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Development of WEPO – Water Extraction and Particle formation On-line

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Populärvetenskaplig sammanfattning

Det finns många nyttiga ämnen i naturen runt oss, ämnen som t.ex. antioxidanter. Antioxidanter är viktiga för vår hälsa och kan göra att vi håller oss friskare. För att extrahera dessa nyttiga ämnen från sin naturliga källa används oftast organiska lösningsmedel. Dessa lösningsmedel belastar miljön både genom dess produktion och genom dess avfallshantering. Därför har en mer miljövänlig metod utvecklats där man istället använder vatten som lösningsmedel. De flesta antioxidanter är inte lösliga i vatten vid rumstemperatur och atmosfärstryck. Men genom att höja temperaturen och trycket minkar man vattnets polära egenskaper och antioxidanterna blir mer lösliga i vattnet. Detta vatten kallas subkritiskt vatten, dvs vatten som är vätska trots att temperaturen är över kokpunkten.

Ett problem med metoden är att man får de värdefulla ämnena i låga mängder i en vattenlösning och att transportera stora mängder vatten är inte miljövänligt eller ekonomiskt. Därför måste lösningarna torkas och på ett sådant sätt att de ämnen som finns i inte påverkas. För att ta bort vattnet och samtidigt bilda partiklar av de ämnen man extraherat kan man blanda lösningen med superkritisk koldioxid ($scCO_2$). $ScCO_2$ uppstår då man utsätter koldioxiden för tillräckligt högt tryck och temperatur (74 bar och 31 °C) för att nå den kritiska punkten. Koldioxiden blir då en vätska med mycket speciella egenskaper som kan beskrivas som ett mellanting av en gas och en vätska. Om man blandar vattenlösningen med $scCO_2$ och sedan leder blandningen till atmosfärstryck kommer koldioxiden att expandera till en gas och splittra vattnet med ämnena i så att väldigt små droppar bildas. Dessa små droppar kan torkas med varm kvävgas (N_2) varpå partiklar av extraktet bildas.

För att en process ska vara användbar i industrin och i större skala bör den vara enkel och med så få steg som möjligt. Färre steg betyder också mindre hantering av de värdefulla ämnena och mindre hantering av ämnena minskar risken för att de ska gå sönder. I det här projektet har en process för att extrahera värdefulla ämnen med hjälp av subkritiskt vatten och torka dem till partiklar med $scCO_2$ i ett enda steg utvecklats. Processen kallas WEPO och står för water extraction and particle formation on-line. De extraherade ämnena erhålls som partiklar direkt efter extraktionen.

För att utvärdera effektiviteten hos processen har olika analyser gjorts: vattenmängden i partiklarna, antioxidant kapaciteten, storleken och formen av partiklarna samt hur stor koncentration av antioxidanten i fråga som partiklarna innehåller. För att jämföra processen med andra metoder för att torka vattenlösningar frystorkades även extrakt som extraherats på samma sätt som för WEPO-metoden och analyserades med samma metoder. Frystorkning är en metod för att torka vattenlösningar men måste göras i ett separat steg och tar ca 5 dygn.

I det här projektet har en antioxidant från lök extraherats, quercetin. I Sverige slängs stora mängder av lökavfall varje år och används i huvudsak till produktion av biogas. Genom att först extrahera antioxidanterna från löken och sedan producera biogas kan vi ta till vara på mer av naturen och de värdefulla ämnena som finns där.

Abbreviations

WEPO	Water extraction and particle formation on-line
SWE	Subcritical water extraction
PHWE	Pressurized Hot Water Extraction
Q	Quercetin
Q-3	Quercetin-3-glucoside
Q-4’	Quercetin-4’-glucoside
Q-3,4’	Quercetin-3,4’-diglucoside
SC-CO_2	Supercritical CO_2
DPPH	2,2-Diphenyl-1-picrylhydrazyl
SEM	Scanning electron microscopy
CAN-BD	Carbon dioxide assisted nebulisation with a Bubble Dryer®

Abstract

There are many valuable substances present in nature such as antioxidants. The conventional method for extracting the antioxidants employs organic solvents. Subcritical Water Extraction (SWE) is a sustainable method for extraction of high valuable compounds. This method uses subcritical water as a solvent instead of organic solvents but leaves the compounds in a water solution. Keeping the antioxidants in water can decrease their stability and to remove water from a solution is harder then to remove an organic solvent. It is not environmentally friendly to transport a large amount of solvent so the water has to be removed and in a way that avoids decomposition of the valuable compounds. The process developed in this project, WEPO, extracts the antioxidants with subcritical water and mixes the extract with SC-CO_2 to form an emulsion that when led to atmospheric pressure forms an aerosol that can be dried quickly with hot N_2 giving particles. The compound extracted in the project is quercetin, an antioxidant that can be extracted from onion. To evaluate the efficiency of the process the particles formed were analysed to determine the water content, size and morphology, antioxidant capacity and concentration of antioxidants. For comparison the same analysis were done on extracts dried with freeze-drying. Antioxidant capacity measured with a DPPH method showed about the same capacity for the particles produced by WEPO and the freeze-dried extract. SEM pictures taken of the extracts showed hollow spheres from the WEPO particles in the size range of 250 nm – 4 μm. For the freeze-dried extracts no particles were observed. The WEPO particles and the freeze-dried extracts were analysed with an HPLC-UV method and showed about the same concentrations of quercetin species. For particle formation of the extracts the WEPO process is superior to freeze-drying the extracts and matches it in antioxidant activity and concentration of quercetin and derivates.

1. Introduction

There are a lot of valuable compounds in nature that are good for our health or useful in our everyday life. Most of these compounds are conventionally extracted with organic solvents. These solvents are not environmentally friendly both through its production and its destruction. SWE is a sustainable technology that uses water as a solvent and is able to extract high value compounds from natural sources [1], [3]. The water is under high temperature and pressure to enhance the solubility of the compounds in the water. One problem with extracting with water as a solvent is that the antioxidants end up in a water solution. Transporting the compounds in water is not environmentally friendly or economical and keeping them in water solutions can decrease the substances' stability. The solutions have to be dried in a way that avoids decomposition of the substance. For appliance to the industry the entire process needs to be easy and in as few steps as possible.

Carbon dioxide assisted nebulisation with a Bubble Dryer® (CAN-BD) is a process designed for drying water solutions of pure compounds and form particles of those compounds [2]. The CAN-BD process mixes the water solutions with SC-CO_2 and when the mixture is introduced to atmospheric pressure an aerosol is formed. The aerosol can be dried quickly with hot and dry N_2.

The aim of this project is to develop a process that can extract high value compounds and dry them to particles in only one step. The process developed uses subcritical water to extract the high value compounds from its natural source and then dries the extracts using SC-CO_2 assisted nebulisation, all in one step. The extracts of the valuable compounds are delivered as dry particles directly after the extraction, instead of in a water solution.

Antioxidants are compounds that can be extracted from nature and can be of use in for instance the food industry or in cosmetics. One of these antioxidants is quercetin and in this project quercetin has been extracted from yellow onion. Quercetin is an antioxidant and is used in the development of the WEPO process in this project because of previous work done in the group to optimize the extraction parameters [3]. The WEPO process can be applied to the upgrade of waste materials by extracting high value compounds from the waste before it goes on to further treatment. The process could be a step to a sustainable and green technology to enhance our possibilities of using the natural resources to a maximum and in an environmentally friendly way.

1.1 Quercetin

Every year in Sweden there is about 1000-5000 tons of onion going to waste from the food and agricultural industry. The waste is mainly used in animal feed or made into biogas by anaerobic digestion. The latter is becoming more frequent [4]. Onions contain substances that are healthy for the human being such as flavonoids that acts as antioxidants. Antioxidants are important for our health, for example, they help neutralize free radicals that can occur in different oxidation processes in our body, for instant in the electron transport chain. For this and other properties it is widely believed that antioxidants give protection against cancer and heart disease and even ageing [5]. Antioxidants are also used in the food industry and in cosmetics or skincare products to help preserve the products. One of these flavonoids that could be used in different products as an antioxidant is quercetin that is found as an aglycone as well as different glucoside derivates (*Figure 1*). Studies have shown positive effects of quercetin in preventing cancer both through its antioxidant activity and in interactions with proteins and receptors [6]. Quercetin is most frequent in the skin and the outer layers of the

yellow onion and is partly responsible for the yellow colour of the onion. The derivates is more frequent in the fleshy part in the inner layers [5]. Quercetin can also be found in various fruits and vegetables such as kale, broccoli, apple skin, green tea and grapes [3]. The levels of quercetin in onion are about 300 mg/kg and are among the highest levels found in vegetables. Only kale has a higher reported level of quercetin, 450 mg/kg [5].

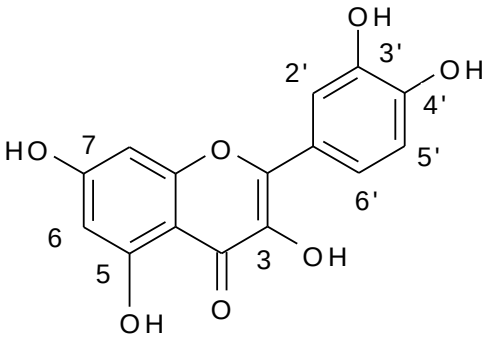

Figure 1. Structure of quercetin. The sites for glucosidation most found in yellow onion is the 3 and 4' carbons.

1.2 Extraction

For extraction of quercetin from onion the conventional method employed is a batch method, solid-liquid extraction, with organic solvents or aqueous methanol [7]. Use of organic solvents in the extraction step can hinder the possibility for biogas production and the harsh conditions (long time of extraction and exposure to light and oxygen) could lead to degradation of the extracts [8].

Subcritical water extraction, also called Pressurized Hot Water Extraction (PHWE), uses liquid water as a solvent at temperatures above the boiling point of water. Compared to the conventional method, which uses organic solvents, it is more energetically and environmentally favourable and matches the conventional method in amount of extracted compounds [3]. Quercetin is more soluble in an organic solvent such as methanol than water but by increasing the temperature of liquid water the dielectric constant decreases and the quercetin becomes more soluble. The glucoside derivates of quercetin are more polar with increasing number of glucose groups attached and therefore more soluble in water at room temperature. From previous work done in the group the optimal temperature for SWE of quercetin species has been determined to 120 °C [3] and is done under pressure enough to keep the water liquid. The extraction gives a liquid sample and has to be dried in order to get rid of the water.

1.3 Particle formation

Particle formation on-line is performed through mixing of the aqueous sample from the SWE with SC-CO_2. Carbon dioxide becomes supercritical at 31°C and 74 bar. The extract and the SC-CO_2 are mixed in a low dead-volume t-connection. Since water is not soluble in scCO_2 an emulsion of the two is created. When the emulsion is introduced to atmospheric pressure the CO_2 expands rapidly splitting the water to small droplets creating an aerosol. The aerosol contains the extract in very small droplets that can be dried quickly with hot and dry N_2. When the heated N_2 hits the aerosol it evaporates the water and forms the particles that can be collected from the vessel. *Figure 2* shows the set up of the WEPO system. In this project the WEPO system has been developed by making particles of extracts from onion. To compare the WEPO process with another drying process, samples from onion extracted with SWE were freeze-dried.

2. Materials and Method

2.1 Chemicals and Samples

Onions were bought in Sweden and chopped by hand to similar sizes (~ 5 x 5 mm) and kept in freezer until use (for the remainder of the project, 10 weeks). Both skin and bulb of the onion were used. Milli-Q water was obtained from a purification system, Merck Millipore (Molshein, France) and degassed before use. Ultrapure CO_2 was provided by Air Products (Amsterdam, Netherlands). Nitrogen 4.5 grade was obtained from Strandmoller (Klampenborg, Denmark). Methanol used for HPLC analysis was of HPLC grade and purchased from Honeywell (Seelze, Germany). The methanol was mixed with formic acid (98-100% purity) from EMSURE (Darmstadt, Germany). Quercetin and quercetin-3-glucoside was purchased from Sigma-Aldrich (Steinheim, Germany). Quercetin-4'-glucoside and quercetin-3,4-diglucoside was obtained from Polyphenols Laboratories AS (Sandnes, Norway). Ethanol (95% purity) was used for cleaning of the equipment. DPPH was purchased from Sigma-Aldrich (Steinheim, Germany).

2.2 WEPO

The WEPO system consists of a home-built apparatus shown in *Figure 2*. The system consists of a HPLC pump (Waters, 515 HPLC pump) delivering water at a constant flowrate through 1/16 inch stainless steel tubings to the extraction cell (Valco, 10 ml) located in a GC oven (Hewlett Packard, 5890A). The oven was set to a constant temperature of 120°C for all of the extractions. From the cell the extracts were led to a t-connection (Valco) were it was mixed to an emulsion with the supercritical CO_2 and exited into atmospheric pressure through a restrictor (Swagelok) and a nozzle (varying brand and inner diameter). CO_2 was pumped by ISCO syringe pump (Model 260D) at a constant pressure of 80 bar. The aerosol of sample and CO_2 was entered into a 2 L glass vessel (custom made) and dried with hot dry N_2. The particles were collected on a filter in the bottom of the vessel or from collection tubes replacing the filter. The system was preheated for 5 min at 120 °C. The extraction was initiated with 5 min of static extraction followed by 40 min of dynamic extraction. The experiments were done with varying temperature, flowrate of water and flowrate of CO_2. The extraction vessel was sonicated in ethanol for 30 min between each run and the system was rinsed with ethanol to avoid carry-overs.

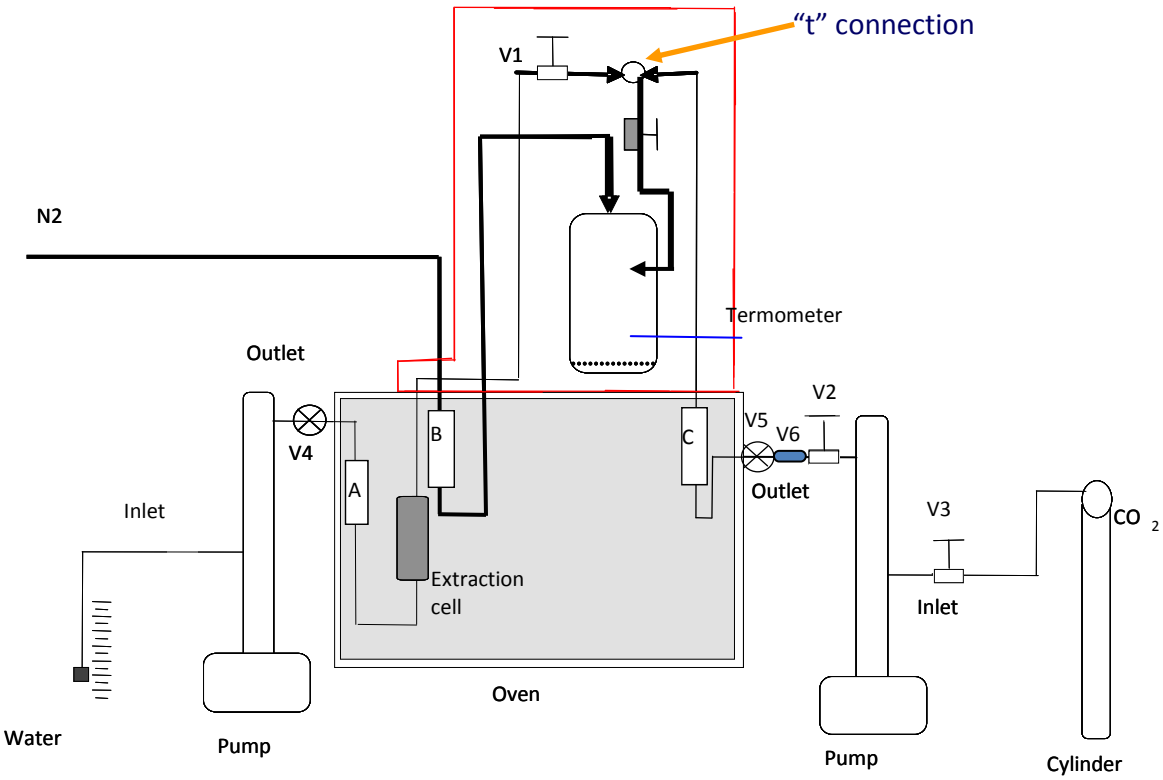

Figure 2. Schematic picture of the WEPO system. V1,V2 and V3: On/Off valves (HIP), V4 and V5: anti-return valves, V6: burstdisk. A, B and C coils. The apparatus inside the red line was heated through heating tapes.

2.3 Dynamic Extraction

In order to roughly optimize the duration of the WEPO process, dynamic extraction of the onions was performed in advanced in a home-built apparatus. For determination of the optimal time of extraction, preliminary experiments were performed with 60 minutes of dynamic extraction were samples were collected every five minutes in vials on ice bath. To shorten the time of the dynamic extraction the extractions were initiated with static extraction. To decide how long the static extraction should be two experiments were done with the dynamic extractions done as described above but once with 5 minutes and once with 10 minutes of static extraction in the beginning. The different extractions were compared to see which gave the highest amount of flavonoids. The extracts were analysed by HPLC-UV.

The extractions that were freeze-dried in order to compare with the WEPO process were extracted under following conditions. The extractions were initiated with 5 minutes of static extraction at a pressure above 80 bar and at a temperature of 120 °C followed by 40 minutes of dynamic extraction under the same pressure and temperature. The flow rate of the water was set at 0.3 ml/min. The samples were collected in a vial on ice bath and stored in a freezer until further analysis by HPLC. After first being analysed by HPLC the samples were freeze-dried for 4 days to remove the water from the extraction generating a dry powder. The dry samples were dissolved in methanol and analysed by HPLC. The oven was preheated at 120 °C for 5 minutes for all of the extractions.

2.4 HPLC analysis

The samples were analyzed by HPLC-UV using a Hewlett Packard (HPLC 1050) system with an Agilent Zorbax SB-C18 column (100 × 2.1 mm, 3.5 µm). Water and methanol (60:40) containing 0.5 v% formic acid was used as a mobile phase in an isocratic method. The flow rate was set to 0.15 ml/min and the injection volume was 10 µl for each sample. The detection was carried out at 350 nm. The flavonoids were identified and quantified in base of retention time and peak area using standards. Data collection time was 80 min for the extractions and 30 min for the calibration curve.

The calibration curve was made from stock solutions of quercetin, quercetin-3-glucoside, quercetin-4'-glucoside, quercetin-3,4-diglucoside and morin (Table 1). Figure 3 shows a chromatogram of the highest concentration of the standard curve.

Table 1. Concentrations of the calibration curve for quercetin (Q), quercetin-3-glucoside (Q-3), quercetin-4'-glucoside (Q-4'), quercetin-3,4-diglucoside (Q-3,4') and morin (M)

Q (µM)	**Q-3 (µM)**	**Q-4' (µM)**	**Q-3,4' (µM)**	**M (µM)**
75	25	75	75	50
56	19	56	56	37.5
37.5	12.5	37.5	37.5	25
19	6	19	19	12.5
1.5	0.5	1.5	1.5	1

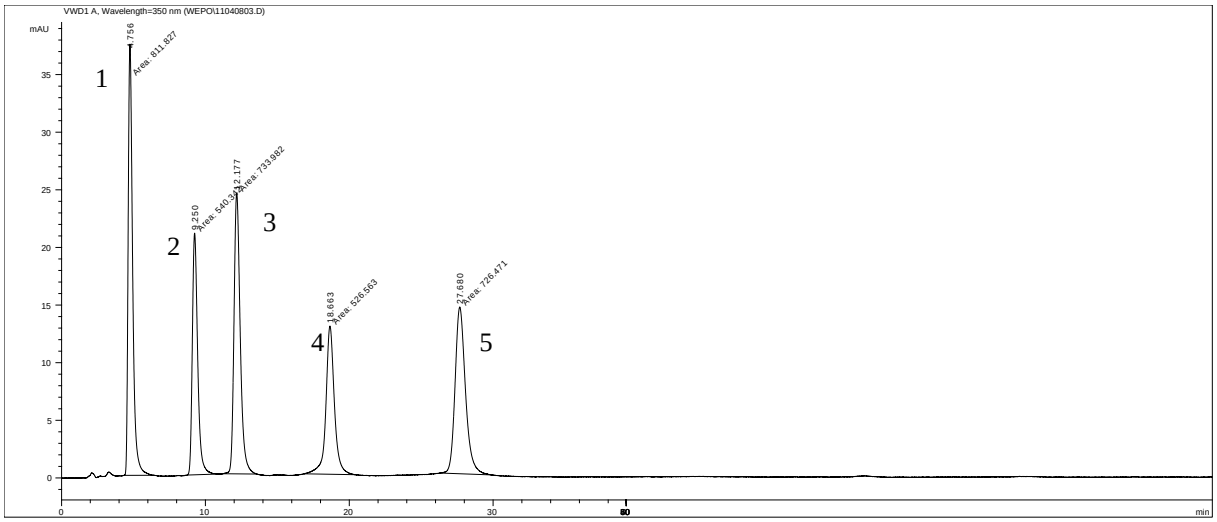

Figure 3. Chromatogram from standard curve of quercetin, quercetin-3-glucoside, quercetin-4'-glucoside, quercetin-3,4-diglucoside and morin. The chromatogram is of the highest concentrations in the standard curve (**Table 1**)

2.5 DPPH

Antioxidant capacity can be measured with a method using the loss of absorbance at 515 nm when the free radical DPPH is neutralised by the antioxidants [9]. Particles from WEPO and dynamic extraction followed by freeze-drying were dissolved in methanol at a concentration of 3 mg/ml. 23.7 mg of DPPH was dissolved in 100 ml of methanol, stock solution. From the DPPH stock solution 10 ml was diluted to 100 ml with methanol, working solution. 25 µl of each sample was diluted with 975 µl of the DPPH working solution. 300 µl of each mixture was put directly to a 96 well microplate and measured in a spectrophotometer (Thermo Multiskan GO, Thermo Scientific) at 515 nm, time 0 measure. The microplate was stored at room temperature covered with aluminium foil to avoid exposure to light, and after 4 h the samples were measured again. The antioxidant capacity was calculated as EC_{50} values according to W. Brand-Williams *et. al.* [9]. The effective concentration, EC_{50}, is the concentration of antioxidant needed to neutralize half of the radicals in the sample. A lower value indicates a greater antioxidant capacity.

2.6 Water content of particles

For determination of the water content of the particles, both WEPO particles (5 mg) and freeze dried extracts (50 mg), samples were weighed in petridishes and put in an oven at 120 °C. After 21 h the petridishes with the samples were weighed again. The weight difference before and after the drying process was interpreted as water present in the particles.

2.7 Scanning electron microscopy (SEM)

Samples from WEPO and dynamic extraction followed by freezedrying were observed in a scanning electron microscope (JEOL, JSM 6700F). Both samples were covered with a thin goldlayer by a sputter coater (Balzers, SCD 004). The samples were scanned with a beam of 10 kV.

3. Results and Discussion

3.1 Extraction

From previous extractions carried out in the group the temperature used for extraction of flavonoids was set to 120 °C [3]. Although later research indicates the better temperature for extraction could be 165 °C [10], but these extractions were only done on the skin of the onion and under static conditions. So due to the risk of caramelisation of the extract and of clogging of the tubings it was decided to use the lower temperature, 120 °C. The pressure for extraction was set to be above 80 bar. The pressure of 80 bar was needed due to the pressure of the SC-CO_2 that the extracts have to meet under the particle formation step of the process. Previous research shows that the pressure of the water during extraction does not influence the extraction significantly as long as it is high enough to keep the water liquid [13]. Remaining parameters was set according to conditions best suited for particle formation (*Table 2*).

The total time of extraction was determined by preliminary studies were the numbers of mol of flavonoids extracted per time were investigated. To decrease the time of the dynamic extractions and hence the amount of CO_2 needed when using the WEPO process it was decided to initiate the extractions with some time of static extraction. To determine how long each of the extractions phases should be, the static and the dynamic, two different experiments were done. One was initiated with 5 minutes of static extraction followed with 60 minutes of dynamic extraction and one was initiated with 10 min of static followed by 60 minutes of dynamic extraction. From the indications received from these experiments a total extraction time of 45 minutes including 5 minutes of static extraction to begin with was determined to be optimal (**Figure 4**). According to the preliminary results the amount of extracted flavonoids received in the time frame of the study never reach zero, but after 40 minutes of dynamic extraction the amount of flavonoids that were extracted was considered too low for the expense of the resources to extract it. When applying the extraction to the WEPO process the longer the time of the extraction the more of CO_2 and N_2 is being consumed. There was no substantial difference in the amount of extracted flavonoids between the two different times of static extraction. Because of this the shorter static extraction time was chosen.

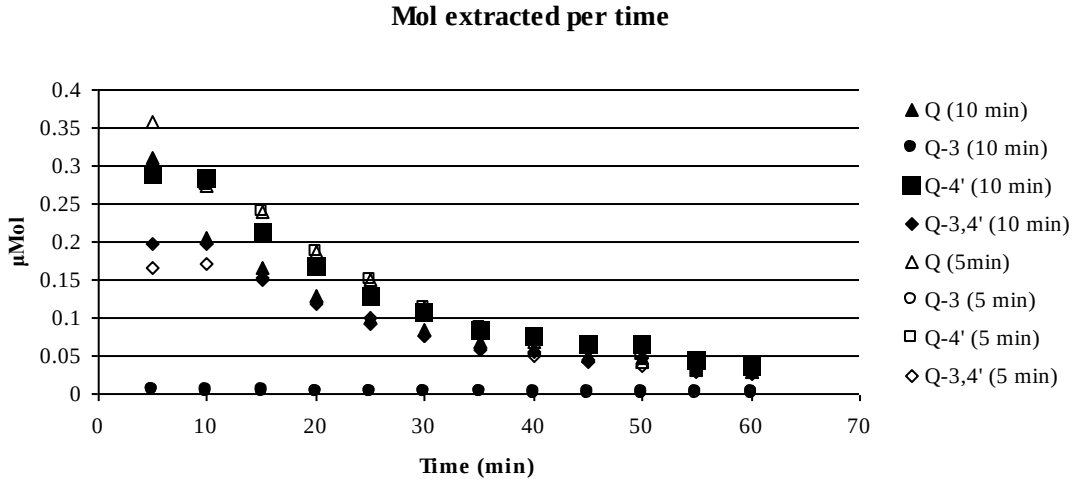

Figure 4. Comparison of the amount of mol extracted per time of dynamic extraction between initiating with 5 min of static extraction (hollow) and 10 min of static extraction (filled).

3.2 Particle formation

Many different parameters, conditions and setups were tried for the WEPO process, temperature, flow rate of the water, flow rate of the CO_2, nozzles and outlets (*Table 2*). The different parameters were altered simultaneously to find the right ones to get particles. The first particles observed were when the vessel for collecting the particles was put under heating. In earlier trials with the system particles were observed without heating, but for those cases the extraction and nitrogen temperature was 200 °C [11] and this was enough to warm the collection vessel. This temperature is too high for extraction of quercetin because of the risk of degradation [3]. The lower temperature of 120 °C is not enough to counter the effect of the cooling of the extract when the gas expands into atmospheric pressure at room temperature. The collection vessel was heated by heating tapes which gave an internal temperature of 70 °C. Another method that employs SC-CO_2 for particle formation is the CAN-BD. The temperature used in the CAN-BD system was 60 °C in the bottom of the vessel and 80 °C at the top [2]. For the WEPO system these conditions on the inside of the collection vessel were not dry enough to collect a significant amount of particles.

It is important to keep the collection vessel under atmospheric pressure to achieve a stable aerosol and to compensate for the high stream of gases flowing in, N_2 (12.5 bar) and CO_2 (10 ml/min), vacuum was applied to the collection vessel.

A big concern with the system was the difficulty to collect the particles formed. Two different filters were tried, paper filter and a cellulose acetate filter. The paper filter did not work very well, all the particles got stuck inside the filter. The cellulose acetate filter did work somewhat better but seemed to attract too much vapour generating wet particles. Another downside of that filter was its fragility. The filter could not be put under vacuum without another filter underneath for support or it would break. There were a lot of particles dispersed on the walls but they were almost impossible to collect due to the shape of the vessel. The particles seemed charged with static electricity and made for another difficulty during the collection step.

The addition of vacuum to the collection vessel was not enough to keep an atmospheric pressure and another outlet was added to the vessel. The outlet was made up out of a plastic tube to be able to see if any particles went out that way. After the outlet was added a substantial amount of particles could be collected (8 mg), mainly from the outlet itself. Instead of the filter and vacuum a second plastic tube outlet was added and from both of the outlets a higher amount could be collected (12 mg).

The WEPO process did deliver the particles directly after extraction and in one step avoiding prolonged exposure of the extracts to light and oxygen. When the dynamic extractions were dried with freeze-drying it took 4 days and the extracts had to be handled in between the extraction and the drying process exposing them to light and oxygen.

Table 2. Parameters altered during particle formation in the WEPO. *Outlet for added to the collection vessel. **Additional outlet added to the collection vessel. Stainless steel (s.s.) and polyether ether ketone (PEEK) were the materials of the nozzle.

Outcome	**T_{tubes} (°C)**	**T_{vessel} (°C)**	**$Flowrate_{water}$ (ml/min)**	**$Flowrate_{CO2}$ (ml/min)**	**N_2 (bar)**	**Nozzle (mm)**	**Vacuum**	**Total extraction time (min)**
No particles	80	-	0.3	10	5-10	0.5 (s.s.)	Yes	Not recorded
No Particles	120	-	0.3	10	10	0.5 (PEEK)	Yes	Not recorded
No Particles	120	-	0.3	3	10	0.13 (PEEK)	Yes	28
Particles observed	120	120	0.3	6	12.5	0.25 (PEEK)	Yes	34
Particles observed	120	120	0.3	7-8	12.5	0.25 (PEEK)	Yes	25
*Particles (8.02mg)	120	130	0.3	10	12.5	0.25 (PEEK)	Yes	27
Particles (9.03mg)	120	130	0.3	7-8	12.5	0.25 (PEEK)	Yes	38
Particles (9.28mg)	120	130	0.3	5-7	12.5	0.25 (PEEK)	No	41
**Particles (12.85mg)	120	130	0.3	10	12.5	0.25 (PEEK)	No	45
Particles (5,61mg)	120	130	0.2	10	12.5	0.25 (PEEK)	No	45

3.3 HPLC-UV analysis

The amount of quercetin and its derivates per dry g of onion determined from HPLC for the WEPO particles were considerably lower then the amount determined for the freeze-dried extractions (*Table 4*). This lower value for the WEPO process is mainly due to the low amount of particles that could be collected, i.e. all particles formed could not be collected. When looking at the concentrations of quercetin species in the samples, both for the WEPO particles and the freeze-dried extracts (*Table 3*), the differences are not big between the two. This leads to the conclusion that the WEPO particles and the freeze-dried powder have approximately the same amount of extracted flavonoids per gram of sample. Therefore, it is how much of the total amount of formed particles that can be collected that makes the difference.

Figure 5 shows a chromatogram from particles from the WEPO process dissolved in methanol.

Table 3. Comparison between the concentration of flavonoids in particles from WEPO and freeze-dried extracts. 2 mg of each was dissolved in 1 ml of methanol.

	WEPO	Freeze-dried extracts
Concentration of Q (mg/ml)	0.008 ± 0.005	0.01 ± 0.003
Concentration of Q-3 (mg/ml)	0.0003 ± 0.00001	0.0004 ± 0.00007
Concentration of Q-4’ (mg/ml)	0.016 ± 0.002	0.018 ± 0.005
Concentration of Q-3,4’ (mg/ml)	0.009 ± 0.0003	0.011 ± 0.002

Table 4. Comparision between the WEPO process and dynamic extraction followed by freezedrying. In both methods the extractions were from 2 g of onion.

	WEPO	**Freezedried extracts**
Mass of dry extract (mg)	12.85	140.5
Amount of quercetin (mg/dry g onion)	0.45 ± 0.26	4.16 ± 1.29
Amount of quercetin-3-glucoside (mg/dry g onion)	0.016 ± 0.002	0.15 ± 0.04
Amount of quercetin-4'-glucoside (mg/dry g onion)	0.89 ± 0.064	7.65 ± 2.46
Amount of quercetin-3,4'-diglucoside (mg/dry g onion)	0.51 ± 0.02	4.55 ± 0.90
Total amount of quercetin and derivatives (mg/dry g onion)	1.87 ± 0.29	16.51 ± 4.67
Water content (w%)	52.5 ± 6.3	28.57 ± 1.5
Antioxidant capacity, EC_{50} (µg/ml)	101.98 ± 3.5	104.33 ± 2.0
Size of particles	250 nm – 4 µm	No particles observed

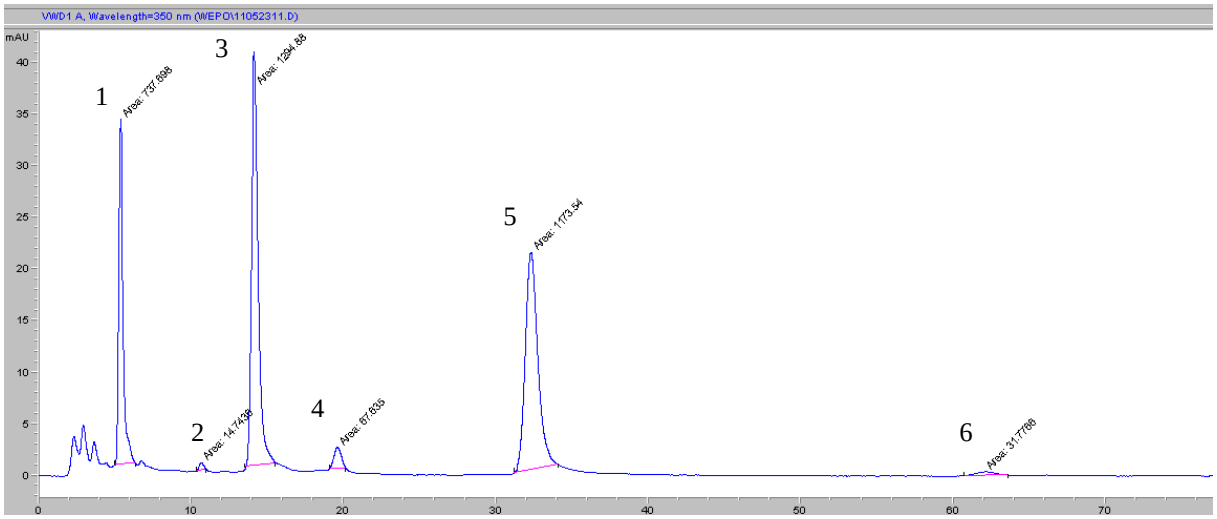

Figure 5. Chromatogram from particles obtained from the WEPO-system dissolved in methanol. Peak 1) Q-3,4', 2) Q-3, 3) Q-4', 4) unknown, 5) Q, 6) unknown.

3.4 Water content of the particles

From the determinations of the water content in the WEPO particles and the freeze-dried samples the dryness of the freeze-dried extracts was considerably higher (*Table 4*). In the WEPO particles the amount of water was 52.5% in weight and the amount in the freeze-dried only 28.6% in weight. Both these results are surprisingly high and give the suspicion that the samples have either absorbed water in the process or storage or that decomposition of the extracts occur and more than water is lost when drying in the oven. When the samples were retrieved from the oven they were dark brown and looked melted. Another way of determining the water content would be desirable but due to lack of time it could not be done in the present work.

3.5 Antioxidant capacity

From the measurements of the antioxidant capacity no substantial difference is detected between the particles from WEPO and the powder from the freeze-dried extracts. The effective concentration, EC_{50} was determined to be around 100 µg/ml for both (*Table 4*).

This shows that both techniques for drying of the samples have the same impact on the extracted compounds' antioxidant capacity.

3.6 Scanning electron microscopy (SEM)

Scanning electron microscopy performed on particles from the WEPO system showed many spherical particles (*Figure 6 A – B*). Damages done by the beam from the microscope, revealing a hollow inside, gives the indication that the particles are in fact hollow (*Figure 6 B*). The size of the particles ranged from 250 nm to 4 µm but most of the particles were in the size range of 1 – 3 µm. Agglomeration was observed, mainly among the smaller particles. From the powder received from the dynamic extraction followed by freeze-drying there were no particles observed. Instead there were a tangled web of sheets and strings (*Figure 6 C – D*). Smaller particles can enhance the solubility and the bioavailability of the sample. When dissolving the samples in methanol, the particles received from the WEPO process were easier to dissolve than the dry samples from freeze-drying. The particles from WEPO were easy to dissolve in water while pure quercetin is not. This fact could be potentially related to an enhance of the bioavailability of the active compound.

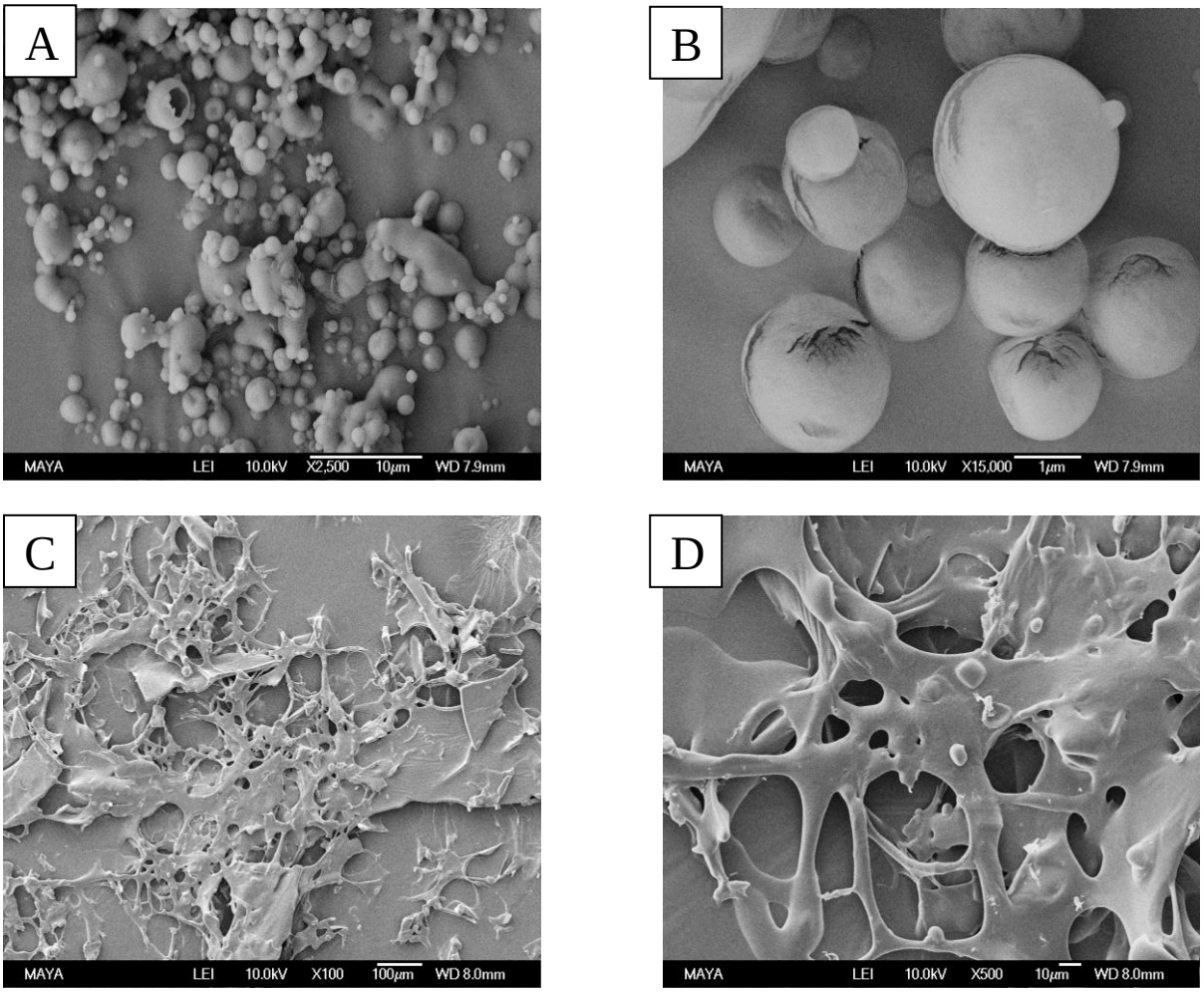

Figure 6. Pictures of extract samples taken by SEM. A) Particles from WEPO-system. B) Close up of the WEPO particles. C) Powder from freeze-dried samples. D) Close up of the freeze-dried sample.

4. Conclusions

The water extraction and particle formation on-line process, WEPO, has been developed. This process combines the subcritical water extraction of plants and drying of the extracts by a particle formation technique based on the use of supercritical carbon dioxide, in one step.

In particle formation of quercetin and the other flavonoids from subcritical water extraction the WEPO is far superior to the freeze-drying process.

The WEPO matches the freeze-dried extracts in antioxidant capacity and concentration of flavonoids in the particles.

However the amount of particles that could be collected from the WEPO is significantly lower then the mass obtained from freeze-drying the extracts. Further improvement of the system is needed in this area.

The water content of the particles is very high but further investigation is needed in the method of measuring the water content to know more.

5. Future work

The biggest issue with the WEPO system is that the amount of particles able to be collected is low. The first change would be to improve the vessel for collecting the particles. A lot of particles get lost because they can not be collected from the walls of the vessel. Another thing is to make the vessel less closed and hence easier to keep at atmospheric pressure. A heating system for the vessel that makes it easier to control the temperature inside and make it more stable would likely improve the method. The inlet for N_2 should be bigger. The current inlet is a tubing of 0.5 mm inner diameter due to the narrow entrance to the vessel. This tubing is working as a restrictor for the N_2, diminishing the possible mass flow.

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