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Bioactive Compounds from Carrot Pomace as Natural Antioxidants to Enhance the Oxidative Stability of Linseed Oil Encapsulated by Particles from Gas Saturated Solutions Technique

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In this work, supercritical fluids were used to (1) extract bioactive compounds from carrot pomaces and (2) to use them as ingredient to test their antioxidant capacity to retard the oxidation of linseed oil produced by an innovative microencapsulation technique, called particles from gas saturated solutions (PGSS). Briefly, the extract was obtained from dried and milled carrot pomaces by supercritical CO_2 extraction. The extraction was run at 30 MPa and 60 °C with a CO_2 flow rate of 2 L/h for 120 min leading a yield ranging from 1 to 2 %. Then, the extracts were mixed with linseed oil and turned into powders by PGSS. This operation consisted of two steps. First, sunflower wax (~80 %), linseed oil (~20 %) and the extract from carrot pomace (from 0.01 to 0.3%) were mixed in a melting chamber saturated with CO_2, for 30 min at a constant pressure of 10, 20 or 30 MPa and 65 °C. Then, the melted mixture was sprayed into a cyclone chamber at ambient temperature and pressure. The depressurization cooled down the droplets and turned them into powder crystals. Interestingly, the lower pressures applied in the melting chamber, greatly increased the encapsulation efficiency, powder density (bulk and tapped) and powder flowability (Carr index). Finally, the oxidative stability of the powders was tested by isothermal calorimetry (at 40°C). The powders prepared at 10 MPa showed an oxidative stability 2 times longer than those prepared at 30 MPa. Also, the addition of carrot extracts (0.3 %) induced an improved oxidative stability to the powders 8 times longer than those prepared without carrot extracts. Overall, our findings highlighted the potential use of supercritical CO_2 to recover bioactive compounds from carrot pomaces and develop powder microcapsules with enhanced oxidative stability by PGSS technique.

1. Introduction

In recent years, the demand for nutritive and healthy foods is drastically increasing. This trend is leading the food industry to carry out more research with the aim to find out natural extracts that, combined with the use of innovative encapsulation technologies, may produce stable ingredients.
Natural extracts are compounds occurring in foods, by-products and plants, which possess antioxidant and antimicrobial attributes. Even though synthetic antioxidants have been used commonly in food industry, due to their safety and risks for human health, natural extracts are considered a competitive alternative to be (Simran et al., 2019). They gain their antimicrobial and antioxidant power due to the presence of a variety of compounds such as simple phenols, phenolic acids, tocopherols, carotenoids, flavonoids, vitamins and anthocyanins.
Among the several sources of natural extracts, those derived from food by-products are gaining increasing attention. Carrot pomace, the by-product derived from the peeling and cutting of carrots for the production of juice or fresh-cut products, represents an important source of bioactive compounds with antioxidant power. The production of carrots worldwide account for more than 37 million tons per year. However, about 11 % of the

carrot is lost during the processing in different forms like peels, tubers and attached flesh (Ranalli et al., 2004; de Andrade et al., 2018). Consequently, there is a high interest to valorise these by-products and utilize the extracted compounds in food, chemicals, cosmetics, pharmaceutical and personal care products (de Andrade et al., 2019).
To preserve the functional properties of the extract, the choice of the technology used for the extraction is very important. Recently, eco-friendly extraction technologies, such as supercritical fluid extraction, are receiving increasing attention. Several solvents can be used in supercritical state. However, for the extraction of bioactive compounds supercritical carbon dioxide (CO_2) is the most used fluid. The reason is that it is abundant, non-flammable, nontoxic, inexpensive, and environmentally inert (Iovine et al., 2020). It dissolves nonpolar organic compounds with high efficiency. It has limitations in the solubility of ionic and polar compounds, which can be overcome by using low quantities of a surfactant or polar solvent. In the literature, published studies show that the technology can be successfully applied for the extraction of carotenoids from carrot pomace (de Andrade et al., 2018; 2019).
Besides the extraction, to fully exploit the functionality of the extracted compounds for food applications, studies are needed to identify the suitable encapsulation technologies able to preserve their bioactivity. The most common techniques applied for the encapsulation of bioactive compounds and oils are: freeze drying, spray drying, coacervation phase separation, and fluidized bed coating (Ndayishimiye et al., 2020; Ahmad et al., 2020). Among these methods, spray drying and freeze drying are the most used ones but they present some limitations such as agglomeration phenomena by the production of fine microcapsules, low protection of the encapsulated compounds, high production costs, long processing times or high-energy use just to list some (Bakry et al., 2016). To overcome these limitations, increasing attention are receiving technologies based on the use of supercritical fluids. One of the methodologies, which has been developed and improved during the last years, is the Particles from Gas Saturated Solutions (PGSS) technique. It is a valuable technique when wall materials soluble in CO_2 are used since the CO_2 dissolution causes the melting point reduction of these compounds. To obtain the encapsulated particles, the supercritical fluid is dissolved in a substrate, which makes the saturated solution. The solution is then rapidly expanded though the nozzle at atmospheric pressure. The rapid release of CO_2 causes an intensive cooling effect leading the formation of solids or liquid particles (Yun et al., 2013). Based on the above considerations, the aim of the present study has been to recover bioactive compounds from carrot pomace and evaluate their antioxidant capacity when used as ingredient to retard the oxidation of microencapsulated linseed oil produced by PGSS technique.

2. Materials and methods

2.1 Carrot pomace preparation

Fresh carrots were purchased from a local market. They were preliminary brushed and washed with water. Then, carrot juice was extracted by using a juice machine (Kenwood juice extractor, Bolzano, Italy) equipped with rotating blades. About 3 kg of carrots were processed to obtain 1.7 kg of juice and 1.3 kg of pomace. The carrot pomace was then dried using a lab-scale freeze drier (Epsilon 2-6D LSC plus freeze-drier, Martin Christ, Osterode am Harz, Germany). After the drying, the carrot pomace was milled with a hammer mill type reaching a particle size lower than 0.8 mm. The final moisture content of the sample was measured by a thermobalance (SARTORIUS Moisture Analyzer, Sartorius Lab Instrument, Germany) resulting equal to 17 ± 2%.

2.2 Extraction by supercritical carbon dioxide

The dried and milled carrot pomace was extracted by supercritical carbon dioxide. The system (Superfluid s.r.l., Padova, Italy) included an extractor and two separators. The extractor was built up with a reactor chamber of 1 liter capacity. Inside the reactor chamber, a stainless-steel extraction cell of 800 mL volume and a diameter of 50 mm was present. The sample was loaded in the extraction basket enclosed in the high-pressure stainless cylinder. To avoid moving of solid particles out of the cylinder, two stainless steel mesh were placed on both sides of the cylinder, which allowed only CO_2 to go through. To secure the reactor, a rubber ring gasket at the top was placed to avoid pressure losses and was sealed with a screwed cap. Thanks to a jacketed heater, the extractor was maintained at constant temperature. From a cylinder tank, the liquid CO_2 was sent to the high-pressure diaphragm pump, which was cooled to 4 °C by using a chiller (Euroklimat R407C, Siziano, Italy). Carbon dioxide was pumped into the high-pressure vessel flowing through the sample from the bottom to the top and interacting with the soluble compounds contained in the solid matrix. By opening the extractor exit valve, the stream exited the first unit and was sent to the separators where the extract was precipitated and collected. The extraction was carried out without the use of a co-solvent at 30 MPa and temperature of 60 °C with a CO_2

flow rate of 2 L/h for 120 min. At the end of the process, the extract was collected in amber flasks and weighted using an analytical balance.

2.3 Particles from gas saturated solution encapsulation

The antioxidant capacity of carrot extracts was tested on the oxidative stability of linseed oil encapsulated by the particles from gas saturated solutions (PGSS) technique using carbon dioxide as solvent. To perform the experiments, about 5 g of sunflower wax, chosen as wall material, was mixed with 1 g of linseed oil at room temperature. The sample was loaded into the high-pressure reactor having a capacity of 0.5 liter, which was previously heated at 65 °C. The system was carefully closed to prevent any gas leakage. Subsequently, CO_2 was pumped inside the vessel until the desired pressure was reached. The oil and the carrier material were left in the reactor at the set operative conditions of temperature and pressure for 30 min. The time was defined based on preliminary experiments assessing the minimum time needed to achieve the melting of the wall material. After this time, the depressurization was carried out by suddenly opening a needle valve. The sample was sprayed trough a nozzle of 600 nm and collected from the bottom part of a cyclone at ambient temperature and pressure. A first set of experiments was carried out to assess the effect of the operative pressure (10, 20, 30 MPa) on linseed oil encapsulation efficiency. In a subsequent set of experiments, carrot pomace extracts were added to the linseed oil (from 0.8 to 16 mg per gram of oil) to study the effect of the extract to protect linseed oil oxidation.

2.4 Encapsulation efficiency and yield

The yield was calculated by dividing the amount of the encapsulated powder, obtained at the end of the process, to the amount of sample loaded inside the vessel. The encapsulation efficiency was defined as the total amount of encapsulated oil divided by the total initial amount of oil (20% w/w), as given by the following equation:

$$EE\ (\%) = \frac{m_{encapsulated\ oil}}{m_{total\ oil}} \quad (1)$$

The amount of total oil of the particles was determined by accurately weighting 5 g of the sample on a filter paper. The sample was first washed with 150 mL of ethanol for three times to achieve the complete oil removal and then dried at 40°C until reaching a constant weight. The amount of total oil was determined by the difference in weight of the sample before and after extraction. The measurements were performed in triplicate and the results expressed as mean values and standard deviations.

2.5 Bulk and tapped density

For the measurement of the bulk density, 10 mL glass cylinder was fully loaded with the sample to have a uniform horizontal level. The weight of the required sample was recorded. Then, the bulk density was measured by calculating the ratio between the weight and the volume of the particles.
For the measurement of the tapped density, a 10 mL size glass cylinder was fully loaded of sample and the weight of the required sample was measured. Then, the cylinder was carefully tapped repeatedly by lifting and dropping it manually at a vertical distance of 10 cm until a constant volume was obtained. The tapped density was determined by calculating the ratio between the weight of the particles and the tapped volume. Measurements were performed in triplicate and the results expressed as mean values and standard deviation.

2.6 Flowability

The flowability of the particles was determined from the value of bulk and tapped densities and indicated as Carr index (CI) using the equation:

$$CI = \frac{\rho_{tapped} - \rho_{bulk}}{\rho_{tapped}} * 100 \quad (2)$$

2.7 Oxidative stability by isothermal calorimetry

To understand the effect of the encapsulation and the addition of carrot extracts, the oxidative stability of linseed oil was assessed by isothermal calorimetry. A micro-calorimeter (Thermal Activity Monitor, Model 421 TAM III, TA Instruments, USA) equipped with 24 micro-calorimetric chambers submersed in an oil bath was used. The analysis was performed by transferring of 200 ± 5 mg of the encapsulated powders into 4 mL glass ampules, which were then closed hermetically with silicon septa. The ampoules were first lowered into the thermal equilibration position and left there for 15 min. Then, they were positioned into the measurement place. The

heat flow developed during the oil oxidation was recorded for up to 3 days at 10-second intervals in isothermal conditions at 40 °C.

2.8 Statistical analysis

The results were statistically evaluated by an analysis of variance (ANOVA) using the XLSTAT software Version 2016.02.28014 (Addinsoft, New York, USA) in order to detect significant differences between values. The significant differences ($p < 0.01$) were analysed by Tukey test.

3. Results and discussion

3.1 Extraction from carrot pomace by supercritical carbon dioxide

The extraction by supercritical carbon dioxide was performed by adding 80 g of freeze-dried powdered carrot pomaces into the extractor. The processing conditions were chosen based on previous published studies (de Andrade et al., 2018). The temperature was set at 60 °C and the applied pressure was 30 MPa. The whole process was set up as a dynamic extraction. Therefore, CO_2 was circulated through the sample at a flow rate of 2 L/h (Ferrentino et al., 2018). Overall, the resulting amount of extract was equal to 1.09 g. The final yield, defined as the percentage of the ratio between the amount of dried extract and the amount of dried pomace used for the extraction, was equal to:

$$Yield\ (\%) = \frac{mass\ of\ extract}{mass\ of\ sample} \cdot 100 = \frac{1.09\ g}{80\ g} \cdot 100 \cong 1.4\% \quad (3)$$

3.2 Effect of pressure on the physical properties of samples encapsulated by particles from gas saturated solutions

Linseed oil was encapsulated into microcapsules by PGSS technique. At this purpose, sunflower wax was used as wall material. The ratio of oil to wall material used for the formation of the particles was equal to 1:5. The temperature was set at 65 °C and the process time to 30 min. The encapsulation process was carried out at 10, 20 and 30 MPa to investigate the effect of the pressure on the physical properties of the powders such as yield, encapsulation efficiency, bulk and tapped densities, flowability and oxidative stability of linseed oil.

Table 1 shows the results of the physical properties of the obtained samples. By increasing the pressure from 10 to 30 MPa, the encapsulation efficiency decreased. This effect was explained by considering the changes in the solubility of both the wall material and the oil in supercritical CO_2. Processing at high pressures promoted a higher solubilization of the oil in the CO_2, which induced a consequently higher separation of the oil from the wall material during the depressurization and micronization steps. As concerns the bulk and tapped densities, both variables decreased with the pressure showing the same trend observed for the encapsulation efficiency. In general, at lower encapsulation efficiencies, a lower amount of oil was retained inside the particles leading to a lower mass and consequently to a lower density. From the bulk and tapped densities, the Carr index was also calculated. Such index takes into account the flowability properties of a powder. Values of Carr index lower than 15 % indicate excellent flow behavior properties, while values higher than 25% are associated to powders with poor flowability. The results revealed that the pressure had a significant effect on the Carr index. Indeed, the particles obtained at 10 and 20 MPa reported a Carr index lower than 25 % thus showing a good flowability behavior.

Table 1: Physical properties of samples obtained by particles from gas saturated solutions at different pressures. Different letters between rows indicate significant differences ($p < 0.01$).

Pressure (MPa)	Yield (%)	Encapsulation efficiency (%)	Bulk density ($g \cdot cm^{-3}$)	Tapped density ($g \cdot cm^{-3}$)	Carr index (%)
10	91.6 ± 3.3^a	92 ± 2^a	0.28 ± 0.04^a	0.32 ± 0.05^a	12.5 ± 0.5^a
20	92.4 ± 3.4^a	87 ± 1^b	0.23 ± 0.01^b	0.28 ± 0.04^b	17.8 ± 0.8^b
30	93.2 ± 3.4^a	86 ± 3^b	0.18 ± 0.02^c	0.24 ± 0.02^c	25.1 ± 0.3^c

3.3 Oxidative stability of linseed oil encapsulated by particles from gas saturated solutions

The oxidative stability of linseed oil encapsulated at different pressures was studied at 40 °C by isothermal calorimetry. The samples were loaded in closed ampoules and the heat produced during the oxidation was

recorded. Figure 1 shows the heat flow curves obtained from the oxidation of linseed oil and the powdered samples as a function of the time. For comparison, in the same graph the behavior of the oil not encapsulated is also reported. For all the samples at the beginning of the storage, the value of the heat was negligible. Then, the signal increased sharply showing an exothermic peak. The onset time, defined as the time at which the heat flow signal started to increase, provided a simple and direct measurement of the end of the sample stability and the beginning of the propagation step of the oxidation reaction. The results showed that linseed oil oxidized in about 5 hours generating an important exothermic peak. However, when the oil was encapsulated in sunflower wax by PGSS technique a delay of the oxidation was observed based on the shift of the time at which the onset time occurred. The results also showed that the oxidation of the encapsulated linseed oil was dependent on the pressure applied for the PGSS process. In details, the powders prepared at 10 MPa showed an oxidative stability 2 times longer than those prepared at 30 MPa**.** This difference was explained by the values obtained for the encapsulation efficiency as a function of the pressure. As shown in Table 1, higher pressures led samples with a lower encapsulation efficiency and, consequently, a higher percentage of oil exposed to the air and easily prone to oxidation.

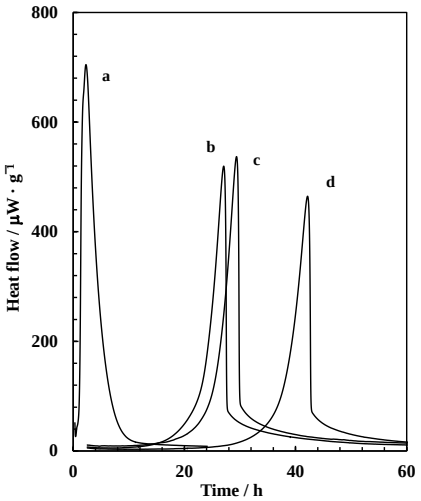

Figure 1: Effect of PGSS encapsulation on the oxidative stability of linseed oil as a function of the processing pressures: a, linseed oil; b, linseed oil encapsulated at 30 MPa; c, linseed oil encapsulated at 20 MPa; d, linseed oil encapsulated at 10 MPa

3.4 Effect of addition of carrot extract on the oxidative stability of linseed oil encapsulated by particles from gas saturated solutions

In this paragraph, the effect of the addition of carrot extract on the oxidation of the encapsulated linseed oil was investigated. The samples were produced at 10 MPa by PGSS with the addition to the oil of different concentration of carrot extracts. The produced powders were then loaded in glass ampoules and the oxidation was monitored at 40 °C by isothermal calorimetry. The results, shown in Figure 2, reported the heat flow curves of the microcapsules obtained with the addition of increasing concentration of carrot extract (from 0.01 to 0.3%) to linseed oil (1 g) and sunflower wax (5 g). The effect of the presence of carrot extract in the formulation was to retard the occurrence of the onset time and the peak signal (time at which the maximum heat flow was recorded) of the heat flow curves. From Figure 2, it is evident that for the control sample (curve a, powder without the addition of carrot extract) the oxidation started after almost 25 hours. When the carrot extract was added (0.8 mg/ g of oil), the onset time occurred after about 36 hours later. At higher concentrations of carrot extracts added (16 mg/ g of oil), the onset appeared after 46 h. Interestingly, the peak time was linearly correlated with the concentration of carrot extract added to the micronized formulation (see inset of Figure 2, R^2 =0.97).

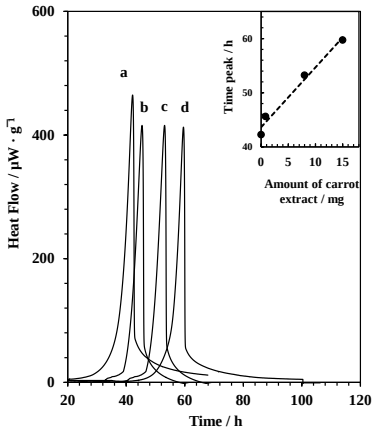

Figure 2: Effect of the addition of increasing amount of carrot extracts on the oxidative stability of encapsulated linseed oil (a): 0.8 mg/ g of oil (b), 8 mg/g of oil (c), 16 mg/g of oil (d).

4. Conclusions

The aim of this study was to recover bioactive compounds from carrot pomace by supercritical CO_2 and to assess the antioxidant capacity of the obtained extracts to retard the oxidation of linseed oil encapsulated by PGSS technique. The efficiency of the technique was assessed by applying three different pressures. The result showed that an increase of the pressure from 10 to 30 MPa decreased the oil encapsulation efficiency from 91.68 % at 10 MPa to 86.23 % at 30 MPa. A similar trend was also observed for the protection of the oil from oxidation. Indeed, microcapsules produced at 30 MPa had a faster oxidation compared to those prepared at 10 MPa. Moreover, the samples produced at 10 MPa and formulated with carrot extracts enhanced linseed oil oxidative stability. Indeed, the addition of carrot extracts (0.3 %) induced an improved oxidative stability to the powders 8 times longer than those prepared without carrot extracts. In conclusion, the results highlighted the potential use of supercritical CO_2 to recover bioactive compounds from carrot pomaces and develop microcapsules with enhanced oxidative stability by PGSS technique.

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