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Original article

Is high-frequency ultrasound a useful process to add value to out of specification strawberries, raspberries and blackberries industrially?

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Summary Bioactive ingredients can be extracted from surplus soft fruits to add value to them as a fortification ingredient in many new products. Ultrasound-assisted extraction and spray drying have been heavily studied in the past, with evidence to suggest the positive uptake of these by the food industry. In this paper, strawberries, raspberries and blackberries were examined using a distilled water 'green' extraction method with assisted high-frequency ultrasound and concentration through spray drying. The results showed that crop year and variety had more impact on bioactive concentration than extraction through high-frequency ultrasound. Two different machines were examined for differences between a cold extraction of water, and a 700 and 2000 Hz industrially relevant probes. Typically, total phenolic content (TPC) was lower in strawberries and blackberries than the control for both methods, however raspberries had a higher GAE mg ml−1 for the 2000 Hz ultrasound than the control. For Radical scavenging (RS) percentage using DPPH Blackberries had higher RS % than the control, whereas strawberries and raspberries had less than the control. These results suggest that ultrasound as a singular method for extracting valuable bioactive ingredients is not suitable with water as the solvent.

Keywords antioxidants, spray drying, total phenolics, ultrasonication.

Introduction

The bioactive compounds present in soft fruits are well known to provide health benefits such as anti-inflammatory, antioxidant potential, antithrombotic, anticarcinogenic, antidiabetic, hepatoprotective and prevention of cardiovascular and degenerative diseases (Rojas *et al.*, 2021). The concentration of these compounds increases with ripening where harvesting period is optimal to ensure ripened fruit are available to consumers. Over-ripened fruit results in waste which is a loss to the economy estimated to be 10 000 tonnes annually just within the UK for one crop (strawberry) (Wrap, 2017). Season fruit such as *Fragaria* × *ananassa* (Strawberries), *Rubus idaeus* (Raspberries) and *Rubus* (Blackberries) are widely cultivated throughout the world and contribute significantly to the global economy. Strawberries are estimated to contribute $29 bn USD, whereas raspberries contribute to around $1.1 bn USD to the global economy (FOA Stat, 2022).

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Ilari *et al.* (2021) described the Life Cycle Assessment of Protected Strawberry Productions in Central Italy and looked at the soilless (common in Scotland) and tunnel aspects of strawberry cultivation, showing soilless had more impact of greenhouse gases than tunnel cultivation in all areas of production.

While retail and consumers have an expectation for product quality in terms of condition of the harvested soft fruit at the point of sale or consumption, in terms of colour, appearance, texture, flavour and aroma, there is a significant amount of wastage. Strawberries, raspberries and blackberries tend to have 3–4 days before they soften and are no longer appealing to consumers, this short life span makes it increasingly difficult to supply (Joo *et al.*, 2011). To prolong the storage of these soft fruits, many manufacturers are looking at drying techniques to reduce the moisture content and keep the products longer. Wastage is estimated to be up to 9% for strawberries, with an estimated UK commercial value and therefore loss of £24 million annually (WRAP, 2017). The principal causes of waste and losses in primary production are due to

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pests and diseases, cosmetic specifications, overproduction, weather-related damage, supply and demand, harvest, storage and processing facilities and changing consumer demand (Beausang *et al.*, 2017). Much of the waste has little sale value and is therefore a net cost to the producers. Recently, the authors have suggested various streams for valorisation (adding value) to raspberries in terms of dried powders (Krivokapić *et al.*, 2021) and seeds (Daud *et al.*, 2022), strawberries into biochar, bioethanol and bioplastics (Cubero-Cardoso *et al.*, 2020) which can reduce financial loss and wastage, but the high cost of producing these is questionable without scale.

A more interesting approach is to utilise or extract natural higher-added value components contained within the soft fruit. Soft fruit can be divided into different 'fractions' to maximise utilisation, which include dietary fibre fractions, lipids from the seeds or bioactive ingredients. Extraction of bioactive compounds is, however, dependent on the downstream processing methods used and should include cost and solvent used, which can lead to the generation of potentially hazardous waste, and quantity of sample to be processed per batch (Sagar *et al.*, 2018). Bioactive extraction using 'green' methods can be a useful addition to the commercialisation process of tunnel strawberry, raspberry or blackberry production and growing literature suggests the use of high-frequency ultrasound could be used as a potential way of extracting bioactive compounds more readily from soft fruits. Golmohamadi *et al.* (2013) found antioxidant activity of extracted phenolics, and monomeric anthocyanins were found to be significantly greater at 20 kHz compared to other 986 kHz for raspberry puree. At 20 kHz, the total phenolic content increased from 1454 ± 29 to 1628 ± 30 mg L^{-1}, whereas the anthocyanin content decreased from 282 ± 3 to 248 ± 8 mg L^{-1}. Krivokapić *et al.* (2021) found that raspberry pomace ultrasound-assisted extraction (UAE) had greater efficiency that conventional macerations for FRAP (1002.72 µmol L^{-1}) and DPPH assays (969.71 µmol ml^{-1} vit C Eq; 567.00 µmol 100 g^{-1} Trolox Equivalence) using mildly acidulated methanol as the solvent medium. Zafra-Rojas *et al.* (2016) suggested that UAE was a useful addition at laboratory scale for blackberry TPC and Anthocyanins extracted using water as the base medium. Spray drying is a method whereby a liquid with particulates dissolved into its medium is atomised through a spray drying nozzle and carried through a heated chamber where moisture is driven off and results in a 'spray' dried powder. Current literature is exploring the optimisation of the process and expanding on carrier performance. Raspberry powders sprayed dried can be optimised to produce powders with higher quality, like improved microbial resistance (low moisture contents and water activity improvements) lower possibility to clumping which can improve the storage life of powders over time (Przybył *et al.*, 2021). Carrier compounds are required to bind to the bioactive compounds so that during the spray drying process the yield can be increased, maltodextrin out preformed agave fructans and showed a high stability for blackberry than raspberry (Farías-Cervantes *et al.*, 2020).

What is lacking within the scientific literature is the upscaling of UAE to better understand the implications in terms of industrial processes and adding value to waste materials from the tunnel growing of raspberries, strawberries and blackberries. This article fills that gaps and specifically looks at the effects of two different methods of UAE to increase total phenolic content and free-radical scavenging capacity of raspberries, strawberries and blackberries suspended in water, which would provide industry with an additional commercial focus.

Materials and methods

Characterisation of soft fruit

Soft fruit were grown in polytunnels on a fruit farm in rural Perthshire, Scotland (A.P Barrie Ltd.). Raspberries, strawberries and blackberries were harvested towards the end of their respective growing seasons, with all fruit harvested between mid-August and the end of October in both 2018 and 2019. These 2 years constitute the harvest years.

Chemicals and sample preparation

All chemicals were purchased from Sigma Aldrich (Surrey, UK) unless otherwise stated.

High-Frequency Ultrasound extraction

Two standard sonicators were used in this study, which are commonly used in bench-top and industrial processing of liquids for homogenisation, emulsification, dispersing, particle fine-milling, lysis and extraction:

iQ700 sonicator (qSonica, Connecticut, USA). This device comprised of a ½-inch probe and operated at a frequency of 20 kHz with a controllable output to a maximum of 700 W.

iiUIP2000hdt sonicator (Hielscher Ultrasonics GmbH, Germany). This device comprised a ½ inch probe which operated at 20 kHz had a maximum power output of 2000 W.

Soft fruit were prepared for extraction by first grinding in a blender (Philips Blender HR3573/91, Netherlands) while frozen. This comprised 5 g samples of fruit per 50 ml ddH_2O. The samples were then added to a glass vessel to a final volume of 500 ml and the sonicator probe was lowered to a depth of 2.5 cm from the base. The glass vessel was held within an ice bath to ensure that the temperature did not exceed 40°C. Samples of extract were withdrawn at the start (0 min) and 45 min. The sonicators were operated at 350 W (50% of maximum power). The total energy input, along with sample extraction temperature was recorded every 15 min.

After sonication, the samples of extract were first filtered through a sieve (mesh size 10), and then through cheese cloth to remove any remaining solids. The samples were then weighed and stored frozen at -20 °C until required for spray drying.

Spray drying

Frozen samples were prepared for spray drying by thawing. Maltodextrin as a carbohydrate carrier was added at a concentration of 10% w:v. This was mixed until dissolved using a magnetic stirrer. Samples were then dried using a bench top Buchi Mini Spray Dryer B-290 (Buchi UK Ltd, Suffolk, UK). This was operated with an inlet temperature of 140 °C and outlet temperature below 70°C. The liquid sample flow rate was 7 ml min^{-1} while the aspirator flow was 35 m^3 h^{-1}. The spray drying used a nozzle injector, and the system was allowed to stabilise for 5 min first using water before using the liquid extract. Control maltodextrin samples were also generated at this stage, with a solution of 10% w:v maltodextrin/ddH_2O generated and spray dried. Between each run, the powder was collected in the collection chamber, weighed into a foil container to determine drying efficiency and then 100 mg samples decanted into 2 ml Eppendorf tubes. These tubes were kept in the dark and at room temperature until they were analysed for TPC and DPPH, which was within 48 h. Typically the water activity of each powder ranged from 0.2–0.3 aw and kept air tight prior to being used again.

Total phenolic content assays

A modified Folin assay based on the protocol of Blainski *et al.* (2013) was used. Using a 96 well plate, the samples were analysed simultaneously, and to ensure continuity between assays. This involved re-suspending 100 mg of powder in 1 ml of ddH_2O. First, 25 μL of ddH_2O was added to each well, before 6 μL of sample was pipetted. To this, 6 μL of Folin–Ciocalteu reagent (Sigma Aldrich) was added, before samples being allowed to stand for 5 min. To this 65 μL of sodium carbonate (Na_2CO_3) was added, before a further 200 μL of ddH_2O was added. Samples were left to sit for 1 h in a dark environment before spectrophotometer readings at 760 nm were taken. Results were expressed as mg Gallic Acid Equivalents (GAE)/100 g fruit, as an 8-point standard curve up to 1 mg ml^{-1} Gallic acid was used. All samples were run in triplicate.

2,2-diphenyl-1-picrylhydrazyl Assay

A modified assay (Sánchez-Moreno 2002) method was used to evaluate the free radical scavenging activity and adapted onto a 96 well plate and read in a plate reader (Anthos Labtec Plate Reader Type 10 550, Netherlands). The samples for this were re-suspended in 1 ml of Methanol (Fisher Scientific UK). The sample was mixed using a bench top vortex for 1 min before resting on the bench top for 20 min to allow any undissolved maltodextrin precipitating to the bottom of the Eppendorf. Then, 10 μL, 20 μL and 30 μL of sample were pipetted in triplicate into the microplate, before each of the wells being made up to 200 μL final volume with 100% methanol. To this, 100 μL (1 mmol) DPPH (Sigma Aldrich, UK) was added to all wells. Twelve control wells were set up containing 200 μL Methanol, with 100 μL DPPH and 12 blank wells were set up containing 300 μL 100% methanol. The plate was covered and kept dark for 30 min before spectrophotometric (Jenway™ Visible Spectrophotometer, UK) readings were taken at 515 nm.

Statistical analysis

Statistical analysis was completed using SPSS (IBM Corp. Released 2021. IBM SPSS Statistics for Windows, Version 28.0. Armonk, NY: IBM Corp) using a three-way between subjects ANOVA. The yield experiment was subjected to a three-way ANOVA to determine if treatment, crop year and variety of soft fruit influenced the yield of spray-dried powder. A three-way ANOVA was used to better understand the interaction of crop year, soft fruit variety and treatment of ultrasound on the gallic acid equivalence (GAE) mg ml^{-1}. A three-way ANOVA was conducted with treatment, crop year and variety of fruit for the DPPH assay.

Results and discussion

Yield

Figure 1 shows the recovery of spray dried bioactive compounds from the ultrasonic treatment and variety of fruit (A), crop year and treatment (B) and crop year and fruit variety (C). Raspberries within the control show a larger marginal mean compared with yield from treated raspberries, and none from the treated other soft fruits (Fig. 1 (A)). The addition of ultrasound to strawberries and blackberries has very little effect on the recovery of bioactive compounds. Very little differences were observed for the treatments and

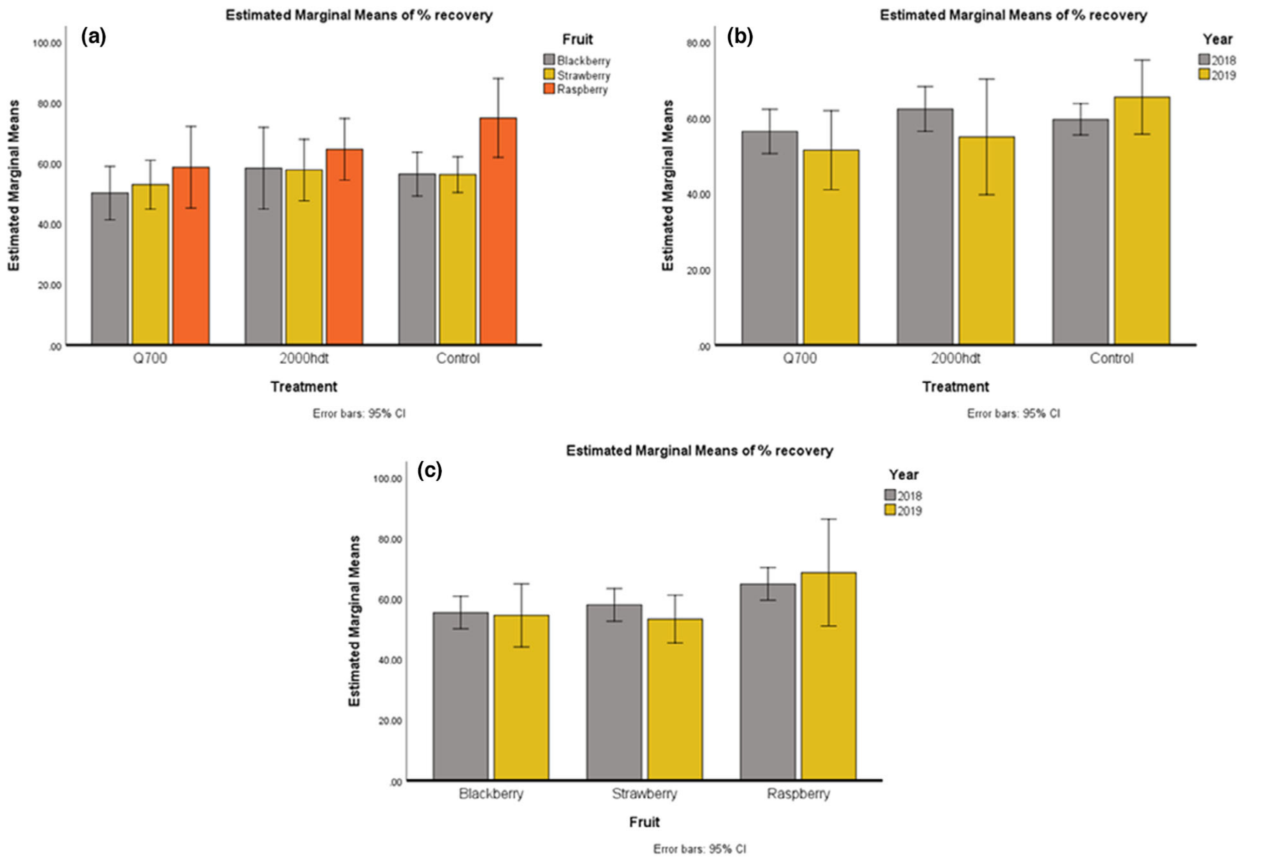

FIGURE 1 Yield % of extracted bioactive fruit from crop year, variety of fruit (strawberry, blackberry and raspberry) and treatment with or without ultrasound.

crop year (B), whereas raspberries seem to have higher yield than the other soft fruits within this study. Previously, ultrasound has been shown to significantly increase the yield of bioactives within fruits and vegetables (Kumar *et al.*, 2021). There were no significant three-way interactions observed F (3, 78) = 0.525, $P = 0.666$.

Extraction of bioactive ingredients from pomegranate peel have shown that ultrasound power of 400 and 600 W was adequate to see a small increase in phenolics, however combined with pressure increases by 20% (Santos *et al.*, 2019). This is contrary to the results presented here, where the control showed higher recovery than the ultrasound treatments, however the treatment energy is different in the two experiments as well as the fruit, which could have explained the results. Obtaining a higher yield of bioactive ingredients is an important marker for adoption into industrial processes, however, if the yield in mg g^{-1} is not affected by treatment, then the treatment should be useful in terms of ensuring that these bioactive ingredients are more readily available within the biological system.

TPC

A more complex picture is shown with bioactive ingredients being treated with ultrasound for industrial processes (Fig. 2) on the variety of fruit (A), crop year (B) and finally the crop year and variety of fruits (C). The treatment of raspberries showed a higher GAE mg ml^{-1} than the control and considerably higher than the smaller probe (Fig. 2a). Strawberry appears to have very little differences in GAE mg ml^{-1} with or without either treatment, whereas for blackberries the treatments had a detrimental effect on the GAE mg ml^{-1} for both treatments. Crop year has a large difference between the smaller probe (2018 data considerably higher than 2019 data) for the Q700 probe, but when upscaled with the larger probe this effect is diminished (Fig. 2b). The control samples showed higher GAE mg ml^{-1} than the treatments for both crop years. GAE mg ml^{-1} for variety and crop year was static, except for raspberries in 2019 (Fig. 2c). The results showed there were no significant three-way interactions observed F (3, 78) = 0.354, $P = 0.786$.

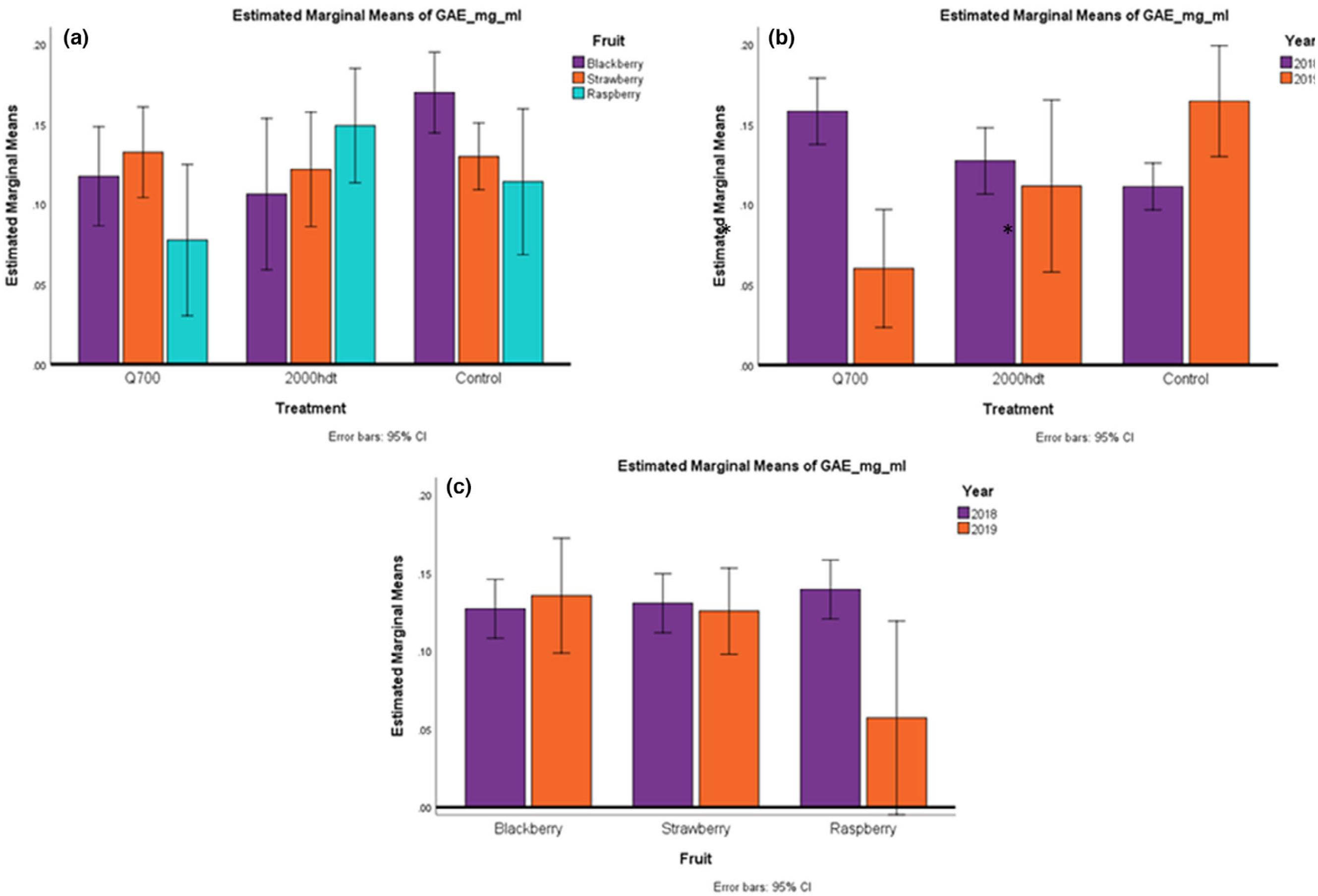

FIGURE 2 Gallic acid equivalent mg ml^{-1} of fruit from crop year, variety of fruit (strawberry, blackberry and raspberry) and treatment with or without ultrasound.

Interestingly, ultrasound treatment and crop year had a two-way interaction (F (2, 78) = 14.475, $P = 0.000$).

Ultrasound treatments have been shown to influence increasing GAE, previously studies with Citrus latifolia waste have shown an 80% increase in total phenolic content in 50% ethanol (Medina-Torres *et al.*, 2019), increases in ethanol extractants of date palm (Almusallam *et al.*, 2021) and ethanol extractants of jabuticaba peel (Rodrigues *et al.*, 2015). Interestingly, these articles all used ethanol or an ethanol as a base for the extractants, and therefore it is difficult to correlate these results well.

The amplitude of ultrasound may break cells open to release the stored antioxidants, but in this case the higher amplitude could be decaying carotenoids, flavonoids and other bioactive compounds. Ultrasound-assisted clay-assisted clay absorption technique was shown to reduce DPPH activity in sea buckthorn pulp (Ma *et al.*, 2021), and similarly, this could be the mechanism at which this reduced rate is observed.

Within the literature ethanol or methanol is generally used as a way of extracting optimal bioactive ingredients from within the fruit pomace. Within this study, water is the only solvent used, and therefore it is expected to have slightly lower concentrations than other studies. For ‘green’ extraction to work industrially water was deemed as the obvious choice, but if this extraction medium is not performing at the optimal level, further extraction is required. In addition, these samples were spray dried and then rehydrated to analyse, the spay drying process may be the reason for very little differences with TPC concentrations, although contrary to other authors looking at cranberry juice (Zhang *et al.*, 2020) and lemongrass leaf extract (Tran & Nguyen, 2018).

DPPH

Total phenolic content was not improved using ultrasound in both a small and industrial scale, however

TPC is not the only measurement for bioavailability within the biological system. Radical scavenging activity can be a useful tool for understanding how active the bioactive ingredients are against a standard unstable molecule. In this study, the scaling up of the larger ultrasound probe had marginal effect on the radical scavenging percentage of raspberry, strawberry and blackberries (Fig. 3a). The small-scale bench top treatment (Q700) had more of an effect on the radical scavenging percentage than the 2000hdt or control. Raspberries showed the largest difference between the larger machine and the bench top machine and had a lower scavenging percentage than the control for the 2000hdt machine. Those fruits grown in 2019 where of higher radical scavenging percentage than those grown in 2018 (Fig. 3b), interestingly those 2019 fruits with the larger scale treatment (2000hdt) showed slightly higher radical scavenging percentage than those with the smaller treatment and control. The same effect is seen with crop year on the variety of fruits, where crop year 2019 has higher radical scavenging percentage than 2018 (Fig. 3c).

A three-way ANOVA was conducted with treatment, crop year and variety of fruit, which showed no significant interactions F (3, 78) = 0.152, $P = 0.928$. Interestingly in this study, significant differences were observed for Crop Year (F (1, 78) = 31.467, $P = 0.000$) and Variety of Fruit (F (2, 78) = 7.164, $P = 0.001$).

The addition of the larger scale ultrasonic probe showed little difference to the smaller laboratory scale probe in this study, as well as the control and it is of little wonder as to why this type of equipment is used within the soft fruit industry. Previously, there has been an increase in radical scavenging percentage through treatments with ultrasound in other fruits, like date palms (Almusallam *et al.*, 2021) as well as increasing radical scavenging in leaves of *Lobelia nicotianifolia* (Zimare *et al.*, 2021). Ultrasound has been shown to reduce DPPH activity previously (Wang

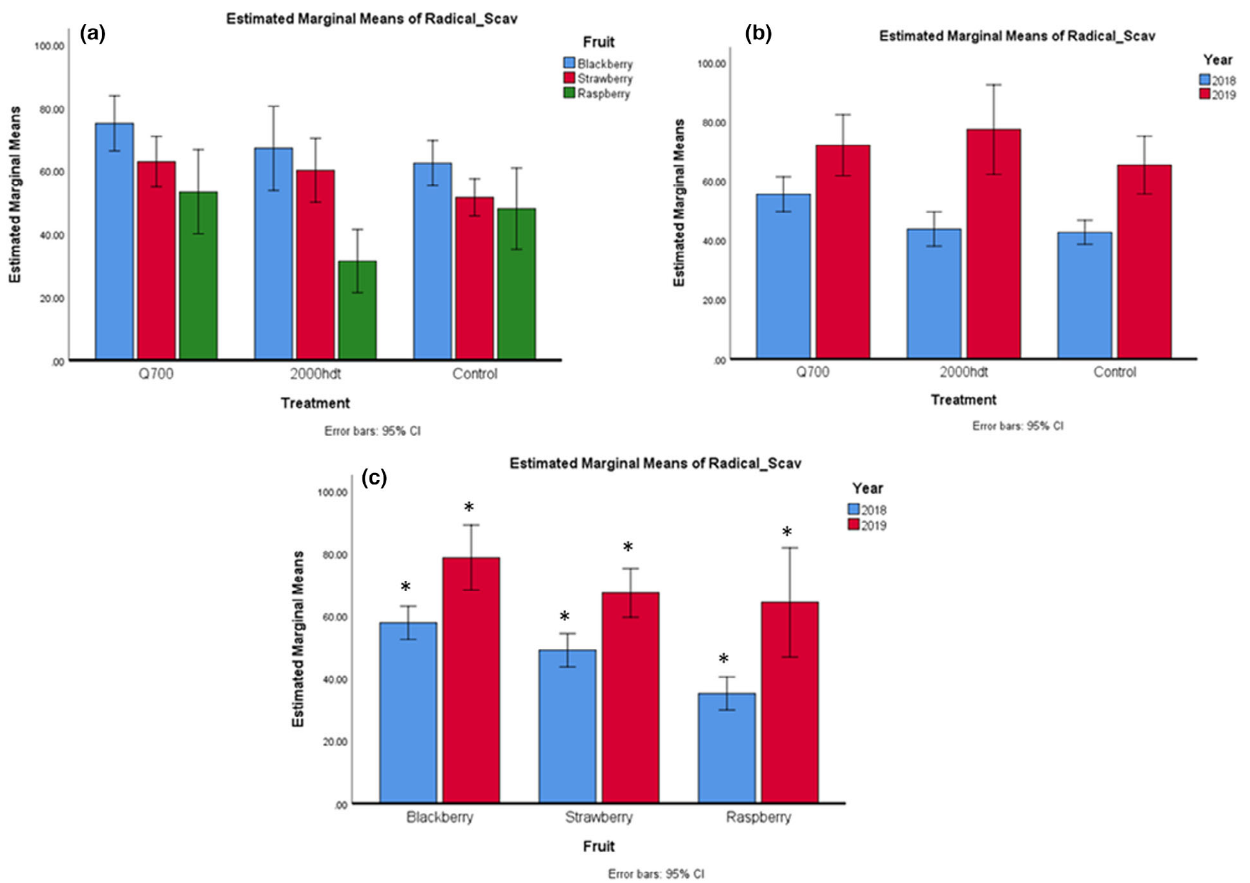

FIGURE 3 Radical scavenging percentage of fruit from crop year, variety of fruit (strawberry, blackberry and raspberry) and treatment with or without ultrasound.

et al., 2018) through destruction of carotenoids, tocopherols and other bioactive ingredients. Interestingly, Tran & Nguyen (2018) found spray drying to decrease the TPC content while increasing Radical Scavenging content, which is similar to this study.

Conclusion

In this study, crop year and variety of fruit have a larger interaction with radical scavenging and total phenic content than the treatments with two ultrasound probes at different amplitudes. In addition to this, ultrasound treatments had very little differences in the yield of bioactive ingredients once spray dried. In this study, two different sonication extractors were compared against no sonication, and the use of sonication deemed less effective in raspberries for radical scavenging and blackberries and strawberries for total phenolic content. Very little differences were observed for the treatments of either sonication, and it is assumed that the extraction medium played more of a role than the extraction itself.

It is the recommendation of this study that industrial processors of strawberries, raspberries and blackberries should reconsider using ultrasound combined with water as a green extraction method for bioactive ingredients from berry wastes and focus on using methanol or ethanol extractants with ultrasound as these are previously reported to more beneficial to increasing bioactives.

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Conflicts of interest

The authors have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics statement

This project was approved by the Abertay University Ethics committee, and as no human samples were used within this project, it went through the 1st pathway.

Author contributions

Jonathan Wilkin: Conceptualization (equal); formal analysis (equal); funding acquisition (equal); methodology (equal); supervision (equal); writing – original draft (equal); writing – review and editing (equal). **Matthew Hooper:** Conceptualization (equal); data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); writing – original draft (equal); writing – review and editing (equal). **Alison McNeilly D:** Conceptualization (equal); funding acquisition (equal); supervision (equal); writing – review and editing (equal). **Carl Schaschke:** Investigation (equal); methodology (equal); writing – review and editing (equal).

Peer review

The peer review history for this article is available at https://publons.com/publon/10.1111/ijfs.15994.

Data availability statement

The data that support the findings of this study are openly available in Abertay University Repository at https://rke.abertay.ac.uk/.

References

a. This reference can be found in the introduction area of our research article, and we thought it was a useful addition to speak about in terms of raspberry pomace (particularly in tunnel grown fruits). In this article, the author discusses both TPC and DPPH which aligns nicely to what we have discussed; and shows that UAE raspberries increases bioactive compounds compared of to normal maceration.

a. In their review article, they discussed in a concise and easy to understand manner the effects of UAE on bioactive compounds of a range of different fruits and vegetables, from mangos to olive leaves. This paper was cited as it's a review paper and had the information on an array of different crops, which showed an increase in extractants – most notably due to the solvent used.

a. In this paper, the authors looked at better utilising UAE to progress the formation of chitosan bioactive nanoparticles, however, the data they showed was a useful addition to correspond some of the findings we have within this paper. The showed a different in TPC using UAE compared to us, and this was the reason behind the addition of this reference.

a. Found in the introduction area is a nice paper regarding spray drying the use of AI to better optimise the process. The authors noted that lower drying temperatures increased particle size of the powders whilst increasing the moisture content and water activity, they also optimised the addition of a carrier. In our research article, we used maltodextrin, and these authors agree that both inulin and maltodextrin are useful carriers for spray drying bioactive compounds from raspberries.

a. This is an interesting paper on UAE with gas expansion which showed different ultrasound power (which we used in this study), but these differences were with the application of gas expansion as well, so it is unknown if this is due to either or the combined motion of both.

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