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## Clean recovery of antioxidant flavonoids from onions: Optimising solvent free microwave extraction method

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### Abstract

A solvent free microwave hydrodiffusion and gravity extraction (MHG) of flavonol content from onion (*Allium cepa L.*) was studied. Effectiveness of this innovative method in extraction of onion total phenolic content, total quercetin (TQ), quercetin aglycon (QA), quercetin-3,4'-diglucoside (QDG), quercetin-4'-monoglucoside (Q4G), quercetin-3-monoglucoside (Q3G), kaempferol (KMF) and myricetin (MRT) have been evaluated and compared with conventional solvent extraction. Microwave extraction offers important advantages like shorter extraction time (23 mins), cleaner feature (no solvent or water used) and extraction of valuable onion crude juice retaining fresh organoleptic properties with higher phenolic content (58.29 mg GAE/g DW) at optimized power (500W). Microwave extraction resulted significant yield (81.5%) with 41.9% of flavonol contents, with better retain of remaining flavonoids (55.9%) in residues of onions. QDG (239.7 mg/100g DW) and Q4G (82.55 mg/100g DW) have been reported the main flavonol in this study. Minor quantities of QA (traces), Q3G (4.22 mg/100g DW) and KMF (3.99 mg/100g DW) were also detected in microwave onion extracts.

**Keywords:** Microwave, extraction, onion, flavonoids, solvent free.

## 1. Introduction

Human biological system is vulnerable to the attack of extremely reactive oxygen species (ROS), which are produced continuously as a result of endogenous enzymatic reactions and also by exogenous sources [1, 2]. The formation and activity of these ROS are believed to be responsible for degenerative diseases and their associated complications like cancers, cardiovascular diseases and accelerated aging of organisms [3]. Increased consumption of diets rich in fruits and vegetables are associated with low prevalence of degenerative diseases as they provide a great amount of antioxidant phytochemicals and literature proved antioxidants as one of the defence mechanisms within the organism against ROS [4]. These phenolic antioxidants act as free radical scavengers and offer protection against cellular damage by retarding oxidative stress. Among vegetable polyphenols the flavonoids group generally dominate and found relatively in higher concentration as sugar conjugates as studied by Miean and Mohamed [5] in 62 edible plants and detected abundant amount of quercetin glucosides in different vegetables.

Onion (*Allium cepa L.*) a versatile vegetable of *Allium* family is appreciated worldwide not just for its distinctive taste and flavour but also as a significant source of many beneficial compounds. Several studies revealed the presence of various dietary flavonoids in different varieties of onions along with other bioactive compounds [6]. The main flavonol are based on quercetin among which quercetin diglucoside and quercetin monoglucoside are the major components [7-9] and almost all of these are mainly localized in the abaxial epidermis of scales [10]. The amounts of quercetin glucosides are much larger in onion bulbs in comparison with other vegetables [11, 12] and they possess very high antioxidative [13] and antiproliferative activities [4]. While considering the usefulness of antioxidants against cardiovascular disease and colorectal cancers, it's necessary to examine their extraction processes from different vegetables for obtaining maximum health effects.

Efficiency of extraction process and mass of release components depend on degree of vegetal cell disintegration which have been achieved previously by conventional solid-liquid extraction, with assistance of processes like heating, boiling, pressing, blending, maceration and mechanical fragmentation of plant material [14-17]. Leaching or organic solvent extraction is the most extensively used process for obtaining plant phenolic components from many decades. Current literature also shows the use of conventional solvent extraction

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74 supplemented by intensive processes like steam distillation from onion sprouts [18] and  
75 Soxhlet extraction of onion peel [3]. Phenolic extracts are also purified and extracted by using  
76 ion exchange resins from onion and lettuce [19, 20].

77 Use of these traditional methods not only resulted in degradation of different phenolic  
78 contents due to intensive mechanical disruption but the involvement of long extraction  
79 timings, severe heating conditions and extensive usage of toxic organic solvents favours the  
80 liberation of oxidative enzymes and also promotes these degradation reactions. The use of  
81 ultrasound, as an upcoming extraction technique, also has been reported for onion phenolic  
82 compounds extraction [21]. The cavitation effect of ultrasound provides greater penetration  
83 of solvent into cellular materials which helps in improving the release of cell contents into  
84 bulk medium at room temperature, but its main disadvantage is again its extensive  
85 requirement of solvents and their incomplete separation from extract.

86 Microwave hydrodiffusion and gravity (MHG) is a novel technology that has massive  
87 potential for variety of extractive applications as the extraction of essential oil have been  
88 performed from rosemary leaves [22] and from Spearmint (*M. spicata L.*) and Pennyroyal  
89 (*M. pulegiom L.*) plant [23]. But for antioxidants extraction, it was the first time we have  
90 utilized the efficiency of this innovative technology. The use of microwaves influence textural  
91 properties of plant material and increase secondary metabolites diffusion by improving tissue  
92 softness and increasing cell permeability. It emerges as an energy saving technology as  
93 microwaves are also being able to enhance cell disruption due to their high penetration power  
94 resulting in enhancement of mass transfer within and outside the plant tissues [24]. MHG not  
95 only appeared as an efficient and economical technology but its chief advantage is its  
96 environmental friendly approach as it works without using any solvent just under effect of  
97 microwaves and earth gravity at atmospheric pressure [25].

98 In this case common yellow onion, a vegetable of huge economic importance grown  
99 all over the world, loses its water content more rapidly when treated with MHG under  
100 controlled temperature. This innovative method proved itself as an ideal alternative extraction  
101 method by producing juice with retention of fresh organoleptic qualities and also by retaining  
102 increase content of valuable phenolic components. This article illustrates the efficiency of  
103 MHG for extraction of flavonoid content of yellow onion at optimized power with  
104 combination of control temperature in comparison with conventional solid liquid extraction  
105 and also focussed on onion nutritional attributes in terms of total phenol content and flavonol

106 contents (total quercetin (TQ), quercetin aglycon (QA) quercetin-3,4'-diglucoside (QDG),  
107 quercetin-4'-monoglucoside (Q4G), quercetin-3-monoglucoside (Q3G), kaempferol (KMF)  
108 and myricetin (MRT).

## 109 2. Experimental

### 110 2.1. Raw material

113 Raw yellow onions (*Allium cepa* L.) were purchased from a local supermarket in  
114 Avignon province (South France). Onion bulbs which exhibit a diameter of 50-70 mm and  
115 were apparently free of external damages was selected and peeled manually for their  
116 following processing.

### 117 2.2. Chemicals

118 All solvents used for chromatographic purposes were HPLC grade. Methanol and Formic  
119 acid were from Merck (Darmstadt, Germany) and Acetonitrile was from Fisher Scientific Ltd.  
120 (Bishop Meadow Road, Loughborough, UK). The HPLC grade flavonol standards quercetin-  
121 3,4'-diglucoside and quercetin-4'-glucoside (spiraeoside) were purchased from Extrasynthese  
122 (Lyon, France). Quercetin, quercetin-3-glucoside, kaempeferol and myricetin were purchased  
123 from Sigma Chemicals Chimie (Fallavier, France).

### 124 2.3. Determination of moisture content

125 Moisture content determination of onion was carried out firstly by conventional Dean-  
126 Stark distillation apparatus according to the American Oil Chemist' society (AOCS) official  
127 method [26], and also with an electric oven at 80°C. The average moisture content measured  
128 by both processes was  $88.5 \pm 0.5\%$ .

### 129 2.4. MHG apparatus and procedure

130 Microwave hydrodiffusion and gravity has been performed in a Milestone EOS-G  
131 microwave laboratory oven illustrated in Fig. 1. This is a multimode microwave reactor 2.45  
132 GHz with a maximum delivered power of 900W variable in 10W increments. Time,  
133 temperature, pressure and power can be controlled with the "easy-WAVE" software package.  
134 The extraction vessels are made from Pyrex and have a capacity of 1000 mL. During  
135 experiments temperature was monitored by temperature sensor optic fibers which were

136 inserted in the centre and outer layer of sample and also in the sample reactor. Temperature  
137 variations in different parts of plant material and reactor were measured continuously and data  
138 was saved automatically. This feedback helped in controlling the temperature by microwave  
139 power regulator.

140 MHG procedure was performed at atmospheric pressure; 500 g of fresh entire onion  
141 bulbs was heated using a fix power density without addition of solvents or water. The direct  
142 interaction of microwaves with biological water favours the release of compounds trapped  
143 inside the cells of plant material. These compounds thus move naturally by diffusion along  
144 with hot water or crude juice out of the cells of plant material and move thus naturally  
145 downwards under the effect of earth gravity on a spiral condenser outside the microwave  
146 cavity where it condensed. The crude juice was collected continuously in a graduated  
147 cylinder. The extraction was continued until no more juice was obtained or overheating was  
148 detected. Extracted crude juice was collected and was freeze-dried.

## 149 **2.5. Conventional solid liquid extraction**

150 In conventional solid liquid extraction fresh onion scales were used, onion bulb was  
151 peeled and cut manually and 5g onion scales were homogenised with 50mL of 80% methanol  
152 in an ultrahomogeniser at 8000 rpm for 45mins. After that the mixture was filtered and  
153 supernatant was collected and made upto 50mL of methanol. This filtered solution was used  
154 directly for HPLC and TPC analysis. Final concentrations of different flavonoids were  
155 calculated in mg/100g DW (dry weight).

## 156 **2.6. HPLC analysis**

157 HPLC analyses were performed using a Waters (Milford, MA) HPLC system  
158 consisting of a Waters 600E pump, a Waters 717 manual injector rheodyn, a Waters 2996  
159 photodiode array detector. The HPLC pumps, manual injector rheodyn, column temperature,  
160 and diode array system were monitored and controlled by using Waters Empower 2  
161 Chromatography Data software program. The wavelength used for the quantification of the  
162 onion flavonoids with the diode detector was 360 nm. The chromatographic separation was  
163 carried out on a Purospher Star RP-18 end-capped column (250 mm × 4 mm I.D.; 5 µm  
164 particle size from VWR), with a RP18 guard column (4 mm×4mm I.D.; 5µm particle size also  
165 from VWR). The end-capped column and guard column were held at 37°C and the flow rate

166 was set at 1mL/min. The mobile phase consisted of two solvents: (A) acidified water (0.5%  
167 formic acid) and (B) 100% acetonitrile. The solvent gradient used was the following: 0 min,  
168 (A) 95% and (B) 5%; 20min, (A) 60% and (B) 40%; 30min, (A) 0% and (B) 100%; 45min,  
169 (A) 95% and (B) 5%. The injection volume was 20  $\mu$ L and all analyses were performed at  
170 least three times and only mean values were reported. Identification of flavonoids was done  
171 by comparing the elution order and UV-visible spectra. Quantification was carried out by  
172 using the external standards of known concentration. Peak areas were used to quantify the  
173 compounds in the sample. A linear regression analysis was carried out on the data of the peak  
174 area versus concentration. Linear calibration curves of the standards ranging from 10 to 100  
175 mg/L were obtained with good linearity and  $R^2$  values which were more than 99.5% accurate  
176 for all the standards. Extraction was performed three times and final concentrations of  
177 different flavonoids were calculated by using the mean values, expressed in mg/100g DW.

## 178 2.7. Total phenolic content (TPC)

179 Total polyphenols were estimated colorimetrically using the Folin-Ciocalteu method  
180 [27], with a kit (SEPPAL (Isitec-lab), France) especially suitable for TPC measurement of  
181 food products. This kit includes reagent A (modified Folin-Ciocalteu reagent), reagent B  
182 (alkaline buffer) and a gallic acid solution (3g/L). A small volume (20  $\mu$ L) of H<sub>2</sub>O (blank),  
183 gallic acid solution (standard) and 200  $\mu$ L the extract (sample) was mixed with reagent A (2  
184 mL). After 1 min, 1 mL of reagent B was added in both water and gallic acid standard and  
185 850  $\mu$ L in sample. The mixtures were allowed to stand for 30 min in the dark at room  
186 temperature. Then, their absorbance was measured at 760 nm with a diode-array Hewlett-  
187 Packard 8453 spectrophotometer [28]. TPCs were calculated by using the following formulae:  
188  $TPC = 3 \times (\text{sample absorbance} - \text{blank absorbance}) / (\text{standard absorbance} - \text{blank}$   
189  $\text{absorbance})$ . TPC measurements were performed thrice and mean values, expressed as mg  
190 gallic acid equivalent/g of dry weight (mg GAE/g DW), were reported.

## 191 3. Results and discussion

192 Efficient performance of MHG for onion antioxidants extraction depends on different  
193 factors like moisture of plant material, irradiation power, temperature and time. To determine  
194 optimal reaction conditions for obtaining significant results a preliminary study consisting of  
195 various experiments was carried out.

## 196 3.1. Microwave heating phenomenon

197 Fig. 2 shows the heating phenomenon proceeding in the centre of onions at different  
198 powers from 300W – 900W detected by temperature sensor optic fibre. Different phases in  
199 development of temperature can be distinguished (Fig. 2). The first phase corresponds to the  
200 heating phase (A), a rapid increase in temperature was observed from initial temperature  
201 (20°C) of onions to the boiling point of water (100°C). Heating rates observed in this phase  
202 were proportional to the different applied powers: 8.5°C/min (300W), 13.5°C/min (400W),  
203 29.7°C/min (500W), 32.2°C/min (600W), 36°C/min (700W), 40°C/min (800W) and  
204 45.9°C/min (900W). During this phase, in situ water of plant material was heated up, when  
205 irradiated with microwaves and diffused out of plant material and moved downward under the  
206 influence of earth gravity. This phase was ended with appearance of first drop of water  
207 outside the microwave cavity.

208 At this point, temperature maintained to a plateau region and remained in this steady  
209 state at 100°C until the complete extraction of non bounded water. It corresponds to the  
210 extracting phase (B) of process, and when there was only tightly bound water remained, the  
211 temperature increased very quickly and led to the burning phase (C) which leads to the end of  
212 extraction. Heating rates of burning observed at different powers during this phase were:  
213 5.14°C/min (300W), 6°C/min (400W), 8.5°C/min (500W), 9.6°C/min (600W), 10.3°C/min  
214 (700W), 12°C/min (800W) and 16.4°C/min (900W). Here, we can observe that the initial  
215 heating rates were more rapid than the heating rates of burning. Perhaps, it was due to the less  
216 free water content, inside the onion during the last phase of heating.

217 Similar phases were also detected by using optic fibers in outer layer of onion and also  
218 in reactor but the heating efficiency were in descending order from centre to outer layer of  
219 onion and then the reactor. Heating rates of phase A and phase C observed in the outer layer  
220 of onion and also in reactor were proportional to the applied powers but were less quick in  
221 comparison to the rates observed in centre of onion. Behind this, heating phenomenon of  
222 microwaves works, which are distributed volumetrically and heat transfer occurs from centre  
223 of samples to the outer colder environment. This cause an important difference when  
224 compared to conventional heating in which heat transfer occurs from outer layer to centre of  
225 onion (Fig. 2). While considering the mass transfer, it occurs from inside to the outside of  
226 plant material both in microwave and conventional heating [29]. In conventional heating, heat  
227 transfer depends on thermal conductivity, on the temperature difference across the sample,



228 and for fluids on convection currents. As a result, the temperature increase is often rather  
229 slow. By contrast, in microwave heating, due to volumetric heating effect, much faster  
230 temperature increases can be obtained, depending on microwave power and the dielectric loss  
231 factor of the material being irradiated.

## 232 3.2. Extraction kinetics

233  
234 In order to carry out the study on extraction kinetics of onion extracted juice, volume  
235 of onion juice obtained at different powers was plotted as function of time. Extraction curves  
236 obtained at different powers in Figure 3 shows three diverse stages of extraction.

237 Stage 1 corresponds to the induction time, during this phase no recovery of water was  
238 occurred. It ends with emergence of first water drop.

239 Stage 2 represent the constantly increasing flow rate of extract as illustrated in Figure 3 by  
240 linear curves at different powers. All the easily exchangeable water of onion was extracted in  
241 this phase. During this phase the gradient of curves increases with increase of power.

242 Stage 3 marks the end of extraction process as represented by horizontal line on graph. At  
243 initiation of this phase, onions were almost dry with no more further extraction. At this point,  
244 burnt smell was generated as a result of prolonged heating.

245 Finally, the extracted crude juice was collected freeze dried and yield was expressed in  
246 percentage (%).

## 247 3.3. Extraction yield

248 The aim of this part of study was to examine the impact of MHG on extraction yield  
249 of crude onion juice at various powers. Actual yield of four medium sized onions was taken  
250 until the time at which moisture collection was completely stopped due to overheating. No  
251 remarkable difference in onion juice yield was observed at different powers. A slight decrease  
252 in juice volume was observed while moving from lower (300W) to higher (900W) power. The  
253 percentage of crude juice yields calculated at different powers was: 82.7% (300W), 82.1%  
254 (400W), 81.5% (500W), 81.1% (600W), 80.8% (700W), 80.3% (800W) and 79.7% (900W),  
255 which is close to the actual moisture percentage (88.52%) of fresh onion.

256 The water content of onion is not an alone factor for determining the final yield. The  
257 onion crude juice also holds some soluble compounds like sugar, acids and polyphenols. The  
258 dry extracts weight which was taken, after removal of water content of crude onion juice by  
259 the process of freeze drying, at different powers was also in descending order from lower to  
260 higher powers. The weight of dry extracts yields we obtained at different powers, 4.70%  
261 (300W), 4.40% (400W), 4.28% (500W), 3.41% (600W), 2.87% (700W), 2.19% (800W) and  
262 1.95% (900W), proved that the efficient extraction of soluble solids significantly depends on  
263 applied powers. As it vary remarkably among very lower and very high power.

### 264 **3.4. Microwave extracted onion polyphenol contents**

#### 265 266 **3.4.1. Total phenolic content**

267 The amount of total phenolic content (TPC) varied in the onion extracts obtained at  
268 different powers as shown in Table 1. Highest phenolic content (58.29 mg GAE/g DW) was  
269 found at power of 500W and lowest content was observed at 900W (29.94 mg GAE/g DW).  
270 Initially TPC increased with increase of power from 300W (47.54 mg GAE/g DW), and a  
271 maximum amount was detected at 500W but with further increase of power, phenolic content  
272 concentration started to decrease and lowest concentration was observed at very high power  
273 (900W). TPC results obtained at 500W were not only significant in comparison with  
274 conventional solvent extraction (64.81 mg GAE/g DW) but also correlate with previous data.  
275 Our detected range of TPC falls in the range (4.6-74.1 mg GAE/g DW) observed in different  
276 varieties and layers of *Allium cepa*, including varieties contain very high level of phenolic  
277 content (red onion) to very low level (white onion) [30]. These results are also found in good  
278 concentration in comparison to the TPC values studied by Nuutila *et al.* [31] in the dry outer  
279 skin of red (80.0 mg GAE/g) and yellow onions (26.0 mg GAE/g). TPC values of microwave  
280 extracted (at 500W) residue was also observed after its conventional solvent extraction, in  
281 order to check the remaining amount of phenolic contents in residue, which was 21.60 mg  
282 GAE/g DW, these results shows that with MHG we have extracted a good percentage of  
283 phenolic compounds along with “in situ” water content of plant material.

#### 284 **3.4.2. Flavonoid content of onion extracts obtained at different powers**

##### 285 286 **3.4.2.1. Total quercetin and major flavonoids**

287 Total quercetin presented in Table 1 is the sum of concentration of free quercetin and  
288 different forms of quercetin present in conjugation with carbohydrates mainly as glucosides  
289 like QDG, Q4G and Q3G. QDG and Q4G provide a good estimation of level of total  
290 quercetin in the sample as they are representing about 90% of overall flavonol content [32].  
291 QDG was detected in highest concentration in comparison to other quercetin glucosides  
292 followed by Q4G identified as second major flavonol compound. Quantification of all these  
293 compounds has been done by comparing the retention time and absorbance of peaks at 360nm  
294 with the use of authentic standards. In preliminary study, extraction efficiency of different  
295 powers for flavonol contents was tested. Higher levels of total quercetin (326.5 mg/100g DW)  
296 was calculated at 500W (Table 1) compared to other applied powers, which correspond well  
297 to the previous published data (414 mg/100g DW found by Aoyama and Yamamoto [14] in  
298 yellow onion; 348mg/100g DW quercetin content in yellow onion illustrated in Danish results  
299 by Justesen *et al.* [33], 507 mg/100g DW by Hertog *et al.* [11] and 280 mg/100g DW by  
300 Mogren *et al.*, [34] ). All the analyzed flavonols have shown almost similar behaviour to TQ,  
301 as the highest levels of QDG (239.7 mg/100g DW) and Q4G (82.55 mg/100g DW) were also  
302 found at 500W. These results not only fall in the range reported by Cardí *et al.* [32], (QDG:  
303 153-404 mg/100g DW, Q4G: 58-286 mg/100g DW) among different onion varieties but Q4G  
304 was also found in good concentration as compared to the results determined by Roldán-Marín  
305 *et al.* [9] (282 mg/100g DW concentration of QDG and 43.9 mg/100g DW of Q4G) in high  
306 pressure processed onion. Bonaccorsi *et al.* [8] have also found QDG in highest concentration  
307 in red onion variety (254-274 mg/100g DW), our results also correspond well with these  
308 results as yellow onion ranked after red onion as a good source of quercetin flavonoid  
309 contents. Concentration of QDG (581.8 mg/100g DW) and Q4G (187.5 mg/100g DW), in  
310 fresh onion samples treated with conventional solvent extraction was also detected in higher  
311 amount in comparison to other flavonols. TQ concentration determined by conventional  
312 solvent extraction method was 782.6 mg/100g DW which falls in the range of flavonols  
313 content of yellow onion (270-1187mg/100g DW) reviewed by Slimestad *et al.* [35]. For  
314 analysing the effect of microwave on stability of flavonoid content and their extraction  
315 efficiency, concentration of flavonoids retained in microwave extracted (at 500W) onion  
316 samples was also calculated by conventional solvent extraction. TQ retained in the residue of  
317 microwave extracted onions was found 440.7 mg/100g DW which is 56% of the TQ content  
318 determined by conventional solvent extraction of fresh onion. Amount of TQ observed at  
319 500W is 42% of the concentration determined by conventional method. Our results showed  
320 2% losses of TQ by microwave extraction in comparison to conventional fresh onion

321 extraction. Concentration of major flavonols, QDG (342.4 mg/100g DW) and Q4G (95.2  
322 mg/100g DW), retained in residue of onions was found almost comparable with microwave  
323 extracted content along with water content of onions. These results showed that there was no  
324 remarkable loss or degradation of flavonoid compounds occurred at 500W and onion bulbs  
325 still retained a good amount of these major compounds after microwave extraction of water  
326 content. Similar to the findings of Rodrigues *et al.* [36], as they have been determined no loss  
327 of QDG and Q4G at mild (450W) microwave heating in comparison to untreated onion but  
328 16% (QDG) and 18% (Q4G) losses were detected with increase of power (750W). Similarly,  
329 losses of these compounds were also observed with increase of power, in comparison to the  
330 highest concentration observed at 500W. A rapid decrease in concentration was observed at  
331 intense powers as shown in Table 1 and finally lowest amount of QDG was determined at  
332 900W (101.6 mg/100g DW) with complete absence of detectable amount of Q4G, as more  
333 intense treatments resulted with loss of quercetin components. But very low power (300W)  
334 was also not an effective and efficient power for flavonoids extraction, along this it also  
335 consumes more time for complete extraction of onion water content. QDG exhibited the  
336 lowest loss (degradation or conversion into quercetin aglycon) as it is still present at very  
337 drastic condition at 900W in comparison to Q4G, Q3G and free quercetin which were not  
338 detected at 900W. In QDG glucose is attached at 3 and 4' and due to blockage of the two  
339 positions it showed much greater stability than Q4G in which position 3 is not conjugated.  
340 Makris and Rossiter [37] have also observed the lowest loss in QDG concentration (8.4%)  
341 and Q4G and QA content declined by 37.6% when subjected to heating treatments. Our  
342 results are also supported by the work of Kana *et al.* [38] who has selected microwave  
343 roasting without water as a better cooking method to retain flavonoids in onion tissue. But the  
344 concentration of TQ varied with power. This shows that microwave with a mild power is an  
345 efficient method for extraction of quercetin components without remarkable degradation.  
346 With MHG we can extract more than 40% of the flavonol components along with the "in situ"  
347 water content of onions also possessing fresh organoleptic properties.

### 348 3.4.2.2. Minor flavonoids

349 Free quercetin was also quantified in conventional and microwave extracted samples  
350 at retention time of 24.352min by HPLC. The content of free quercetin showed a low  
351 percentage in comparison with QDG and Q4G. Any measurable quantity of QA was not  
352 detected at low powers 300W and 400W. At 500W just traces of QA were observed, similar

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353 to the results obtained by Patil *et al.* [39] in four yellow onion varieties and one pink and red  
354 onion variety. They have detected not more than 0.4 mg/100g DW of free quercetin in all the  
355 onions. Zielinska *et al.* [40] also mentioned only 1.1% of free quercetin in yellow onion  
356 bulbs. At 600W a good concentration (5.25 mg/100g DW) of QA was detected but then a fall  
357 in concentration was observed with increase of power. In fresh onions normally there is  
358 always a low concentration of QA but as a result of some treatment or processing breakdown  
359 of glycosidic bonds in QDG and QMG, a good concentration of QA can be detected. Perhaps  
360 similar is the case here with decrease of quercetin glycosidic forms, an increase in  
361 concentration of QA observed at high powers of 600W and 700W. But with further increase  
362 of power, phenomenon of degradation was more profoundly expressed in free quercetin  
363 which has both sides exposed. With conventional solvent extraction method (80% methanol)  
364 a very light concentration of QA was observed (1.32 mg/100g DW) and in the residues we  
365 have also detected just traces of QA.

366 Along with QA the other minor components which are representing almost less than  
367 5% of total amount of flavonols were analysed. Very small peaks with retention times of  
368 18.115min and 27.289min have been identified as Q3G and kaempferol, respectively, by  
369 comparison with their standards. Similar to the already discussed major compounds highest  
370 concentration of Q3G have been detected at 500W (4.22mg/100g DW) which is almost 1.27%  
371 of the total amount of analysed flavonols. This value is in good agreement with the results of  
372 Zielinska *et al.* [40] who have determined 1.4% of Q3G in Sochaczewska onion variety which  
373 is a typical onion with a yellow-brown bulb colour. Similar to other flavonoids, it was also  
374 detected in minor amounts at vary high powers.

375 Kaempferol presence in comparison to quercetin content in different varieties of  
376 onions has been reported in minor quantities [39]. Kaempferol content identified and  
377 quantified at 400W (4.01mg/100g DW) and 500W (3.99mg/100g DW) were not significantly  
378 different from each other, and these values were similar to the previously reported values of  
379 3-7 mg/100g DW by Bilyk *et al.*[41] in the outer and inner skins of bulbs of different  
380 varieties. A slightly higher content of kaempferol has been reported by Sellappan and Akoh  
381 [42] in five varieties of onion which ranged from 15.4 - 19.8 mg/100g DW. In contrast to the  
382 findings of Sellappan and Akoh [42], myricetin was not detected in yellow onion variety  
383 which we have extracted and analysed. The presence of different flavonoids varied among

384 different varieties and their concentration also depends on different factors like climatic  
385 conditions and stages of maturity.

### 386 3.4.3. Flavonoid contents at optimized power

387 Impact of different microwave irradiation powers, from 300W-900W, were examined  
388 in terms of heating and burning rate, yield, extraction rate, flavonoid contents. With  
389 increasing power no better results were obtained, as at high powers (600W-900W), high  
390 speed of extraction was observed but resulted with less total dry extract yield due to  
391 degradation of phenolic compounds. On the other hand low power resulted more yield but  
392 with slower extraction rate and are also inefficient for complete extraction of flavonoids. An  
393 irradiation power of 500W was selected as an optimum power for later experiments. At  
394 optimized power, the yield and flavonoids composition were examined after each five  
395 minutes (A=0-3min, B=3-8min, C=8-13, D=13-18, E=18-23, F=23-27.5).

396 Initially, in situ water was heated at the rate of 27.9°C/min and it takes almost 3mins  
397 (A) to reach the extraction temperature 100°C, resulted with appearance of first drop. Almost  
398 comparable yields of water content were obtained after each five minutes: 14.7% (B), 17.6%  
399 (C), 16.9% (D), 16.4% (E), and 15.9% (F), until the end of extraction process completed in  
400 27.5mins. But the dry extract yield obtained after freeze drying was in inverse proportion to  
401 the time, as with increase of time, the percentage of extracted component decreased (Fig 4).  
402 Highest yield of flavonoid components obtained in part C: QDG (108.5mg/100g DW), Q4G  
403 (41.9mg/100g DW), Q3G (1.80mg/100g DW). Traces of QA were also detected only in part  
404 C which also showed the highest content of total phenols (20.1mg GAE/g DW) in comparison  
405 to other parts of extraction process: 18.9mg GAE/g DW (B), 16.1mg GAE/g DW (D) and  
406 15.9mg GAE/g DW (E). Highest dry extract yield was observed in part B as shown in Figure  
407 4. but the lower content of flavonoids were observed in this part in comparison to part C;  
408 QDG (89.18mg/100g DW), Q4G (30.9mg/100g DW), Q3G (1.74mg/100g DW). After part C  
409 the next extracted parts D and E resulted with further decrease in dry extract yield with  
410 minimum content of flavonoids. In part D only the two major components: QDG  
411 (44.6mg/100g DW) and Q4G (7.16mg/100g DW) were detected. Kaempferol was detected in  
412 part E of extraction along with minimum quantity of these above mentioned major flavonoid  
413 components QDG (16.4mg/100g DW) and Q4G (1.12mg/100g DW). Onion juice extracted in  
414 first 23mins just before the onset of burning was analysed with HPLC (Fig. 5) and rejected

415 the last part of juice which was extracted during burning, as the content of flavonoids have  
416 already been decreased.

### 417 **3.5. Cost, cleanliness and scale-up of the method**

418  
419 The reduced cost of extraction is clearly advantageous for the proposed MHG method  
420 in terms of time and energy. Conventional solvent extraction required organic solvent, long  
421 extraction time, evaporation of the solvent is needed and the purification of the extract. The  
422 MHG method required irradiation for 20 min only. MHG is proposed as an “environmentally  
423 friendly” extraction method which avoids the use of large quantities of solvent and  
424 voluminous extraction vessels.

425 MHG could also be used to produce larger quantities of extracts by using existing  
426 large-scale microwave extraction reactors ([www.archimex.com](http://www.archimex.com)). These microwave reactors  
427 are suitable for the extraction of 10, 20, or 100 kg of fresh plant material per batch. These  
428 reactors could be easily modified and used for MHG isolations.

### 430 **4. Conclusion and perspectives**

431 MHG is a novel technology which has been used first time for extraction of onion  
432 flavonol content, whose antioxidant activity relates to human health promoting effects. With  
433 MHG its possible to extract 330.46mg of flavonol content from one kilo gram of fresh plant  
434 material (yellow onion variety), which is 41.9% of the flavonoid content extracted with  
435 solvent (80% methanol) from fresh plant material, along with crude onion juice content  
436 retaining organoleptic properties similar to fresh unprocessed product. MHG is an attractive  
437 novel technology that clearly offers opportunities for food processing industries to meet the  
438 growing demand of consumers for healthier food products as it works in absence of any  
439 solvent. Along with paying its role in creation of green environment, this technique is also  
440 offering a quicker alternative extraction process which allows substantial saving in energy and  
441 cost.

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