**Fractionation**

Fractionation was carried out using a laboratory-scale SC-CO2 system (Thar Model SFE-500F-2, Thar Technologies Inc., Pittsburgh, PA, USA) that consisted of a high pressure CO2 pump, heat exchanger, automatic back pressure regulator, three 500 cm3 stainless-steel vessels (raffinate, first and second fractionation columns) and a thermostated circulating bath (Thermo Scientific K10 model, Karlsruhe, Germany). Approximately 100 g of melted milkfat was placed into the raffinate vessel for each process. The fractionation schemes performed in this study are presented below. Three sequential processes were followed for the fractions using same milk fat.

|  |  |  |  |
| --- | --- | --- | --- |
| Process  | Pressure (bar) | Process time (hr) | Number of sample |
| Run 1  | 100 bar | 6 | 5 |
| Run 2  | 150 bar | 6 | 5 |
| Run 3  | 200 bar | 6 | 6 |

The fractions were continuously collected every 1 h for 6 h at each pressure. For run 1, the pressure inside the raffinate vessel was maintained at 90 bar for 30 min to allow sufficient contact of the milkfat with the supercritical CO2. It was then kept at 100 bar for the first fractionation run by using an automated back pressure regulator. Afterwards, the pressure in the first fractionation vessel was adjusted to 50 bar, at which point a valve between the first and second vessels was opened along with a vent to keep the pressure and CO2 flow constant.

Fractions were collected hourly from the bottom of the first vessel at the operational pressure. The procedure was repeated with pressures of 150 and 200 bar. Each run was completed in 6 h at a 50 mL/min CO2 flow rate. In all, sixteen fractions plus raffinate were collected at 40 °C from the same starting batch sample. Rate of fractionation, fatty acid composition and melting behavior were determined for fractions.