

A volume of 0.5 mL microemulsion sample was diluted 1:1 with methanol in a 1.5 mL Eppendorf tube and vortexed for a few seconds. Then, the tubes containing the mixture were centrifuged at 10,000 rpm for 10 min (20 °C) (Ortoalresa Digicen 20-R, Los Frailes, Spain) and supernatants were collected for DPPH and HPLC analysis.

3.7 Radical scavenging activity determination

The radical scavenging activity of selected microemulsions was determined by the using the DPPH radical method following our previous methodology (Kaltsa *et al.*, 2021). Briefly, 975 μL of DPPH solution (100 μM in methanol) was mixed with 25 μL of each supernatant and kept in the dark to react. The absorbance of the mixture was measured after 30 min at 515 nm (Shimadzu UV-1700, Shimadzu Europa GmbH, Duisburg, Germany). The lower the absorbance of the reaction mixture, the higher the scavenging activity. Simple aqueous solutions containing 3, 6, 9, 12 and 15 wt% of wine waste extract served as control samples. Results were expressed as percentage inhibition (A_{AR}%) against blank according to Equation (1):

$$A_{AR}\% = 100 \times \left(1 - \frac{A_s}{A_0}\right) \quad (1)$$

where, A_s and A_0 are the absorbance at 515 nm of the sample and the blank respectively.

2.8 HPLC analysis

A Shimadzu CBM-20A liquid chromatograph (Shimadzu Europa GmbH, Duisburg, Germany) was used for HPLC analysis, coupled with an SIL-20AC autosampler and a CTO-20AC column oven. The detector was a Shimadzu SPD-M20A, which was connected to the computer *via* Shimadzu LC solution software. In a previous work (Alibade *et al.*, 2021), the characteristics of the column and the elution program were described in detail. Based on the calibration curves (1–50 μg mL^{-1}) constructed with catechin ($R^2 = 0.9999$), caftaric acid ($R^2 = 0.9999$), rutin ($R^2 = 0.9990$), and quercetin ($R^2 = 0.9999$), the flavanols, hydroxycinnamates, and flavonols were quantified at 280, 320 and 360, respectively. Samples were analyzed in triplicate.

2.9 Statistical analysis

Statistical analysis of the results was performed with Statgraphics Centurion XV (Statgraphics, Rockville, MD, USA) and the LSD test was applied in order to compare the mean values of microemulsion and extract solution properties (A_{AR}% and content of phenolic constituents by HPLC) at 99% level of confidence.

3 Results and discussion

3.1 Phase diagram

A mixture of non-ionic surfactants, Tween 80 and Span 20 in 4:1 ratio was selected because it has been shown that this

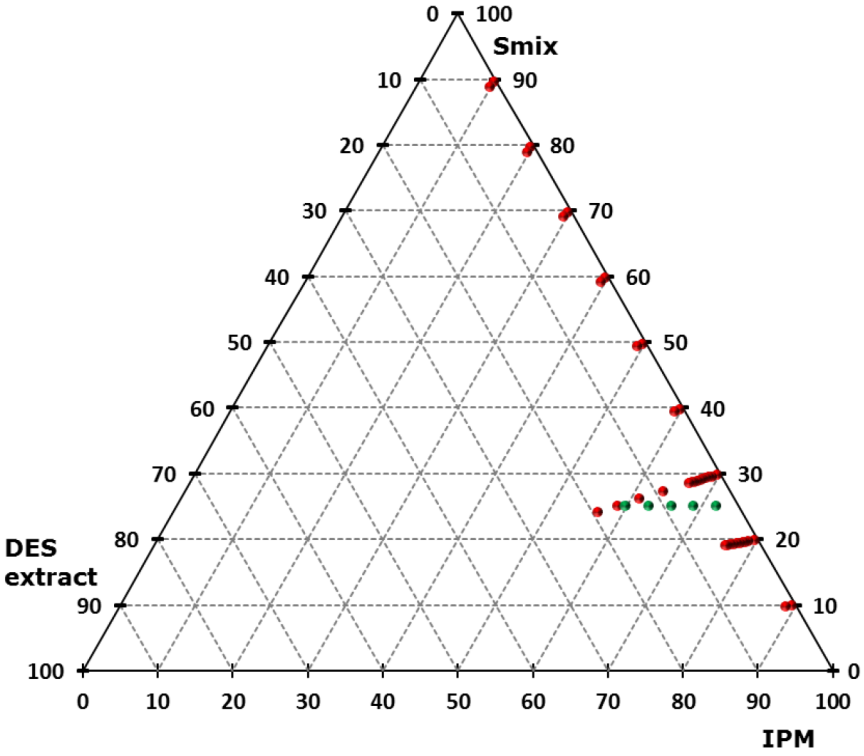


Fig. 1. Pseudoternary phase diagram for obtaining DES-in-oil microemulsions. Black dots represent the area of microemulsion formation and green dots selected formulations (25 wt% S_{mix}: 3, 6, 9, 12 or 15 wt% red wine pomace extract).

analogy was able to entrap high amounts of resveratrol diluted in DES composed by similar ingredients (~50 wt%; Sakuragi *et al.*, 2020). Isopropyl-myristate used as the oil phase finds many applications in cosmetics as well as in food grade delivery systems (Li *et al.*, 2017; Chen *et al.*, 2019; Mitsou *et al.*, 2019).

The pseudo-ternary phase diagram of the wine waste DES extract: S_{mix}: IPM system is depicted in Figure 1. As can be seen, a moderate/narrow microemulsion formation area was detected based on the optical translucency of the samples. The main microemulsion area was located within the region of S_{mix} concentration between 20 and 30 wt%. According to our results, the above S_{mix} ratio at the given concentration (~22 wt%) was able to solubilize the RGPE at concentrations as high as ~20 wt%. However, this sample presented phase separation after a few days of storage and hence it was omitted from further investigation. Therefore, formulations marked with green dots, representing samples with a fixed 25 wt% total surfactant concentration and varying wine waste extract concentration (3, 6, 9, 12 and 15 wt%), were selected to assess their physical and chemical stability.

3.2 Centrifugal and physical stability during storage

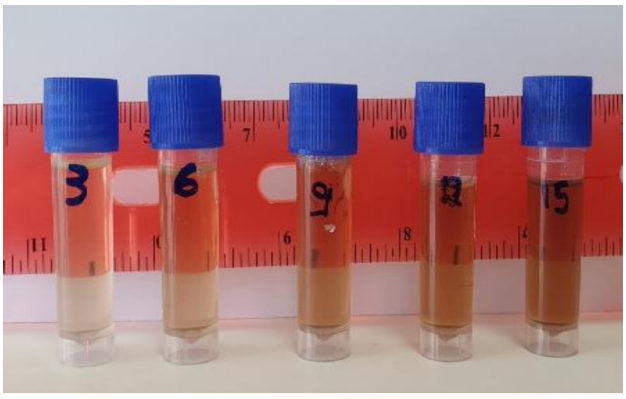
The appearance of freshly prepared microemulsion samples was red hued translucent, relatively low viscosity liquids that were free to flow when reversed. Upon the effect of centrifugal forces neither of the samples was stratified (Fig. 2a). All microemulsion formulations remained stable as well during storage under different temperature conditions (25 or 37 °C) with no visible turbidity, phase separation, aggregation, or sedimentation of the components at the bottom during 30 days of storage in the dark (Fig. 2b and c). Therefore, the long-term gravitational stability of the formulations is ensured even when elevated storage temperatures may occur.

3.3 Effect of storage on radical scavenging activity

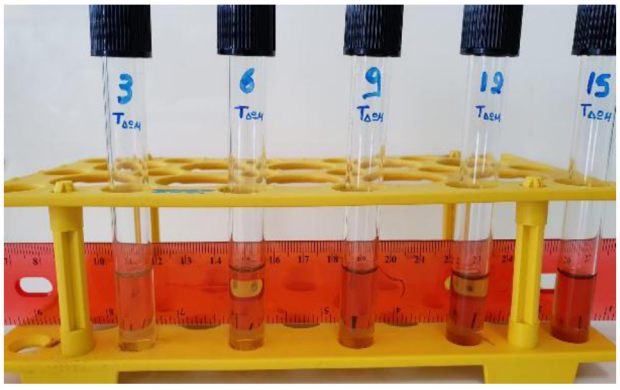
DPPH assay is a simple, versatile, and low-cost method for assessing the total radical scavenging activity of phenolic extracts.

In Fig. 3a, b , the effect of concentration and storage temperature on the radical scavenging activity of encapsulated and non-encapsulated/crude/free RGPE is presented. Extract concentration increase from 3 to 15 wt% caused a positive linear dose-dependent response to initial DPPH inhibition values, which varied between 12.59 to 55.82% and 12.7 to 59% for microemulsions and non-encapsulated extracts accordingly. However, similar values of scavenging activity were observed in most cases between microemulsions and plain extract homologues ($P < 0.01$).

Temperature had also a profound effect on the antioxidant activity of both types of samples during storage. For presentation reasons, data on the effect of different storage temperatures on microemulsions and extract solutions containing 15 wt% RGPE that presented the highest initial radical scavenging activity are displayed. DPPH values of microemulsions or extract samples (15% RGPE) stored under room temperature conditions (25 °C) remained practically unaffected even after 30 days of storage (Fig. 3b; $P > 0.01$). On the other



(a)



(b)

(c)

Fig. 2. Centrifugal and gravitational stability of PGPE microemulsions: (a) freshly prepared samples after centrifugation (3,000 rpm/20 min), (b) after 30 days at 25 °C and (c) after 30 days at 37 °C.

hand, elevated storage temperatures (37 °C), gradually decreased the scavenging activity of non-encapsulated extract. Fortunately, emulsified RGPE (RGPE MEs) was more resilient against storage and similar values were observed between freshly prepared and stored samples (day 30) ($P > 0.01$). It should be mentioned that the protective effect of emulsification against temperature and time degradation has an onset around 15–18 days, before which no statistical differences are detected between microemulsions and extract solutions. In total, a drastic reduction in scavenging values of almost 23% was estimated for non-emulsified 15 wt% extract solutions at 37 °C, whereas the scavenging activity of microemulsions was reduced only by ~2%.

Temperature is a key factor when it comes to the stability of microemulsions which serve as delivery systems of antioxidant compounds. However, the extent of its influence cannot be easily predicted, as formulations and extract antioxidant properties vary considerably. In general, temperature elevation is accompanied by a loss of the antioxidant potential of phenolic substances. The loss of antiradical activity of phenolic compounds caused by temperature is associated to their chemical degradation (Sólyom *et al.*, 2014; Slavu *et al.*, 2020; Zapata *et al.*, 2021). For instance, the main derivative of gallic acid decarboxylation occurring at high temperatures (105–150 °C) is pyrogallol (Boles *et al.*, 1988). Quercetin glycosides under roasting conditions (180 °C) form the quercetin aglycone (Rohn *et al.*, 2007), whereas when heated at milder temperatures, protocatechuic acid is identified as the major product of cleavage (Buchner *et al.*, 2006). The overall degradation phenomenon of phenolic antioxidants is enhanced over time even at temperatures under room temperature, whereas refrigerated storage conditions suggest an effective strategy for preserving both TPC content and radical scavenging activity (Gollücke *et al.*, 2009; Tsantili *et al.*, 2011).

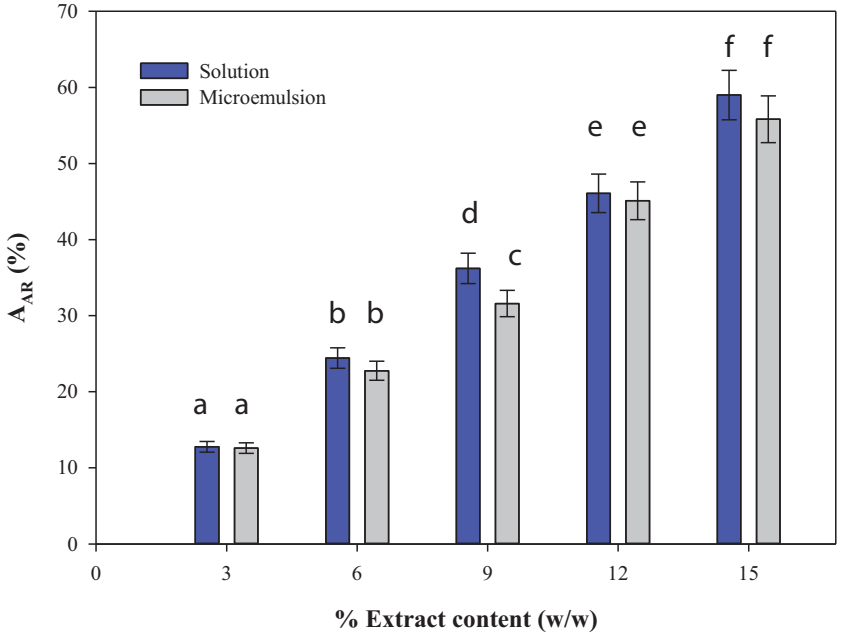
Our findings are in line with other studies on antioxidant compounds embedded in microemulsions. The encapsulation of curcumin in O/W microemulsions significantly improved its antiradical properties (Li *et al.*, 2017). Enhanced antiradical properties of *Cymbopogon citratus* essential oils encapsulated in microemulsions by 47% and 184% as assessed by ORAC (oxygen radical absorbance capacity assay) or PSC (peroxyl radical scavenging capacity assay) compared to the direct use of the oil have also been reported.

As shown by Mostafa *et al.* (2014) the retention of polyphenols in microemulsions stored at room temperature was improved by 8% compared to those stored at 40 °C after 3 months of storage. In another study, no significant differences on the antioxidant activity of curcumin loaded O/W microemulsions when stored at lower temperatures (4 or 25 °C) were observed (Amuti *et al.*, 2021).

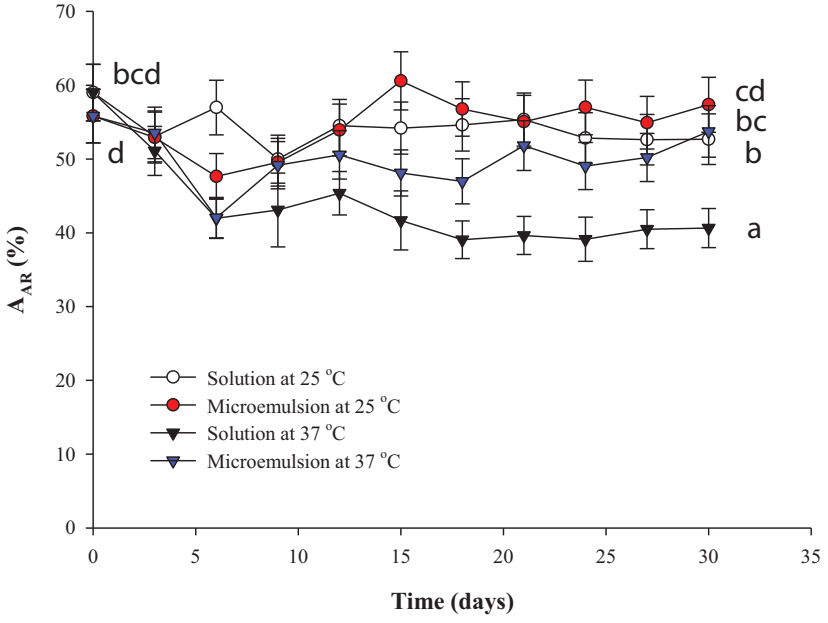
Hence, room or lower temperatures are suggested as an efficient means for prolonging their antioxidant stability of encapsulated RGPE.

3.4 Effect of storage temperature on the content of major phenolic constituents

The quantification of the non-pigment phenolic compounds conducted by HPLC analysis is demonstrated in Figure 4. The samples tested are the initial free RGPE used as 15 wt% aqueous solution, and stored samples of the extract and its emulsified homologue after 30 days at room temperature (25 °C) or at 37 °C. As shown in our previous work (Alibade *et al.*, 2021), catechin is one of the main abundant compounds identified (17.74 $\mu g\,g^{-1}$), followed by quercetin (6.68),



(a)



(b)

Fig. 3. Radical scavenging activity of free and emulsified RGPE: (a) as affected by concentration (freshly prepared samples) and (b) samples containing 15 wt% RGPE as affected by storage. Different letters indicate significant differences among samples ($P < 0.01$).

caftaric acid (6.14) and rutin (1.48). In general, the emulsification of the RGPE enhanced the chemical stability of the polyphenols identified and this was more evident in the case of samples stored at elevated temperature (37 °C) ($P < 0.01$). More specifically, catechin was not detected in samples of the free extract after being stored for 30 days at room temperature, whereas in microemulsions it was depleted almost by 66% compared to the initial concentration ($P < 0.01$). In free extract samples stored at 37 °C, both catechin and quercetin could not be detected. On the contrary, in the form of microemulsions – with the exception of catechin –, the degradation of polyphenols was moderate (13.1% or less). Hence, the loss observed on the antioxidant capacity of free extracts at elevated temperature is possibly

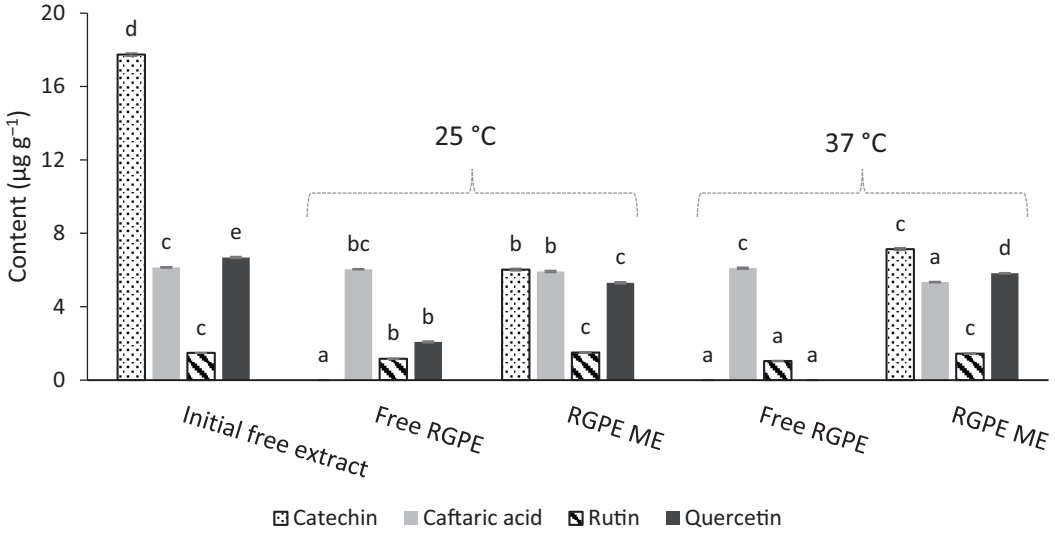


Fig. 4. Concentration of main phenolic compounds of free and encapsulated red grape pomace extract after 30 days of storage under different temperatures. Different letters for the same property (catechin, caftaric acid, rutin or quercetin content) indicate significant differences among samples ($P < 0.01$).

associated to the complete degradation of these major phenolic components, catechin and quercetin. Slightly different findings are reported by Tolun *et al.* (2020) who described the effect of storage conditions on the stability of grape pomace extract in polymer microcapsules. Their study revealed that the content of caftaric acid, catechin and rutin in free extract is reduced after 75 days by ~70–80%, 57–65% and 60–80% respectively depending on relative humidity levels. On the contrary, when encapsulated in maltodextrin-gum Arabic spray dried microspheres the overall reduction in the concentration of the specific antioxidants was restricted to 2 up to 15%. However, they do report that catechin was the most affected compound among encapsulated samples and rutin the most resilient one, that is in line with our findings. Similar degradation levels are also reported for other maltodextrin microspheres containing red wine phenolics (Galmarini *et al.*, 2013). Of course, direct comparisons cannot be made with the present study, but the fact that the polymer beads seemingly act more protectively towards the degradation of phenolic compounds could possibly be ascribed to the low mobility of the core compounds within the solid-like polymer matrices. The complexation of antioxidants with polysaccharides (Tolun *et al.*, 2020) may also provide an additional barrier against their decomposition.

4 Conclusions

In this study, a new type of microemulsion (DES-in-oil) has been introduced for the successful emulsification of red grape pomace extract, which allowed the entrapment of high amounts of polyphenolic antioxidants with a relatively low concentration of low molecular weight surfactants. The incorporation of RGPE in microemulsions was more efficient in maintaining the antiradical activity of the extract during storage at elevated temperature (37 °C), as evidenced by practically unaffected A_{AR}% values. Catechin followed by quercetin were the most sensitive phenolic compounds, since their concentration totally depleted after 30 days of storage at 37 °C in the free extract. Emulsification and storage at room temperature ensured the chemical stability and long-term antiradical activity of the red grape pomace extract.

Author contributions

S.I.L., D.P.M. and A.C., experimental design, supervision, monitoring of research work, manuscript reviewing; O.K. laboratory work, analysis, statistical analysis, writing; A.A., V. A., and D.P., laboratory work, analysis, editing; E.B.: manuscript revision. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare that they have no conflicts of interest in relation to this article.

References

Alibade A, Lakka A, Bozinou E, Lalas SI, Chatzilazarou A, Makris DP. 2021. Development of a green methodology for simultaneous extraction of polyphenols and pigments from red winemaking solid wastes (pomace) using a novel glycerol-sodium benzoate deep eutectic solvent and ultrasonication pretreatment. *Environments* 8(9): 90.

Amuti A, Wang X, Zan M, Lv S, Wang Z. 2021. Formulation and characterization of curcumin-loaded microemulsions: evaluation of antioxidant stability and *in vitro* release. *J Mol Liq* 336: 116881.

Antonić B, Jančíková S, Dordević D, Tremlová B. 2020. Grape pomace valorization: a systematic review and meta-analysis. *Foods* 9(11): 1627.

Apostolopoulou AA, Flouros AI, Demertzis PG, Akrida-Demertzi K. 2005. Differences in concentration of principal volatile constituents in traditional Greek distillates. *Food Control* 16(2): 157–164.

Boles JS, Crerar DA, Grissom G, Key TC. 1988. Aqueous thermal degradation of gallic acid. *Geochim Cosmochim Acta* 52(2): 341–344.

Buchner N, Krumbein A, Rohn S, Kroh LW. 2006. Effect of thermal processing on the flavonols rutin and quercetin. *Rapid Commun Mass Spectrom* 20: 3229–3235.

Cecchi L, Piazzini V, D'Ambrosio M, *et al.* 2020. Formulation of a phenol-rich extract from unripe olives (*Olea europaea* L.) in microemulsion to improve its solubility and intestinal permeability. *Molecules* 25(14): 3198.

Chatzidaki MD, Mitsou E, Yaghmur A, Xenakis A, Papadimitriou V. 2015. Formulation and characterization of food-grade microemulsions as carriers of natural phenolic antioxidants. *Colloids Surf A Physicochem Eng Asp* 483: 130–136.

Chen L, Lin X, Xu X, *et al.* 2019. Self-nano-emulsifying formulation of Sonchus oleraceus Linn for improved stability: implications for phenolics degradation under *in vitro* gastro-intestinal digestion: food grade drug delivery system for crude extract but not single compound. *J Funct Foods* 53: 28–35.

de Oliveira WP, Biasoto ACT, Marques VF, *et al.* 2017. Phenolics from winemaking by-products better decrease VLDL-cholesterol and triacylglycerol levels than those of red wine in wistar rats. *J Food Sci* 82: 2432–2437.

El Gaamouch F, Liu K, Lin HY, Wu C, Wang J. 2021. Development of grape polyphenols as multi-targeting strategies for Alzheimer's disease. *Neurochem Int* 147: 105046.

Ferri M, Bin S, Vallini V, *et al.* 2016. Recovery of polyphenols from red grape pomace and assessment of their antioxidant and anticholesterol activities. *N Biotechnol* 33(3): 338–344.

Fregapane G, Cabezas Fernández C, Salvador MD. 2022. Emulsion and microemulsion systems to improve functional edible oils enriched with walnut and pistachio phenolic extracts. *Foods* 11 (9): 1210.

Galmarini MV, Maury C, Mehinagic E, *et al.* 2013. Stability of individual phenolic compounds and antioxidant activity during storage of a red wine powder. *Food Bioproc Technol* 6: 3585–3595.

Gollücke PBA, Ramos Catharino R, de Souza JC, Nogueira Eberlin M, de Queiroz Tavares D. 2009. Evolution of major phenolic components and radical scavenging activity of grape juices through concentration process and storage. *Food Chem* 112(4): 868–873.

Ilyas T, Chowdhary P, Chaurasia D, Gnansounou E, Pandey A, Chaturvedi P. 2021. Sustainable green processing of grape pomace for the production of value-added products: an overview. *Environ Technol Innov* 23: 101592.

Jin Q, Wang Z, Feng Y, *et al.* 2020. Grape pomace and its secondary waste management: biochar production for a broad range of lead (Pb) removal from water. *Environ Res* 186: 109442.

Juškaitė V, Ramanauskienė K, Briedis V. 2015. Design and formulation of optimized microemulsions for dermal delivery of resveratrol. *Evidence-Based Complementary and Alternative Medicine* 2015: 540916.

Kaltsa O, Alibade A, Batra G, Bozinou E, Makris DP, Lalas SI. 2021. Fortification of chocolate using *Moringa oleifera* extract encapsulated in microemulsions. *OCL* 28: 38.

Kaltsa O, Grigorakis S, Lakka A, Bozinou E, Lalas S, Makris DP. 2020. Green valorization of olive leaves to produce polyphenol-enriched extracts using an environmentally benign deep eutectic solvent. *AgriEngineering* 2(2): 226–239.

Li Y, Yokoyama W, Xu S, Zhu S, Ma J, Zhong F. 2017. Formation and stability of W/O microemulsion formed by food grade ingredients and its oral delivery of insulin in mice. *J Funct Foods* 30: 134–141.

Milinčić DD, Stanisavljević NS, Kostić AZ, *et al.* 2021. Phenolic compounds and biopotential of grape pomace extracts from *Prokupac* red grape variety. *LWT* 138: 110739.

Mitsou E, Dupin A, Sassi AH, *et al.* 2019. Hydroxytyrosol encapsulated in biocompatible water-in-oil microemulsions: how the structure affects *in vitro* absorption. *Colloids Surf B Biointerfaces* 184: 110482.

Mostafa DM, Ammar NM, Abd El-Alim SH, El-Anssary AA. 2014. Transdermal microemulsions of *Glycyrrhiza glabra* L.: characterization, stability and evaluation of antioxidant potential. *Drug Deliv* 21(2): 130–139.

Muñoz-Bernal ÓA, Coria-Oliveros AJ, de la Rosa LA, *et al.* 2021. Cardioprotective effect of red wine and grape pomace. *Food Res Int* 140: 110069.

Oh JK, Kim SJ, Imm JY. 2006. Antioxidative effect of crude anthocyanins in water-in-oil microemulsion system. *Food Sci Biotechnol* 15(2): 283–288.

Pasinetti GM, Wang J, Ho L, Zhao W, Dubner L. 2015. Roles of resveratrol and other grape-derived polyphenols in Alzheimer's disease prevention and treatment. *Biochim Biophys Acta – Mol Basis Dis* 1852(6): 1202–1208.

Rohn S, Buchner N, Driemel G, Rauser M, Kroh LW. 2007. Thermal degradation of onion quercetin glucosides under roasting conditions. *J Agric Food Chem* 55(4): 1568–1573.

Rosenzweig T, Skalka N, Rozenberg K, *et al.* 2017. Red wine and wine pomace reduced the development of insulin resistance and liver steatosis in HFD-fed mice. *J Funct Foods* 34: 379–389.

Sakuragi M, Yano R, Binti S, Hasnol M, Kusakabe K. 2020. Evaluation of microemulsions containing a large amount of a deep eutectic solvent as a potential transdermal carrier of resveratrol. *Jpn J Appl Phys* 59: 095004.

Sirohi R, Tarafdar A, Singh S, *et al.* 2020. Green processing and biotechnological potential of grape pomace: current trends and opportunities for sustainable biorefinery. *Biores Technol* 314: 123771.

Slavu M, Aprodu I, Milea ȘA, *et al.* 2020. Thermal degradation kinetics of anthocyanins extracted from purple maize flour extract and the effect of heating on selected biological functionality. *Foods* 9(11): 1593.

Sólyom K, Solá R, Cocero MJ, Mato RB. 2014. Thermal degradation of grape marc polyphenols. *Food Chem* 159: 361–366.

Spigno G, Marinoni L, Garrido GD. 2017. State of the art in grape processing by-products. In: Galanakis CM, ed. Handbook of grape processing by-products. Cambridge (Massachusetts): Academic Press, pp. 1–27.

Tayengwa T, Chikwanha OC, Raffrenato E, Dugan MER, Mutsvangwa T, Mapiye C. 2021. Comparative effects of feeding citrus pulp and grape pomace on nutrient digestibility and utilization in steers. *Animal* 15(1): 100020.

Tolun A, Artik N, Altintas Z. 2020. Effect of different microencapsulating materials and relative humidities on storage stability of microencapsulated grape pomace extract. *Food Chem* 302: 125347.

Tsantili E, Konstantinidis K, Christopoulos MV, Roussos PA. 2011. Total phenolics and flavonoids and total antioxidant capacity in pistachio (*Pistachia vera* L.) nuts in relation to cultivars and storage conditions. *Sci Hortic* 129(4): 694–701.

Yutani R, Teraoka R, Kitagawa S. 2015. Microemulsion using polyoxyethylene sorbitan trioleate and its usage for skin delivery

of resveratrol to protect skin against UV-induced damage. *Chemical and Pharmaceutical Bulletin* 63(9): 741–745.

Zapata JE, Sepúlveda CT, Álvarez AC. 2021. Kinetics of the thermal degradation of phenolic compounds from achiote leaves (*Bixa orellana* L.) and its effect on the antioxidant activity. *Food Sci Technol* [online]. Available from https://scholar.google.com/scholar?hl=el&as_sdt=0%2C5&q=Kinetics+of+the+thermal+degradation+of+phenolic+compounds+from+achiote+leaves+%28Bixa+orellana+L.%29+and+its+effect+on+the+antioxidant+&btnG=.

Cite this article as: Alibade A, Kaltsa O, Bozinou E, Athanasiadis V, Palaiogiannis D, Lalas S, Chatzilazarou A, Makris DP. 2022. Stability of microemulsions containing red grape pomace extract obtained with a glycerol/sodium benzoate deep eutectic solvent. *OCL* 29: 28.