Antioxidant activity of membrane-fractionated coffee extracts in dependence of the storage conditions

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Abstract. Present paper aims at one of the important aspects of the application of products with antioxidant activity: namely the preservation and change of their properties during the storage in different conditions, as well as their reliable characterisation.

The tests of antioxidant properties were conducted with membrane-separated coffee extracts, isolated using a “Microdyn Nadir NP030P” type of commercial nanofiltration membrane (30% retention of NaCl; MWCO~400). Prepared coffee permeates and retentates were stored 0÷10 days in cool/warm conditions, with/without air access and at different illumination conditions. The kinetics of content changes was evaluated according to Folin-Ciocalteu method of total phenolic/reducing content determination.

1. Introduction

The effects of storage conditions on the stability of different herbal, biological, medical, nutritional etc. extracts are widely researched; with objects of interest being: from plants and mushrooms, foods, flavourings, blood and tissues, DNA extracts, to house dust mites and many more [1-5]. Nowadays a lot of attention is attracted to the membrane-derived plant products, especially in aspect of their usage as alternative natural remedies and healthy food supplements [6-7]. As regards the application of products with antioxidant activity, here the preservation and as minor as possible change of their properties during the storage in different conditions plays a very important role. Any research of biologically-active extracts, solutions, samples etc. and their deterioration products relies on a reliable and possibly simple characterisation.

With present work we exhibit a practical application of the nanomembrane filtration, aimed at the important task of durability assessment for the herbal extracts and their nanofiltration derivatives. Analytical part is comprised by a determination of the antioxidant efficiency of coffee extracts under storage, implementing the Folin-Ciocalteu’s oxidative method and spectrophotometric evaluations.

2. Experimental part

2.1. Materials

All reagents used were of analytical grade quality: Folin & Ciocalteu’s phenol reagent, gallic acid, anhydrous sodium carbonate from Sigma-Aldrich, Taukirchen, Germany. Syringe filters of 0.45 μm
Distilled water (water still GFL Typ 2004, Burgwedel, Germany) was used throughout the work.

2.2. Coffee extract preparation

A short brew of coffee type “Carraro” (Belogia Espresso Machine) was used for the preparation of the initial extract. An attempt to eliminate hampering the subsequent membrane filtration colloidal coffee ground particles through centrifugation at 3000 min\(^{-1}\) for 20 minutes turned out to be insufficient. Therefore as a primary treatment filtration using “Boeco” 391 filter paper (2-3 μm porosity) was applied, followed by 0.45 μm syringe filters. Filtered this way extract was finally diluted twentyfold and directed to the nanomembrane separation.

2.3. Nanomembrane filtration

150 mL of the diluted coffee extract (further: Feed; F) were subjected to a nanofiltration (set-up according to 2.6.; filtration procedure as described previously [6]). The membrane separation continued until 76 mL of permeate (P) were isolated, with 74 mL of retentate (R) being the rest. The nanomembrane performance is shown in the subsection 3.1.

2.4. Preparation of samples for “durability kinetics” tests

Samples were divided in four major groups and two sets (figure 1): 1) storage with presence of air in cool conditions (samples F\(_{1S}\), P\(_{1S}\) and R\(_{1S}\)); 2) storage without air presence in cool conditions (samples F\(_{1Sn}\), P\(_{1Sn}\) and R\(_{1Sn}\)); 3) storage with presence of air in warm illuminated conditions (samples F\(_{1T}\), P\(_{1T}\) and R\(_{1T}\)); 4) storage without air presence in warm illuminated conditions (samples F\(_{1Tn}\), P\(_{1Tn}\) and R\(_{1Tn}\)). Before mentioned samples represented also the first set, which was incubated until the sixth day from the deposition; next set was researched on the tenth day (samples F\(_{2S}\), P\(_{2S}\) and R\(_{2S}\); F\(_{2Sn}\), P\(_{2Sn}\) and R\(_{2Sn}\); F\(_{2T}\), P\(_{2T}\) and R\(_{2T}\); F\(_{2Tn}\), P\(_{2Tn}\) and R\(_{2Tn}\)). The samples for no air presence were packed in 2-mL HPLC vials; those for air presence had 5-mL samples poured in 50-mL plugged volumetric flasks. The storage for cool conditions was executed at 8°C in refrigerator in dark; the storage for warm conditions was at room temperature around 20°C, near a window open to light as shown on the figure 1.

![Figure 1. Samples of coffee extract (from left): feed, permeate and retentate prepared for durability tests](image)

2.5. Total phenolic content (TPC) determination

A spectrophotometric assay of TPC was applied using a modified method of Folin-Ciocalteu method [8-9]. The assay in sum was following: corresponding amount of the phenolic/reductive coffee extract (0.200 to 1.000) was brought to a volume of 7 mL through appropriate dilution with distilled water. 0.5 mL of of Folin–Ciocalteu’s reagent was subsequently added, and after thorough shaking 10 minutes of dead time was applied. Then 2.5 mL of 10% (w/w) aqueous sodium carbonate solution was added. The colourisation reaction developed for 20 min at room temperature. Afterwards the specific absorbance at 760 nm was finally measured at room temperature with a UV–Vis spectrophotometer.
an identical mixture without coffee extract was used as a blank sample. The total phenolic/reducing contents were rated as mg kg\(^{-1}\) gallic acid equivalents (GAE), through a calibration curve of gallic acid (figure 2) and normalised per kg of initial extract sample.

![Standard curve GALLIC Acid in water (Folin-Ciocalteu)](image)

\[ y = -0.0038 + 0.0098 \times x \]

**Figure 2.** The standard curve for the gallic acid standard. Rsqr = 0.9999

2.6. Instruments
Following instrumentation was used throughout the work:

**Membrane filtration.** “Dead-end” filtration cell of type “METcell” (Membrane Extraction Technology, London, UK), fitted with nanofiltration membrane “Microdyn Nadir” NP 030 P (Wiesbaden, Germany); processing at 10 bar (compressed nitrogen).

**Spectrophotometry.** An S-22 UV/Vis type of spectrophotometer (Boeco, Germany) was used for determination of the maximal spectral absorption at wavelength of 760 nm.

**Centrifugation.** “Janetzki” T32a centrifuge (Berlin, Germany).

3. Results and Discussion
Extracts having different antioxidant properties and degree of degradation were researched with the course of our work. In addition to the different duration and conditions of each procedure, the samples’ response was specific also for each type of used feed or nanofiltration product.

3.1. Nanomembrane filtration of initial coffee extract
Membranes used in the “production scale” filtration procedures are exclusively operated under crossflow filtration mode [10]. For the laboratory practice though membranes are mostly operated under “dead-end” filtration mode, simpler and faster approach with no parallel feed flows onto the
membrane surface. Under the dead-end filtration mode, all the colloidal particles and high-molecular compounds contained in the feed coffee extract deposit on the membrane surface, in addition to the potential concentration polarisation. Both phenomena result in permeate flow rate reduction with working solutions, compared to filtration runs with pure solvent. This effect is illustrated in figure 3 for the used nanomembrane type “Microdyn Nadir” NP 030 P, where a cubic regression was the best fit for coffee extract nanofiltration kinetics. The membrane rejection in regard to the antioxidant contents of the feed extract was calculated in two different ways [6], with rejected compounds mainly over 350-400 Da molecular weight:

\[
R_1 = (1 - \frac{C_p}{C_f}) \times 100 = 77.2\%, \text{ or}\]
\[
R_2 = \left( \log \left( \frac{C_r}{C_f} \right) \right) \times 100 = 75.8\% \quad (2)
\]

The membrane performed within the manufacturers specifications for water permeate flux: 2.4913 LMH/bar (L/(m²hbar)) before the coffee extract filtration and 1.8519 LMH/bar after it; with manufacturers’ data being >1 LMH/bar.

![Figure 3. Kinetics of the NP 030 P nanofiltration of pure water and coffee extract: cumulative permeate volume per unit membrane surface versus the time of collection](image)

3.2. The “Durability Kinetics”

According to the Folin-Ciocalteu tests, the primary short “Carraro” coffee brew contained 3722 mg kg⁻¹ GAE total polyphenolic and reducing content of antioxidants. Diluted feed extract reached \(C_f=182.4\) mg kg⁻¹ GAE, with permeate and retentate immediately after nanofiltration \(C_p=41.6\) and \(C_r=308.5\) mg kg⁻¹ GAE. After six days of incubation period relatively limited changes with the antioxidant capacity were observed; with bigger downshift for both of permeates and especially for the \(P_{IT}\) which was kept in warm illuminated conditions. Excluding the latter, less than 2% RSD dispersion was registered for this first set (Table 1 and figure 4).
Table 1. Kinetics of the antioxidant contents in feed coffee extract, permeate and retentate at sixth and tenth day of storage; RSD here depicts the degree of changes

| Sample type | AIR | No AIR | RSD,% | Sample type | AIR | No AIR | RSD,% |
|-------------|-----|--------|-------|-------------|-----|--------|-------|
| F₁T         | 173.5 | 175.9 | 0.97  | F₂T         | 165.4 | 169.9 | 1.90  |
| P₁T         | 33.4  | 39.7  | 12.19 | P₂T         | 27.9  | 38.3  | 22.22 |
| R₁T         | 286.8 | 293.8 | 1.71  | R₂T         | 191.3 | 289.3 | 28.84 |
| F₁S         | 183.7 | 185.6 | 0.73  | F₂S         | 173.9 | 182.9 | 3.57  |
| P₁S         | 38.8  | 39.9  | 1.98  | P₂S         | 36.4  | 39.4  | 5.60  |
| R₁S         | 301.0 | 301.9 | 0.21  | R₂S         | 282.2 | 297.8 | 3.80  |

Figure 4. Comparative kinetics for the two sets of samples.

Significant changes were logged at the tenth day of incubation period though. Exposed and kept in warm samples showed rapid deterioration, except the feed extract. A possible reason for the feed extract’s stability is the preliminary sterilization during the brewing process. For both of retentates some mold cultures started to develop into the air-containing flasks, more abundant for R₂T, where TPC value plummeted with a third. Despite of the mold presence, the TPC for the deposited in cold R₂S was not significantly affected. The growth of the mold was most likely promoted by the retained carbohydrates in R-samples with the nanofiltration; but this must be further specified.

4. Conclusions
With this work we studied the nanofiltration of the coffee brew extract, and changes of the derived products under different duration and storage conditions. Membrane filtration here exhibited a strong necessity of preliminary micro- and ultra-filtration steps, in order nanomembrane fouling to be avoided.

All in all, the extract samples showed small antioxidative changes until the sixth day of storage irrespective of the conditions; further storage could be considered reasonable only at lower temperatures and especially without air in presence.
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