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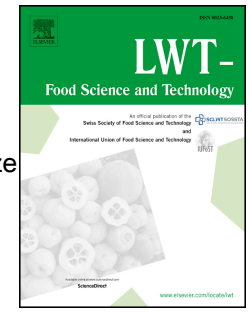

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Impact of ultrasound and blanching on functional properties of hot-air dried and freeze dried onions

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ABSTRACT: The aim of this study was to investigate the effect of ultrasonic treatment and blanching prior to hot-air drying and freeze drying of onions on the retention of bioactive compounds (total phenolics, total flavonoids, and quercetin). Onion slices were treated either with ultrasound at 20 kHz and different amplitude levels (24.4-61 µm) for 1, 3 and 5 min or with blanching using hot water at 70^{o}C for 1, 3 and 5 min. The ultrasound treatment improved the retention of bioactive compounds (especially quercetin) and accordingly the antioxidant activity in onion slices dried either by freeze drying or hot-air drying. This is ascribed to the destruction of the original tissue structure by ultrasound and thus higher extraction ability of the studied phytochemicals. Comparing ultrasound treated samples, freeze dried onions had a higher retention of bioactive compounds than hot-air dried ones. Blanched and ultrasound treated dried onions exhibited similar colour change. Therefore, ultrasound treatment is a potential alternative to conventional blanching before drying of onion slices.

Keywords: Ultrasound treatment; Thermal blanching; Antioxidant activity; Drying; Colour.

1. INTRODUCTION

Dried onions are found in different forms – flaked, minced, chopped and powdered – of extensive demand in several parts of the world (Sarsavadia, Sawhney, Pangavhane, & Singh, 1999).

Sonication is a promising non-thermal technology in the food industry (Tiwari et al., 2010). Ultrasound treatments (US treatments) are used to induce desirable chemical and physical changes in foods and can support several processes, such as drying, osmotic dehydration, extraction, mixing, emulsification, filtration, crystallization, thawing and freezing (Marcuzzo, Peressini, Debeaufort, & Sensidoni, 2010). Ultrasonic waves cause rapid compressions and expansions to plant cells, which leads to the formation of bubbles in the sonicated sample and its surroundings. The resulting rapid and short pressure and temperature shifts in the product leads to changes of viscosity and surface tension, destroying cell walls, forming microscopic channels and free radicals, and producing sonochemicals. Scientific evidence exists to support both the positive and the negative impacts of ultrasound treatment on the retention of bioactive compounds in various fruit and vegetables, although the particular effect depends on the process conditions and specificity of the material involved (Mieszczakowska-Frąc, Dyki, & Konopacka, 2016). Advantages of power ultrasound include reduction in processing time, the effective removal of occluded oxygen in juices, and lower energy consumption (Knorr, Zenker, Heinz, & Lee, 2004).

The responses of plants to abiotic stresses, such as US, associated with the production of stress signalling molecules (i.e. reactive oxygen species – ROS) activate

the expression of genes involved on the primary and secondary metabolism of the plant (Jacobo-Velázquez, González-Agüero, & Cisneros-Zevallos, 2015). These genes are associated with an increase in the activity of enzymes related with the biosynthesis of secondary metabolites and with the accumulation of secondary metabolites (Jacobo-Velázquez et al., 2015). For this reason, US can be used as an approach to increase the extractability of bioactive compounds (Nowacka & Wedzik, 2016), for instance, found a 12.5% higher extractability of carotenoid from carrots after the application of US at 21 kHz. Ultrasound has also shown higher extraction rates of phenolic compounds from carrot pomace and strawberries (Jabbar et al., 2015). Power ultrasound has also potential as a means of preservation due to the microbial inactivation ascribed to cavitation, as the resulting pressure shifts contributes to cell disruption. Ancillary chemical effects, such as the formation of free radicals as a consequence of the sonochemical reaction, also contribute to the microbial cell disruption (Kadkhodaee & Povey, 2008).

The most popular drying methods for onions are hot-air drying and freeze drying. Hot-air drying involves exposure of the product to a continuously flowing hot air stream. It produces dehydrated products with a shelf life of up to one year, but their quality is usually lower than that of the original foodstuff (Ratti, 2001). Freeze-drying is based on dehydration by sublimation of water from a frozen product. Due to the absence of liquid water and the low temperatures required for freeze drying, most of the deterioration and microbiological reactions are retarded resulting in a final product of high quality (Rawson et al., 2011). However, the quality of a dehydrated product

67 depends also on the pre-treatments employed before drying (Negi & Roy, 2000).
68 Hot-water blanching (heating of a product with hot water for a short period) has also
69 been reported to reduce drying time up to a certain operation temperature. Similarly to
70 other thermal processes, blanching affects the concentration of some bioactive
71 compounds in vegetables (Rawson et al., 2011).

72 Given the possible detrimental effects of blanching on the quality of onions, it is
73 necessary to develop alternative pre-treatments to replace blanching. Despite power
74 ultrasound has been extensively reviewed in fruits, its effects on quality parameters
75 have not been studied in thin sliced onions.

76 The present study investigated the effect of ultrasonic and blanching
77 pre-treatments prior to hot-air drying and freeze drying on the retention of bioactive
78 compounds (total phenolics, total flavonoids, and individual flavonoids), colour and
79 antioxidant activity of onions.

80

81 **2. MATERIALS AND METHODS**

82 **2.1 Chemicals**

83

84 Gallic acid, methanol, acetonitrile, ethanol, potassium acetate, aluminium chloride
85 ($AlCl_3$), ferric chloride, 2,2-Diphenyl-1-picrylhydrazyl (DPPH),
86 2,4,6-tripyridyl-s-triazine (TPTZ), hydrogen chloride (HCl),
87 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and trifluoroacetic
88 acid (TFA) were obtained from Sigma (Sigma Aldrich, Arklow, Ireland). Quercetin

4'glucoside (Q 4' G), quercetin 3,4' diglucoside (Q 3,4' D) and quercetin (Q) standards were purchased from Extrasynthese (Geney Cedex, France).

2.2 Sample preparation

Fresh organic onions were obtained from the Kinsealy Systems field trial carried out at Teagasc, Kinsealy (53° 25N, 6° 10W), Dublin, Ireland and stored at 4^{o}C for a maximum of 24 h prior to analysis. After hand-peeling, onions were vertically sliced (5 mm thickness) using a Berkel 800 meat slicer (Berkel company, Indiana, USA).

2.3 Ultrasound and blanching pre-treatments

One kg of fresh organic onion slices (thickness of approximately 1 cm) were obtained from 10 skin-peeled onion bulbs (variety: Hyskin). In each treatment, 50 g of onion slices were mixed with 100 mL of distilled water at 70^{o}C in a 200 mL beaker.

Ultrasound (20 kHz) was irradiated to 50 g of onion slices mixed with 100 mL of water at 70^{o}C with an ultrasonic probe (Ø19 mm) connected to an ultrasonic generator (VC 1500, Sonics and Materials Inc., USA). The energy input was controlled by setting the amplitude of the sonicator probe. Extrinsic parameters of amplitude (power output of 40%, 60% and 80%, equivalent to 24.4, 42.7 and 61 µm) and processing time (1, 3 and 5 min) were varied with pulse duration of 5 s on and 5 s off. The ultrasound probe was submerged to a depth of 25 mm into the sample. All treatments

were carried out in triplicate. The ultrasound densities ranged between 0.06 and 0.59 W/mL.

For the blanching pre-treatment, carried out alternatively to the-US treatment, 50 g of onion slices were mixed with 100 mL of distilled water at 70^{o}C for 1, 3 and 5 min. All treatments were carried out in triplicate.

2.4 Preparation of extracts from dried onions

Control (fresh), sonicated and blanched slices were either freeze-dried or hot-air dried. Hot-air drying of sonicated, blanched and untreated (control) samples was carried out in a laboratory scale hot-air drier (SG96⁄06⁄333, Gallenkamp, UK) at 60^{o}C and 0.3 m/s for 8 h. Pre-treated and control samples of 50 g were placed in a perforated basket (300 x 400 mm; perforation size of 5 x 5 mm), which was inserted in the drying chamber. Each sample was dried separately. Freeze-drying was carried out in a Cuddon freeze-drier (FD80, Cuddon Freeze Dry, Blenhein, New Zealand) at 0.064 mbar for 72 h. After freeze dried or hot-air dried, the samples were vacuum-packed in polypropylene bags and stored at -20^{o}C until analysis.

The leaching water resulting from the ultrasound and blanching pre-treatments were also freeze-dried or hot-air dried, according to the drying method selected for the onion slices. The dry weights were used to calculate the transfer of material from the onions into the cooking water. For this, the dried onions were blended by a kitchen blender (Kenwood Ltd, Havant, UK). Then, 1 g of the blended sample was mixed

with 10 mL of methanol (80%) and homogenised at 24,000 rpm using an Omni-prep multi-sample homogeniser (Omni International, USA). The homogenized sample suspension was shaken overnight with a V400 Multitude Vortexer (Alpha laboratories, North York, Canada) at 1500 rpm at room temperature. The sample suspension was centrifuged (MSE Mistral 3000i, Sanyo Gallenkamp, Leicestershire, UK) at 3000 g for 15 min and immediately filtered through 0.22 μm polytetrafluoethylene filters. The extracts were kept at -20^{o}C until further analysis.

2.5 Analysis of total phenolics (TPC)

The total phenolic content was determined using the Folin-Ciocalteau method with slight modifications (Singleton, Orthofer, & Lamuela-Raventós, 1999) using a spectrophotometer (Shimadzu UV-1700, Shimadzu Corporation, Kyoto, Japan) at 735 nm. Aqueous gallic acid (10-400 mg/L) was used as standard. The results were expressed as gallic acid equivalents per dry weight of sample (mg GAE/g DW).

2.6 Analysis of total flavonoid content (TFC)

The total flavonoid content was determined by the method described by Lin and Tang (2007) using a spectrophotometer at 415 nm. Quercetin (Q) was used to build the standard calibration curve. The total flavonoid content was expressed as

154 milligrams of quercetin equivalents per gram of dry weight (DW) (mg quercetin/g
155 DW).
156
157 **2.7. Analysis of antioxidant activity**
158
159 **2.7.1 Ferric Reducing Antioxidant Power (FRAP) assay**
160
161 The FRAP assay was carried out based on the method by Stratil et al. (2006) with
162 slight modifications. The FRAP solution was freshly prepared on the day of use by
163 mixing acetate buffer (pH 3.6), ferric chloride solution (20 mM) and TPTZ solution
164 (10 mM TPTZ in 40 mM HCl) in a proportion of 10:1:1, respectively. Subsequently,
165 the FRAP solution was heated, while protected from light, until a temperature of 37°C.
166 Appropriate dilutions of onion extracts were prepared using methanol. The sample
167 extract (100 μL), or blank (100 μL methanol) and Trolox standard dilutions (100 μL
168 Trolox of appropriate concentration) were mixed with 900 μL of FRAP solution in a
169 micro-centrifuge tube. The tubes were stirred and left to rest at 37°C for 40 min, and
170 the absorbance was measured at 593 nm using a spectrophotometer. The antioxidant
171 activity of the samples was expressed in mg of Trolox equivalent per gram of dry
172 weight sample (mg Trolox/g DW).
173
174 **2.7.2 DPPH Antioxidant Power Assay**
175

The DPPH (2, 2-diphenylpicrylhydrazyl) scavenging activity assay was performed following the method described by Goupy et al. (1999). DPPH was dissolved in methanol to a concentration of 0.238 mg/mL in a conical flask. The reagent was prepared 2 hours prior to use, to ensure that the DPPH was fully dissolved and stabilised. The flask containing the DPPH solution was covered with aluminium foil to protect it from the light and stored in a refrigerator. For the actual measurements, a 1:5 dilution of the DPPH stock was prepared using 10 mL of the stock and making up to the 50 mL with methanol. Trolox (1-10 µg/mL) dissolved in methanol in appropriate dilutions were used to build the standard curve. This experiment was carried out in three replicates for both samples and standard. In each replicate, 500 µL from the appropriately diluted sample extract was added to 500 µL of DPPH solution. Experiments were carried out to determine the exact dilutions required. In the control, 500 µL of methanol was added in place of the sample extract with an equal volume of DPPH solution. As for the blank, 500 µL of sample extract was mixed with 500 µL of methanol. The absorbance was measured at 515 nm in a spectrophotometer. The radical scavenging activity was expressed in terms of mg of Trolox equivalent per gram of dry weight (mg Trolox/g DW).

2.8 HPLC analysis of the extracts

Reversed phase high performance liquid chromatography (RP-HPLC) of the filtered sample extracts was carried out according to the method of Tsao and Yang

198 (2003). Flavonols were separated on a ZORBAX SB-C18 column (4.6 mm x 150 mm,
199 5 μm particle size, Part no. 883975-902). The mobile phase consisted of HPLC grade
200 water with 0.05 % trifluoroacetic acids (TFA) (A) and acetonitrile with 0.05 % TFA
201 (B). The gradient involved a linear increase/decrease in the amount of solvent B in A,
202 which was set as follows (% B): 0-15 min, 12-21 %; 15-25 min, 21-100 %; 25-35 min,
203 100-12 %. The flow rate was 1 mL/min. Samples of 10 μL were injected into the
204 column and the separation took place at 30^{o}C. The data was presented in the
205 SHIMADZU EZ START Version 7.3 software. The identification of compounds was
206 achieved by comparing their retention times and UV-Vis spectra with those of
207 authenticated quercetin standard, and the UV absorbance was measured at 360 nm.
208 Quercetin and quercetin glucoside concentrations were calculated against authentic
209 calibration standards (quercetin 4' glucoside, quercetin 3,4' diglucoside and
210 quercetin).
211
212 **2.9 Colour**
213
214 Three onion slices were randomly selected from fresh and dried samples to
215 determine colour at both sides (internal and external) of each slice using a colorimeter
216 (D25A DP-9000, Hunter Lab, Reston, VA, USA). The samples were evaluated for
217 colour (L*, a* and b*) at room temperature. L* represents luminosity and ranges from
218 black at 0 to white at 100. The chromaticity coordinate a* indicates red when positive
219 and green when negative, and b* indicates yellow when positive and blue when

220 negative (Doymaz, Tugrul, & Pala, 2006). The colour change, ΔE was calculated by
221 Eq. 1 (Vega-Gálvez et al., 2012):
222
223 $\Delta E = \sqrt[2]{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2}$ (1)
224
225 where L_0^*, a_0^*, and b_0^* are the values for fresh onion samples.
226
227 **2.10 Statistical analysis**
228
229 All experiments were carried out in triplicate and average values were reported as
230 means ± standard deviation. The experimental data were statistically analysed using
231 the software SAS V.9.1 (SAS Institute, NC, USA). The Tukey-Kramer test was
232 applied for multiple comparisons among means at a 95% significance level (p<0.05).
233
234 **3. RESULTS AND DISCUSSION**
235 **3.1 Change of total phenolic content**
236
237 The ultrasound and blanching treatments influenced the total phenolic content
238 (TPC) of onion slices (Table 1). Blanching applied for 1 min and ultrasound applied
239 for 1-3 min in general increased the TPC of dried onions. After 3 min of ultrasound
240 treatment at 42.7 μm and 61.0 μm, for example, there was a 17%-21% TPC increase
241 in freeze dried onions (p<0.05). Samples treated by ultrasound at 61.0 μm for 1 min

242 followed of hot-air drying had a 10% increase (p<0.05) compared to the untreated
243 dried samples. The application of sonication techniques to assist in the extraction of
244 bioactive compounds is in fact widely reported (Keenan et al., 2012). On the contrary,
245 blanched freeze dried (BFD) and blanched hot-air dried (BHD) (3 and 5 min) samples
246 had lower retention of phenolics compared to the control (p<0.05). Turkmen, Sari,
247 and Velioglu (2005) also reported that blanching decreased the total phenolics in
248 squash, peas and leek.

249 Samples subjected to UFD (ultrasound + freeze drying) at 24.4 µm for 3 min and
250 UHD (ultrasound + hot-air drying) at 61.0 µm for 1 min resulted in greater retention
251 of phenolics than samples blanched for the same time. Also, blanching caused
252 phenolics to leach into the cooking water nearly 1-3 times more than during the
253 ultrasound treatment (Table 1). In agreement with this finding, Rawson et al. (2011)
254 reported higher retention of carotenoids and polyacetylenes in dried carrots subjected
255 to a 10 min-pre-treatment with a US-probe under pulsed mode than in dried carrots
256 blanched at 80^{o}C for 3 min.

257 However, the relatively high temperature and longer holding time related to the 5
258 min-ultrasound treatment led to more severe oxidative and thermal degradation than
259 the other ultrasound treatments. The main mechanism involved in the loss of
260 phenolics during US treatment might be the formation of microchanels during
261 cavitation, which facilitate the transport of food constituents, especially soluble
262 nutrients (Mothibe, Zhang, Nsor-atindana, & Wang, 2011). In fact, Opalić et al. (2009)
263 reported that prolonged US pre-treatment in samples with the same geometry led to a

decrease in total phenolics and flavonoids and accordingly in the antioxidant capacity of dried apples. The degradation trend during ultrasonic processing may be also related to the formation of free radicals, resulting in a potential increase in the oxidation pathways (Pétrier, Combet, & Mason, 2007). The degradation related to the some of the US treatments may point to additional contributory factors. The ultrasound probe had direct contact with the sample, with the vessel opened to the atmosphere (i.e. it was not a closed system). Therefore, oxidation could freely occur at the liquid⁄atmosphere interface during processing. This effect would be increased in samples processed for longer periods (i.e. 5 min).

3.2 Change of total flavonoids content

There was a significant difference of TFC (p<0.05) between ultrasound-treated and blanched onions after drying compared to dried samples without pre-treatment, considering either freeze-dried or hot-air dried (Table 1).

TFC in dried (freeze drying and hot-air drying) onion slices treated with ultrasound for 1-3 min in general increased compared to the control dried samples. Lower ultrasound amplitudes (24.4 μm) combined with freeze drying and higher amplitudes (61 μm) combined with hot-air drying resulted in better retention of TFC compared to other ultrasound treatment conditions or dried samples not submitted to pre-treatment (Table 1). Such increase in the retention of TFC may arise from an enhanced extractability of the compounds. Improved extraction efficiency following

286 sonication has been attributed to the propagation of ultrasound pressure waves,
287 induced cavitation and high shear forces resulting in increased mas transfer (Rawson
288 et al., 2011). There was also a significantly (p<0.05) higher retention of flavonoids in
289 UFD (24.4 μm for 3 min) and UHD (61.0 μm for 1 min) than BHD (1, 3 and 5 min)
290 samples. Regarding blanching, as higher the process time, lower was the retention of
291 flavonoids.
292
293 **3.3 Change of antioxidant activity during pre-treatment**
294
295 The antioxidant activity of pre-treated and untreated (control) dried onion slices
296 are presented in Table 1. Sonicated samples processed at the highest amplitude (61μm)
297 for the longest time (5 min) and then freeze-dried as well as sonicated samples
298 processed at the lowest amplitude (24.4 μm) for 5 min and then hot-air dried had the
299 lowest (p<0.05) antioxidant activity. Generally, onions sonicated at lower amplitudes
300 followed of freeze drying had the highest antioxidant activity (FRAP and DPPH),
301 while longer US-times reduced the antioxidant activity (Table 1).
302 The DPPH and FRAP values were similar and indicate that blanching generally
303 resulted in lesser preservation of antioxidant compounds compared to fresh and
304 sonicated samples. The exception was the 1 min-blanching, which resulted in
305 enhanced antioxidant activity. Some studies have suggested that blanching is
306 generally regarded as being destructive to antioxidant components (Krishnaswamy &
307 Raghuramulu, 1998). On the contrary, Halvorsen et al. (2006) reported increased

antioxidant activity for several vegetables such as carrots, spinach, mushroom, asparagus, broccoli and cabbage after thermal treatment. Dewanto, Xu and Liu (2002) found similar results in thermally processed tomatoes compared with fresh controls. These authors hypothesised that higher antioxidant activities may be related to an increase in extractability of antioxidant components following thermal processing.

3.4 Changes of quercetin and quercetin glucosides

The levels of the 3 major quercetins – quercetin 3,4'diglucoside (Q 3,4' D), quercetin 4'glucoside (Q 4' G), and quercetin (Q) – in dried onions are presented in Fig.1-3.

In general, the retention levels of Q 3,4' D and Q for US-freeze dried and US-hot air dried samples were higher compared to the samples dried without any pre-treatment. This can be ascribed to the increased extractability induced by cavitation of US-treated samples (Rawson et al., 2011).

In BFD and BHD onions slices (1 min), the retention levels of Q were higher compared to the control (p<0.05). Blanching in fact does not always result in the destruction of bioactive compounds. In some cases, thermal treatments can induce the formation of novel compounds and improve the antioxidant capacity (Xu & Chang, 2008). Bunea et al. (2008) suggested that the increase in the concentrations of certain bioactive compounds after thermal treatment may be explained either by their better release from the food matrix as a result of breakdown of supramolecular structures

containing functional groups or their thermal stability. However, in BFD and BHD samples (3 and 5 min), the retention levels of Q were lower compared to the control (p<0.05). This is most likely due to the relatively high temperatures required for blanching (70℃ sustained for 3-5 min), which could lead to oxidative and thermal degradation (Rawson et al., 2010).

Regarding the freeze drying, the ultrasound treatment at 24.4 µm for 3 min resulted in significantly higher retention levels of Q 3,4' D and Q compared to BHD (1-5 min) samples. With regard to the hot air drying, there were significantly higher retention levels of Q 4' G and Q after US treatment at 61.0 µm for 1 min compared to BHD (1-5 min) samples.

3.5 Phenolic compounds and antioxidant activity in water

Blanching retained greater amounts of phenolic compounds than ultrasound (p<0.05). The losses could be attributed to water soluble phenolics leaching into the cooking water as well as breakdown of phenolics during thermal processing. These significant losses could be attributed to water soluble phenolics leaching and transferred into the cooking water as well as breakdown of phenolics during thermal processing, which rendered water a good source of dietary phenolics (Table 2).

However, degradation of phenolics in onion slices may be a bigger problem than leaching. The percentage loss of phenolics undergoing degradation during the US-treatment was higher than the percentage loss to the cooking water. These results

suggest that the degradation of phenolics after sonication was greater than the losses due to leaching. Some authors have indicated that pressure-cooking enhanced the antioxidant composition and palatability of vegetables (Xu & Chang, 2009). However, higher power could result in greater degradation (Hiemori, Koh, & Mitchell, 2009).

3.6 Flavonoids in water

The total flavonoid content in the cooking water revealed a trend similar to that described for the TPC (Table 2). The flavonoid losses could be a result of degradation or decomposition of flavonoids (Ioannou, Hafsa, Hamdi, Charbonnel, & Ghoul, 2012). The ultrasound treatment resulted in a higher percentage of flavonoids being degraded than retained in the cooking water ($p<0.05$). There was a transfer of especially Q 3,4' D and Q 4' G from onions to water. This suggests that the decrease of flavonoid during ultrasound was predominantly caused by breakdown of flavonoids rather than their leaching. Higher ultrasound amplitudes and longer time resulted in greater leaching of flavonoids.

3.7 Quercetin and its glucosides in water

The amounts of quercetin 3,4’diglucoside and quercetin 4’ glucoside were also measured in water after ultrasound and blanching treatments (Table 2). In the US-treatment water, the quercetin 4’glucoside fraction was greater than the quercetin

374 3,4'diglucoside one. Hirota, Shimoda, and Takahama (1998) observed that the
375 monoglucoside derivative was oxidized more rapidly than its diglucoside form during
376 cooking, and that the difference in the stability between mono and diglucoside was
377 due to the presence or absence of a hydroxyl group at the C-3 position in the
378 glucosides. As the antioxidant power of flavonols substantially depends on the
379 catechol group in the B-ring and on the 3-hydroxyl group (Rodrigues, Pérez-Gregorio,
380 García-Falcón, & Simal-Gándara, 2009), the monoglucoside is likely to have a higher
381 antioxidant capacity than the diglucoside, since in the latter these two basic functions
382 are blocked. In this work, there was a lower content of flavonols in water, which was
383 however enriched with antioxidant monoglucoside forms.

384 Free quercetin was found in the onion slices (Table 2) but only in very small
385 amounts in the cooking water (Table 2), which may correspond to its poor solubility
386 in water and/or stronger binding to plant structures than its glycoside forms. Quercetin
387 was not detected in water after the 5 min-ultrasound treatment, indicating that this
388 compound is not prone to leaching.

389

390 **3.8 Antioxidant activity in water**

391

392 The blanching water had high antioxidant (Table 2), especially for the 1
393 min-treatment, followed by 3 min. The cooking water from US-treated onions had
394 low values of antioxidant activity according to both assays. The sum of antioxidant
395 activity of the cooked onion and cooking water is different from the antioxidant

activity of fresh samples, which may suggest losses in the antioxidant activity due to breakdown or degradation of antioxidant compounds.

3.9 Effect of ultrasound and blanching on colour

Colour has a major impact on the acceptance of a product by the consumer (Kalt, 2005). Fresh onions were characterized by high luminosity (L* = 74.24 ± 2.15), with a tendency to green and yellow (a* = -6.23±0.53 and b* = 22.79±2.8, respectively) (Table 3). The L* of dried samples ranged from 58.3 to 93.74, b* varied from 23.7 to 33.98, and a* varied from -9.73 to -4.36, indicating the dried onions had more intense green and yellow tones than the fresh ones. All dried samples were characterized by high ΔE values, regardless of the ultrasound and blanching conditions (Table 3).

Although luminosity was similar for fresh, blanched-dried and US-dried onions, sonicated samples had higher colour difference (ΔE) than blanched ones (p<0.05). The longer the sonication time (and blanching time as well), the higher was the colour difference, regardless of the ultrasound amplitude. The use of ultrasound as a pre-treatment to onions contributed to a significant colour change. UFD and UHD (highest amplitude applied for 5 min) samples showed significantly (p<0.05) higher ΔE compared to other amplitudes and to BFD and BHD samples. These changes can be explained by the formation of free radicals and sonochemicals as a result of cavitation (Bermúdez-Aguirre, Mobbs, & Barbosa-Cánovas, 2011), which may influence the food properties. The change of coordinate a*, in specific, can be linked

418 to the formation of colour compounds (Vadivambal & Jayas, 2007) related to
419 non-enzymatic browning during treatment. The greatest colour change for the samples
420 treated by ultrasound is also ascribed to the presence of air during processing, leading
421 to enzymatic browning. In the case of blanching, the colour was better preserved as
422 the contact between samples and air was limited.

423 The colour of vegetables is determined by natural colour compounds that can be
424 oxidized during the pre-treatment, and the most important factor accelerating
425 degradation is high temperature and presence of oxygen. Enzymatic browning also
426 plays an important role in colour change due to the brown pigments formed from
427 colourless polyphenols (Maskan, 2001). Table 4 shows that the b* chroma was
428 correlated to TPC and Q 4' G at 5% significance (Table 4) in the hot-air drying, but
429 the colour coordinates had no correlation with the bioactive compounds in freeze
430 drying.

431

432 **4. Conclusions**

433

434 Blanching and ultrasound treatments significantly affected the colour, TPC, TFC,
435 individual phenolic compounds and antioxidant activity of onion slices dried either by
436 freeze drying or hot-air drying. In this work, ultrasound has been identified as an
437 alternative pre-treatment to blanching regarding the enhancement of functional
438 properties in onions. The ultrasound-treatment applied for 1-3 min at any amplitude
439 (24.4-61 µm) increased (1%-20%) the content of phytochemicals regarding phenolic

compounds, flavonoids and quercetin. As a consequence, sonicated onion slices (1-3 min) featured higher antioxidant activity than blanched ones. However, the 5 min-sonication had a deleterious effect (more than 10% degradation) on the bioactive compounds and antioxidant activity. At last, as the leaching water from onions treated with ultrasound and blanching contained high amounts of antioxidants, it may be considered a valuable co-product for the food and nutraceutical industries.

Further research is required to optimize the retention of bioactives by varying ultrasonic processing parameters such as power level, treatment time and temperature, allowing a successful implementation in the food industry.

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Conflicts of interest

The authors declare that there are no conflicts of interest related to this paper.

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Table 1 – Influence of ultrasound and blanching treatments followed of drying on the total phenolics content (TPC), total flavonoid content (TFC) and antioxidant activity of onion slices.

FREEZE DRYING	TPC	Retention (%)	TFC	Retention (%)	FRAP	Retention (%)	DPPH	Retention (%)
Control	9.21±0.82^{cdef}	---	4.10±0.08^{bcd}	---	11.05±0.99^{c}	---	4.42±0.82^{bc}	---
UFD 24.4 µm 1 min	9.65±0.24^{bcd}	104.87%	4.19±0.18^{abc}	102.08%	11.58±0.29^{bc}	104.87%	5.21±0.84^{abc}	117.98%
UFD 42.7 µm 1 min	9.48±0.40^{bcde}	102.99%	4.13±0.07^{abcd}	100.59%	11.38±0.48^{bc}	102.99%	5.12±0.83^{abc}	115.87%
UFD 61.0 µm 1 min	9.31±0.37^{cdef}	101.13%	4.15±0.03^{abcd}	101.15%	11.17±0.44^{bc}	101.13%	5.03±0.8^{abc}	113.78%
BFD 1 min	9.22±0.10^{cdef}	100.18%	4.16±0.10^{abcd}	101.27%	11.07±0.12^{c}	100.18%	4.98±0.84^{b}	112.71%
UFD 24.4 µm 3 min	11.18±1.27^{a}	121.41%	4.47±0.15^{a}	108.93%	13.41±1.52^{a}	121.41%	6.04±0.89^{a}	136.59%
UFD 42.7 µm 3 min	10.81±0.43^{ab}	117.48%	4.42±0.24^{ab}	107.65%	12.98±0.52^{a}	117.48%	5.84±0.88^{ab}	132.16%
UFD 61.0 µm 3 min	9.76±0.56^{abc}	106.06%	4.27±0.56^{abc}	104.06%	11.72±0.68^{bc}	106.06%	5.27±0.85^{abc}	119.32%
BFD 3 min	8.19±0.11^{defg}	88.96%	3.81±0.11^{bcde}	92.83%	9.83±0.14^{d}	88.96%	4.40±0.76^{bc}	100.08%
UFD 24.4 µm 5 min	8.09±0.07^{efg}	87.91%	3.76±0.06^{cdef}	91.71%	9.71±0.09^{d}	87.91%	4.37±0.75^{abc}	98.90%
UFD 42.7 µm 5 min	7.68±0.06^{g}	83.45%	3.49±0.10^{ef}	84.96%	9.22±0.07^{de}	83.45%	4.15±0.70^{c}	93.88%
UFD 61.0 µm 5 min	7.33±0.14^{g}	79.61%	3.15±0.06^{f}	76.75%	8.79±0.17^{e}	79.61%	3.96±0.63^{c}	89.56%
BFD 5 min	7.86±0.15^{fg}	85.41%	3.57±0.30^{def}	86.98%	9.43±0.18^{de}	85.41%	4.25±0.71^{c}	96.08%
HOT-AIR DRYING	TPC	Retention (%)	TFC	Retention (%)	FRAP	Retention (%)	DPPH	Retention (%)
Control	7.76±0.39^{abc}	---	3.34±0.36^{bcde}	---	9.31±0.47^{b}	---	3.82±0.67^{bc}	---
UHD 24.4 µm 1 min	6.50±0.37^{def}	83.84%	3.35±0.20^{bcde}	100.12%	7.80±0.45^{ef}	83.84%	3.36±0.70^{de}	87.93%
UHD 42.7µm 1 min	7.67±0.47^{abc}	98.88%	3.66±0.18^{bc}	109.43%	9.20±0.56^{bc}	98.88%	3.96±0.73^{b}	103.70%
UHD 61.0 µm 1 min	8.58±0.44a	110.65%	4.34±0.27^{a}	130.04%	10.30±0.53^{a}	110.65%	4.43±0.87^{a}	116.05%
BHD 1 min	7.93±0.14^{ab}	102.24%	3.90±0.31^{b}	116.71%	9.52±0.17^{b}	102.24%	4.09±0.78^{ab}	107.23%
UHD 24.4 µm 3 min	6.69±0.65^{cde}	86.26%	3.45±0.34^{bcd}	103.15%	8.03±0.78^{e}	86.26%	3.45±0.69^{cd}	90.47%
UHD 42.7 µm 3 min	7.34±0.26^{bcd}	94.58%	3.79±0.35^{bc}	113.57%	8.80±0.31^{cd}	94.58%	3.79±0.76^{bc}	99.19%
UHD 61.0 µm 3 min	7.74±0.27^{abc}	99.83%	3.83±0.14ab	114.63%	9.29±0.33^{b}	99.83%	4.00±0.79^{b}	104.70%
BHD 3 min	6.23±0.17^{def}	80.27%	3.10±0.33^{cdef}	92.67%	7.47±0.21^{fg}	80.27%	3.21±0.62^{de}	84.19%
UHD 24.4 µm 5 min	5.50±0.37^{f}	70.94%	2.70±0.17^{f}	80.85%	6.60±0.45^{h}	70.94%	2.84±0.54^{f}	74.40%
UHD 42.7 µm 5 min	6.34±0.26^{def}	81.69%	2.88±0.08^{def}	86.35%	7.60±0.3^{ef}	81.69%	3.27±0.58^{de}	85.67%
UHD 61.0 µm 5 min	7.25±0.23^{bcd}	93.46%	3.34±0.27^{bcde}	100.11%	8.70±0.27^{d}	93.46%	3.74±0.68^{bc}	98.02%
BHD 5 min	5.93±0.14^{ef}	76.46%	2.77±0.32^{ef}	82.84%	7.12±0.17^{g}	76.46%	3.06±0.55^{ef}	80.19%

For each row, values followed by the same letter are not statistically different at $p<0.05$. Values are expressed as mean ± standard deviation in dry weight (%) for n=3. TPC = Total phenolics content (mg of gallic acid equivalents per g of dry weight). TFC = Total flavonoids content (mg of quercetin equivalents per g of dry weight). FRAP and DPPH = Antioxidant activity (mg Trolox/g DW). UFD = ultrasound pre-treatment followed of freeze drying; UHD = ultrasound pre-treatment followed of hot-air drying; BFD = blanching followed of freeze drying; BHD = blanching followed of hot-air drying.
*Blanching was carried out at 70^{o}C, Hot-air drying at 60^{o}C and 3 m/s for 8 h, and Freeze-drying at 0.04 mbar for 72 h.

Table 2 – Effect of ultrasound and blanching treatments followed of drying on the bioactive compounds and antioxidant activity of the leaching water from onion slices.

Treatment	TPC	TFC	Q 3,4' D	Q 4' G	Q	FRAP	DPPH
UFD 24.4 µm 1 min	0.66±0.03^{e}	0.22±0.01^{b}	10.43±0.31^{def}	55.56±5.42^{de}	4.42±0.71^{cd}	0.81±0.04^{d}	0.47±0.03^{abcd}
UFD 42.7 µm 1 min	0.96±0.01^{d}	0.24±0.01^{b}	11.83±0.13d^{e}	61.97±1.24^{d}	4.58±0.26^{cd}	0.79±0.06^{d}	0.46±0.11^{bcd}
UFD 61.0 µm 1 min	1.31±0.07^{c}	0.26±0.00^{b}	17.67±0.04^{d}	92.31±1.31^{c}	4.71±0.47^{c}	0.78±0.05^{d}	0.45±0.12^{bcd}
BFD 1 min	1.52±0.02^{a}	0.71±0.29^{a}	225.05±3.00^{a}	408.37±2.50^{a}	63.0±0.92^{a}	1.1±0.05^{a}	0.60±0.08^{a}
UFD 24.4 µm 3 min	0.43±0.02^{g}	0.06±0.01^{c}	3.67±0.15^{gf}	32.31±2.20^{f}	1.24±0.12^{cde}	0.78±0.18^{d}	0.45±0.16^{bcd}
UFD 42.7 µm 3 min	0.53±0.01^{f}	0.06±0.00^{c}	3.83±0.31^{gf}	35.6±5.94^{f}	1.58±0.83^{cde}	0.93±0.06^{bc}	0.54±0.12^{ab}
UFD 61.0 µm 3 min	0.63±0.02^{e}	0.09±0.01^{c}	7.43±0.02^{efg}	41.97±1.84^{ef}	1.67±0.14^{cde}	0.91±0.08^{c}	0.53±0.15^{abc}
BFD 3 min	1.35±0.02^{b}	0.24±0.01^{b}	208.38±3.60^{b}	325.03±12.43^{b}	38.05±3.38^{b}	0.98±0.07^{b}	0.53±0.12^{abc}
UFD 24.4 µm 5 min	nd	nd	nd	nd	nd	nd	nd
UFD 42.7 µm 5 min	nd	nd	nd	nd	nd	nd	nd
UFD 61.0 µm 5 min	nd	nd	nd	nd	nd	nd	nd
BFD 5 min	1.34±0.03^{c}	0.21±0.00^{b}	175.5±1.60^{c}	310.70±19.10^{b}	35.1±1.58^{b}	0.94±0.10^{bc}	0.51±0.06^{abc}
Treatment	TPC	TFC	Q 3,4' D	Q 4' G	Q	FRAP	DPPH
UHD 24.4 µm 1 min	0.05±0.01^{e}	0.01±0.00^{c}	nd	7.02±1.86^{de}	7.5±1.05^{cd}	nd	nd
UHD 42.7 µm 1 min	0.08±0.02^{e}	0.01±0.0^{c}	nd	7.82±1.31^{d}	17.0±2.1^{b}	nd	nd
UHD 61.0 µm 1 min	1.01±0.03^{b}	0.03±0.00^{c}	nd	11.65±0.22^{c}	16.51±1.95^{b}	nd	nd
BHD 1 min	1.32±0.07^{a}	0.37±0.08^{a}	nd	306.4±23.50^{a}	31.0±2.2^{a}	0.8±0.02^{a}	0.50±0.03^{a}
UHD 24.4 µm 3 min	nd	nd	nd	nd	nd	nd	nd
UHD 42.7 µm 3 min	nd	nd	nd	nd	nd	nd	nd
UHD 61.0 µm 3 min	nd	nd	nd	nd	nd	nd	nd
BHD 3 min	0.41±0.08^{c}	0.26±0.03^{b}	103.86±11.2^{a}	268.7±19.36^{b}	9.55±1.98^{c}	0.39±0.03^{b}	0.42±0.1^{abc}
UHD 24.4 µm 5 min	nd	nd	nd	nd	nd	nd	nd
UHD 42.7 µm 5 min	nd	nd	nd	nd	nd	nd	nd
UHD 61.0 µm 5 min	nd	nd	nd	nd	nd	nd	nd
BHD 5 min	0.29±0.1^{d}	0.20±0.00^{b}	78.23±6.60^{b}	258.60±18.97^{b}	6.2±0.44^{cd}	0.34±0.10^{b}	0.39±0.08^{abc}

For each row, values followed by the same letter are not statistically different at $p<0.05$. Values are expressed as mean ± standard deviation in dry weight (%) for n=3. TPC = Total phenolics content (mg of gallic acid equivalents per g of dry weight). TFC = Total flavonoids content (mg of quercetin equivalents per g of dry weight). Q 3,4' D = quercetin 3,4'glucoside (µg/g); Q 4' G = quercetin 4'glucoside (µg/g); Q = quercetin (µg/g). FRAP and DPPH = Antioxidant activity (mg Trolox/g DW). UFD = ultrasound pre-treatment followed of freeze drying; UHD = ultrasound pre-treatment followed of hot-air drying; BFD = blanching followed of freeze drying; BHD = blanching followed of hot-air drying.

*Blanching was carried out at 70°C, Hot-air drying at 60°C and 3 m/s for 8 h, and Freeze-drying at 0.04 mbar for 72 h.

Table 3 – Colour of freeze dried and hot-air dried onion slices subjected to blanching and ultrasound pre-treatments.

FREEZE DRYING	L*	a*	b*	ΔE
Control	74.24±2.15^{e}	-6.23±0.53^{a}	22.79±2.80^{c}	---
UFD 24.4 µm 1 min	80.8±0.60^{cd}	-8.84±0.62^{ef}	31.07±2.17^{a}	10.88±1.13^{g}
UFD 42.7 µm 1min	81.51±1.21^{bcd}	-9.21±0.19^{fg}	29.78±1.66^{ab}	11.51±1.02^{g}
UFD 61.0 µm 1min	92.41±0.66^{a}	-9.01±0.43^{fg}	29.25±0.78^{ab}	19.47±0.50^{c}
BFD 1 min	86.51±0.38^{bc}	-7.08±0.05^{b}	25.72±0.60^{c}	12.64±0.47^{e}
UFD 24.4 µm 3 min	81.5±1.54^{bcd}	-8.98±0.83^{ef}	31.80±1.09^{a}	11.90±1.15^{fg}
UFD 42.7 µm 3 min	82.35±1.32^{bcd}	-9.32±0.21^{gh}	29.98±0.93^{ab}	12.27±0.82^{ef}
UFD 61.0 µm 3 min	92.41±0.30^{a}	-8.21±0.13^{de}	29.25±0.06^{ab}	19.31±0.16^{c}
BFD 3 min	89.34±0.61^{bc}	-7.28±0.18^{bc}	27.61±0.50^{ab}	15.97±0.43^{d}
UFD 24.4 µm 5 min	91.85±0.45^{a}	-9.30±1.04^{hi}	32.80±2.07^{a}	20.49±1.19^{b}
UFD 42.7 µm 5 min	91.51±1.18^{ab}	-9.73±0.63^{i}	33.97±5.83^{a}	20.87±2.55^{b}
UFD 61.0 µm 5 min	93.74±0.11^{a}	-7.97±0.45^{cd}	33.82±4.76^{a}	22.47±1.74^{a}
BFD 5 min	88.06±0.8^{ab}	-7.44±0.20^{bc}	29.93±0.60^{abc}	15.60±0.53^{d}
HOT-AIR DRYING	L*	a*	b*	ΔE
Control	74.24±2.15^{c}	-6.23±0.53^{b}	22.79±2.80^{de}	---
UHD 24.4µm 1 min	85.8±1.61^{b}	-7.84±0.51^{cd}	30.07±0.98^{a}	10.06±1.030^{k}
UHD 42.7 µm 1 min	82.501±0.36^{b}	-8.21±0.08^{d}	28.78±0.16^{ab}	10.74±0.20^{i}
UHD 61.0 µm 1 min	90.41±0.09^{a}	-6.18±0.08^{b}	28.25±0.51^{ab}	17.06±0.23^{c}
BHD 1 min	59.1±0.34^{e}	-6.04±0.29^{b}	23.71±0.78d^{e}	15.50±0.45^{f}
UHD 24.4 µm 3 min	85.98±0.88^{b}	-7.98±0.48^{cd}	30.51±0.65^{a}	10.46±0.67^{j}
UHD 42.7 µm 3 min	82.85±1.02^{b}	-8.62±0.03^{de}	28.98±0.91^{ab}	10.87±0.65^{h}
UHD 61.0 µm 3 min	90.94±1.37^{a}	-6.43±0.51^{bc}	28.52±0.76^{ab}	17.66±0.88^{b}
BHD 3 min	58.29±0.46^{e}	-5.85±0.22^{b}	25.60±0.22^{bc}	15.70±0.33^{e}
UHD 24.4 µm 5 min	86.28±0.95^{b}	-8.40±0.28^{d}	31.05±1.86^{a}	14.76±1.03^{g}
UHD 42.7 µm 5 min	83.15±0.86^{b}	-8.82±0.38^{de}	29.28±1.29^{a}	15.72±0.84^{e}
UHD 61.0 µm 5 min	91.34±2.36^{a}	-6.74±0.05^{bcd}	28.85±1.18^{ab}	18.15±1.20^{a}
BHD 5 min	64.40±0.88^{d}	-4.36±0.38^{a}	29.29±1.10	15.93±0.79^{d}

For each row, values followed by the same letter are not statistically different at $p<0.05$. Values are expressed as mean ± standard deviation in dry weight (%) for n=3. UFD = ultrasound pre-treatment followed of freeze drying; UHD = ultrasound pre-treatment followed of hot-air drying; BFD = blanching followed of freeze drying; BHD = blanching followed of hot-air drying.

*Blanching was carried out at 70^{o}C, Hot-air drying at 60^{o}C and 3 m/s for 8 h, and Freeze-drying at 0.04 mbar for 72 h.

Table 4 – Correlation matrix of colour and chemical indices of freeze dried and hot-air dried onion slices.

FREEZE DRYING	TPC	TFC	Q 3,4' D	Q 4' G	Q	L	a	b
TPC	1.00	0.83	0.63	0.58	0.21	-0.55	-0.06	-0.23
TFC		1.00	0.52	0.66	0.34	-0.50	-0.03	-0.33
Q 3,4' D			1.00	0.19	0.09	-0.57	-0.01	-0.31
Q 4' G				1.00	0.47	-0.22	-0.20	-0.04
Q					1.00	-0.11	-0.13	-0.08
L*						1.00	-0.07	0.45*
a*							1.00	-0.54*
b*								1.00
HOT-AIR DRYING	TPC	TFC	Q 3,4' D	Q 4' G	Q	L	a	b
TPC	1.00	0.79	0.75	0.83	0.75	0.17	0.05	0.46*
TFC		1.00	0.68	0.81	0.64	0.23	-0.05	-0.27
Q 3,4' D			1.00	0.77	0.67	0.30	-0.25	-0.23
Q 4' G				1.00	0.65	0.01	-0.05	0.52*
Q					1.00	0.51	-0.17	-0.06
L*						1.00	-0.44*	0.64
a*							1.00	-0.32
b*								1.00

Chromameter describes colour in three coordinates: L, lightness, from 0 (black) to 100 (white); a, from -60 (green) to 60 (red); and b, from -60 (blue) to 60 (yellow).

TPC = Total phenolics content (mg of gallic acid equivalents per g of dry weight); TFC = Total flavonoids content (mg of quercetin equivalents per g of dry weight); Q 4' G = quercetin 4'glucoside (µg/g); Q 3,4' D = quercetin 3,4'glucoside (µg/g); Q = quercetin (µg/g).

* Represents significance at 5%.

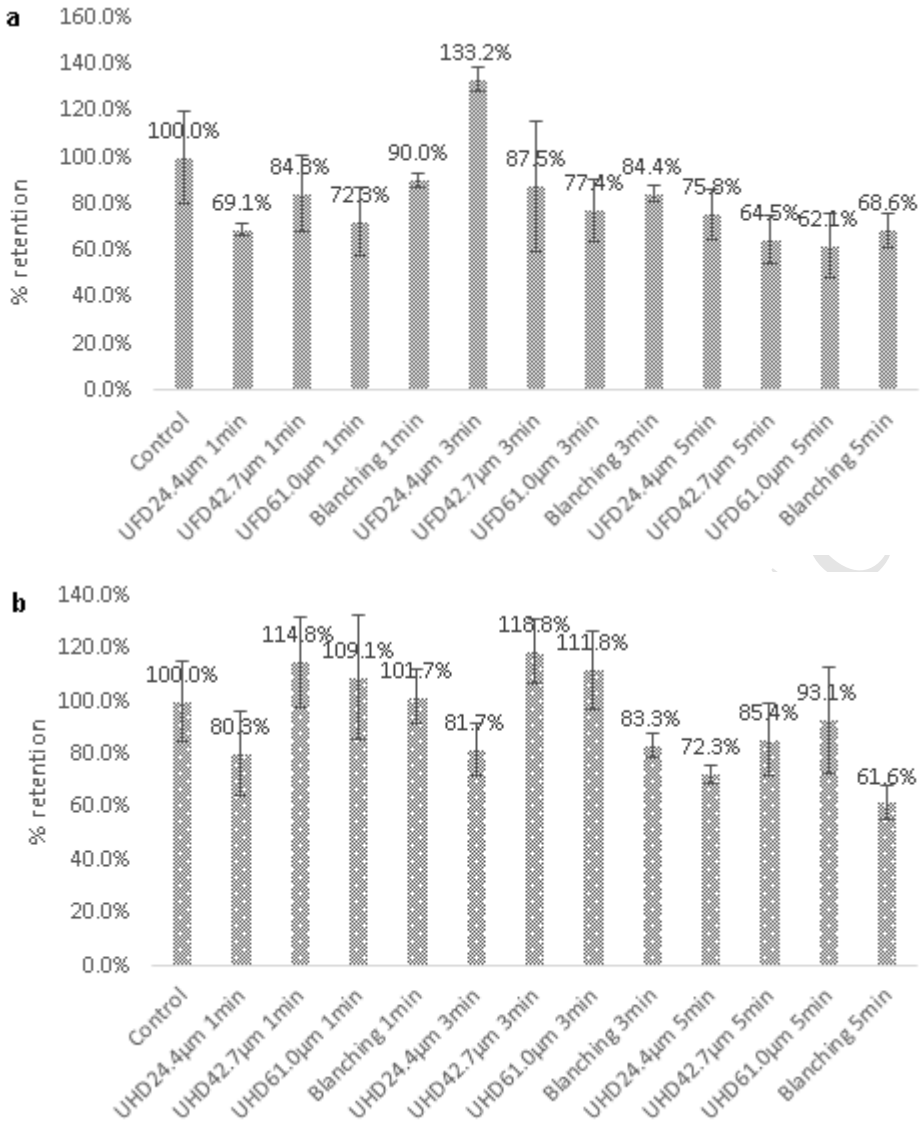

Figure 1 – Retention of quercetin 3,4'-diglucoside after different pretreatments followed of (a) freeze drying and (b) hot-air drying.

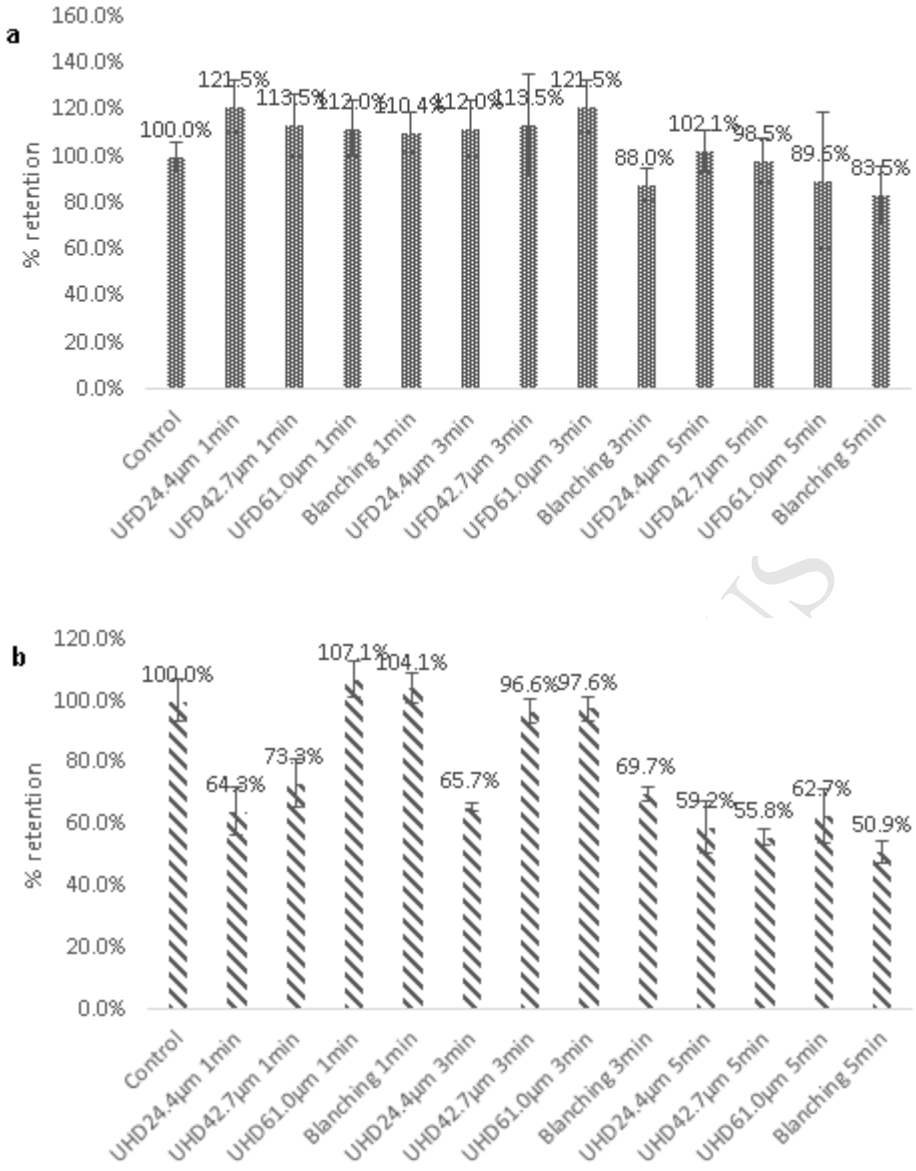

Figure 2 – Retention of quercetin 4'-glucoside after different pretreatments followed of (a) freeze drying and (b) hot-air drying.

Figure 3 – Retention of quercetin after different pretreatments followed of (a) freeze drying and (b) hot-air drying.

Highlights

1. The US-treatment improved the retention of bioactive compounds in dried onions.
2. The colour change was similar between blanched and US-treated dried onions.
3. US-freeze dried onions had higher retention of phenolics than US-hot air dried.
4. Dried onions had higher antioxidant activity when sonicated for 1-3 min.