# The Potential of Supercritical Fluid Extraction to Produce Specialty Oil and Meal Products from Canola

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## Introduction

Supercritical $CO_2$ (SC-$CO_2$) technology has been widely investigated for the processing of fats and oils over the past two decades as an alternative to conventional processes involving organic solvents since it is an environmentally friendly process resulting in solvent-free extracts and residues. In light of the latest rapid growth in the field of functional foods and nutraceuticals, this technology has received renewed interest for the extraction and fractionation of nutraceuticals, including bioactive lipids, on a commercial level since it provides a solvent-free, "natural" product, which has a wide consumer appeal.

A supercritical fluid is at temperature and pressure conditions above its critical point. The critical point defines the highest temperature and pressure at which gas and liquid phases can coexist. Above the critical point, a fluid exhibits physicochemical properties in between those of liquids and gases and becomes an attractive solvent due to its high density like a liquid but faster mass transport capacity due to intermediate viscosity and diffusivity. Carbon dioxide has been the solvent of choice for food applications due to its inert, non-toxic, non-flammable and environmentally friendly nature and moderate critical point (31$^{o}$C, 7.4 MPa). It is readily available in high purity and low cost. Processing in $CO_2$ environment minimizes any undesirable changes due to oxidation. As well, degradation of heat labile components are minimized at processing temperatures just above ambient.

A supercritical fluid extraction process can be carried out by solubilizing the solute at temperature and pressure conditions above the critical point followed by depressurization to recover the solute. Carbon dioxide becomes a gas upon depressurization and loses its solvent properties; thus the solute separates and $CO_2$ gas is pressurized and recycled. Thus, the solvent can be separated with ease and there is no solvent residue in the extract or the solids residue. To maximize extraction efficiency and optimize process design, it is important to have a good understanding of the solubility behaviour of the target solute in SC-$CO_2$ as a function of operating parameters. A recent review by Temelli and Guclu-Ustundag [1] provides a critical analysis of the solubility behaviour of lipid components in SC-$CO_2$ and the implications for extraction and fractionation processes for fats and oils. In general, solubility of lipids in SC-$CO_2$ is determined by $CO_2$ density, which is a strong function of temperature and pressure. A high $CO_2$ density means high solvent power leading to higher solubility of lipids and thus higher extraction yield. Solubility is also determined by solute properties. Solutes of higher vapour pressure and lower molecular weight can be solubilized to a greater extent in SC-$CO_2$. In addition, because $CO_2$ is classified as a non-polar solvent, it is selective for non-polar solutes such as the triacylglycerols that make up a significant portion of fats and oils.

Extraction of oilseeds and specifically canola oil [2-9] using SC-$CO_2$ has been reported in numerous studies in the 80s and 90s. These studies demonstrated the impact of various processing parameters such as temperature, pressure, $CO_2$ flow rate, initial moisture content of feed material, particle size and initial treatment of feed material on the solubility, yield and composition of the oil obtained. In addition, a two-step process was developed where the neutral triacylglycerols were extracted by SC-$CO_2$ first followed by the recovery of polar phospholipids using SC-$CO_2$+ethanol mixture in the second step [7].

With the latest government restrictions on the use of hexane as the traditional solvent for oilseed processing, the industry has been investigating alternative solvents such as ethanol and isopropyl alcohol. SC-$CO_2$ extraction of oilseeds is a viable alternative even though commercial scale processing of oilseeds with SC-$CO_2$ has not been realized yet in spite of the high volume of research carried out. Despite the fact that the overall process based on SC-$CO_2$ extraction would be simpler compared with conventional hexane extraction in terms of eliminating the need for hexane evaporators and meal desolventizer, the high equipment costs associated with the SC-$CO_2$ process and the inability to achieve continuous processing of high volumes of oilseeds have been cited as the major impediments to commercialization of the SC-$CO_2$ process. Therefore, the common belief is that the commercial applications of SC-$CO_2$ technology should be restricted to high-value end products. However, recent developments in equipment and process design have demonstrated that the economics of the SC-$CO_2$ process become favourable at large enough capacities for oilseed processing [10]. In addition, equipment manufacturers are putting in extensive effort to develop continuous processing equipment that couples SC-$CO_2$ extraction with expeller technology. In fact, one equipment manufacturer has developed a pilot system that injects high pressure $CO_2$ into the screw press. Such a press was not tested for canola processing. It is well known that SC-$CO_2$ extracted oil is lighter in color and does not contain phospholipids compared to hexane-extracted oil, requiring less refining and thus reducing refining costs. However, sensory quality of SC-$CO_2$ extracted canola oil has not been reported previously. Therefore, the objectives of this study were to test the new press with $CO_2$ injection for canola, evaluate the chemical composition of canola oils obtained by different techniques and to evaluate the sensory quality of SC-$CO_2$ extracted canola oil in comparison to oil obtained by conventional hexane extraction and refining.

## Materials and Methods

### *Materials*

A summary of the canola oil samples used in this study is presented in Table 1. All oils were extracted from canola flakes provided by a major oilseed processor. Conventional refined oil, stripper, decanter and cold pressed oils were provided by the same oilseed processor. $CO_2$-assisted pressing was conducted at Crown Ironworks, Minneapolis, MN. Supercritical carbon dioxide extraction was conducted at Norac Technologies, Edmonton, AB a division of Newlyweds Foods Inc. SC-$CO_2$ extraction runs were carried out at 300 bar and temperatures ranging from 36 to 59$^{o}$C. The canola oil extract was recovered in two separators, with the first separator set at 120 bar/56$^{o}$C and the second one operating at 49 bar/21$^{o}$C. Thus, the oil was collected in the first separator (referred to as the high fraction) whereas the lighter volatile compounds and water were collected in the second separator (referred to as the low fraction). The yields of the two fractions between the different runs were similar. For chemical analysis of the extracts, the high fraction alone and a combination of the two fractions were analyzed to reflect the total extract obtained by SC-$CO_2$ extraction. The high fraction alone was used for sensory evaluation.

Table 1. Canola oil samples and their descriptions

| # | Sample Name | Sample Description |
|---|---|---|
| 1 | Stripper oil | Hexane-extracted, some desolventization |
| 2 | Decanter oil | Pressed oil, some clarification |
| 3 | Cold Pressed oil* | Pressed, degummed and dewatered |
| 4 | Conventional refined oil* | Refined canola oil |
| 5 | $CO_2$-assisted pressing of press cake | $CO_2$ at 138 bar |
| 6 | $CO_2$-assisted pressing of canola flakes | $CO_2$ at 138 bar |
| 7 | $CO_2$-assisted pressing of canola flakes | $CO_2$ at 207 bar |
| 8 | SC-$CO_2$ Runs 1&2 High Fraction | Extracted at 300 bar, 43$^oC$ |
| 9 | SC-$CO_2$ Runs 3&4 High Fraction* | Extracted at 300 bar, 51$^oC$ |
| 10 | SC-$CO_2$ Runs 5&6 High Fraction * | Extracted at 300 bar, 59$^oC$ |
| 11 | SC-$CO_2$ Runs 7&8 High Fraction | Extracted at 300 bar, 36$^oC$ |
| 12 | SC-$CO_2$ Runs 1&2 High & Low Fractions | Extracted at 300 bar, 43$^oC$ |
| 13 | SC-$CO_2$ Runs 3&4 High & Low Fractions | Extracted at 300 bar, 51$^oC$ |
| 14 | SC-$CO_2$ Runs 5&6 High & Low Fractions | Extracted at 300 bar, 59$^oC$ |
| 15 | SC-$CO_2$ Runs 7&8 High & Low Fractions | Extracted at 300 bar, 36$^oC$ |

*Food-grade samples evaluated by trained panel and analysed by gas chromatography to characterise volatile compounds

*Chemical Composition Analysis*

Volatiles in the headspace of the three oils used in sensory evaluation (cold pressed, conventional refined and SC-$CO_2$ extract) were analyzed by GC-MS.

Peroxide value was determined according to AOCS Official Method Cd 8-53 [11]. Samples were analyzed immediately after receipt from the processor.

Free fatty acid content of the different oil samples was determined according to AOCS Official Method Ca 5a-40 [11]. Samples were analyzed immediately after receipt from the processor.

Sterols were analysed by a modified method of Mounts et al. [12]. An aliquot (0.05 g) of each oil was weighed to 1 mg accuracy into test tubes. Then, 0.5 ml of 50 % w/w KOH, 2 ml of 95% (v/v) ethanol and 1 ml ethanol containing 2.5 mg dihydrocholesterol internal standard were added to each test tube. Samples were heated at 70$^oC$ for 1 hr. To each test tube, 15 ml cyclohexane and 5 ml deionized water were added, samples were vigorously mixed and then centrifuged at 1000 rpm for 10 min. The cyclohexane layer was washed with deionized water until it was neutral and evaporated at room temperature under a stream of nitrogen. To each sample, 0.25 ml each of pyridine and Sylon BFT silyation agent were added and incubated at 70$^oC$ for 30 min. Silyated samples were diluted with hexane and immediately analyzed by gas chromatography.

*Sensory Evaluation by Trained Panel*

Twelve panellists were selected and trained for 12 one-hour sessions to assess flavour, texture and appearance attributes of canola oil following standard protocols. Flavour and aroma profiles for SC-$CO_2$ extract (high fractions of runs 3-6 were combined), cold-pressed and conventional refined canola oils were developed. Oil samples (10 ml each) were heated in capped 16 ml amber glass vials in a 60°C water bath for 10 min before presenting to panellists. Participants were instructed to open the vials while holding them under their noses. They then took three short sniffs and evaluated the aroma attributes of the sample. Oil flavour was evaluated by taking half of the amount of the oil in the vials (about 5 ml) into the mouth, swishing for 5 sec followed by inhaling through the mouth and exhaling through the nose to enhance the perception of volatile aroma compounds. Panellists were instructed not to swallow the samples. Participants cleansed their palates with water, crackers and warm water (55°C) in between test samples. For visual assessments, oil samples were placed in clear plastic petri dishes to a depth of 1 cm and presented under controlled lighting conditions. Colour and lightness/darkness of the oils were evaluated.

All attributes were evaluated on 15 cm unstructured line scales anchored with the cues "Not at all (attribute)" on the left end and "Very (attribute)" on the right end. Reference standards were presented for each of the attributes being evaluated and were anchored on their respective line scales. Trained panel evaluation was replicated three times.

*Statistical Analyses*

Analysis of variance of data was performed at $\alpha=0.05$ using SAS Statistical Software and means were compared using Tukey's multiple comparisons test where significant differences were found.

## Results and Discussion

*Chemical Composition Analyses*

Peroxide value (PV) and free fatty acid (FFA) content of the different canola oil samples studied are presented in Table 2. SC-$CO_2$-extracted and $CO_2$-assisted pressed oils had higher PV and FFA values, indicating that these oils may be less stable than cold pressed and conventional refined canola oils. Canadian standards for fresh refined canola oil is a maximum of 0.05% free fatty acid (by weight) and a maximum peroxide value of 2.0 [13]. As shown in Table 2, the conventional refined canola oil tested within these standard values. For crude canola oil, the maximum acceptable amount of free fatty acid is 1.0% [13]. As expected, free fatty acid content of the SC-$CO_2$ oil that contained both high and low fractions was higher than that of just the high fractions since the more volatile free fatty acids are collected in the second separator.

The higher peroxide value of the $CO_2$-assisted press and SC-$CO_2$-extracted oils may be explained by the fact that these oils have not been refined or treated to increase stability. Yet, cold pressed, decanter and stripper canola oils, which are not further refined, had relatively low peroxide values. The oils were either frozen or analyzed upon receipt, thus any degradation type reactions were minimized prior to analysis. However, some degradation may have occurred during shipping and handling. During $CO_2$-assisted pressing and SC-$CO_2$ extraction, oxidation reactions are minimized in the presence of $CO_2$. Extreme care is needed in handling the oil once it leaves the press or the extractor. It is well known that exposure to oxygen, light, heat and enzymes can promote oxidation reactions, especially in oils containing high levels of polyunsaturated fatty acids.

Sterol analysis results are presented in Table 3. Of the oil samples tested, sterol content was lowest ($p\leq0.05$) in conventional refined canola oil. The stripper, cold pressed, and most of the SC-$CO_2$-extracted oils had significantly higher total and individual sterol contents compared

Table 2. Free fatty acid and peroxide value results

| Sample | FFA (as % oleic acid) | PV (meq peroxide/ kg oil) |
|---|---|---|
| Stripper Oil | 1.23[b] | 3.58[g] |
| Decanter Oil | 0.78[f] | 3.78[fg] |
| Cold Pressed Oil | 0.89[e] | 3.52[g] |
| Conventional Refined Oil | 0.02[h] | 1.39[h] |
| $CO_2$-assisted pressing, Cake Oil (138 bar) | 1.09[cd] | 6.57[d] |
| $CO_2$-assisted pressing, Flake oil (138 bar) | 1.10[c] | 6.67[d] |
| $CO_2$-assisted pressing, Flake oil (207 bar) | 1.14[c] | 17.17[b] |
| SC-$CO_2$ Runs 1&2 High Fraction | 1.13[c] | 6.67[d] |
| SC-$CO_2$ Runs 3&4 High Fraction | 1.02[d] | 5.45[e] |
| SC-$CO_2$ Runs 5&6 High Fraction | - | - |
| SC-$CO_2$ Runs 7&8 High Fraction | 0.91[e] | 3.61[fg] |
| SC-$CO_2$ Runs 1&2 High & Low Fractions | 1.30[a] | 8.57[c] |
| SC-$CO_2$ Runs 3&4 High & Low Fractions | - | - |
| SC-$CO_2$ Runs 5&6 High & Low Fractions | - | - |
| SC-$CO_2$ Runs 7&8 High & Low Fractions | 1.13[c] | 4.29[f] |

[a-g] Means within a column followed by the same letter are not significantly different ($p>0.05$).

Table 3. Results of sterols analyses of canola oil extracted by different methods

| Sample | Sterols (mg/kg) | | | | |
|---|---|---|---|---|---|
| | Brassicasterol | Campesterol | Stigmasterol | β-Sitosterol | Total |
| Stripper Oil | 1071.4[a] | 2966.1[abc] | 58.7 | 4993.0[a] | 9089.1[a] |
| Decanter Oil | 917.5[b] | 2656.0[bcd] | 30.3 | 4421.5[ab] | 8025.2[ab] |
| Cold Pressed Oil | 999.2[ab] | 3146.7[abc] | 62.0 | 5312.1[a] | 9519.7[a] |
| Conventional Refined Oil | 610.1[c] | 2111.8[d] | 0.0 | 3542.8[b] | 6264.7[b] |
| $CO_2$-assisted pressing, Cake Oil (138 bar) | 921.8[ab] | 2655.4[bcd] | 32.1 | 4313.7[ab] | 7923.0[ab] |
| $CO_2$-assisted pressing, Flake oil (138 bar) | 934.8[ab] | 2690.6[bcd] | 34.0 | 4358.0[ab] | 8017.4[ab] |
| $CO_2$-assisted pressing, Flake oil (207 bar) | 929.4[ab] | 2569.8[cd] | 32.2 | 4362.9[ab] | 7894.2[ab] |
| SC-$CO_2$ Runs 1&2 High Fraction | 929.2[ab] | 3252.0[abc] | 15.2 | 4903.7[ab] | 9100.1[a] |
| SC-$CO_2$ Runs 3&4 High Fraction | 855.3[b] | 2964.4[abc] | 19.3 | 4347.5[ab] | 8186.4[ab] |
| SC-$CO_2$ Runs 5&6 High Fraction | 956.5[ab] | 3317.7[ab] | 0.0 | 5021.8[a] | 9295.9[a] |
| SC-$CO_2$ Runs 7&8 High Fraction | 909.8[b] | 3275.9[abc] | 0.0 | 4851.7[ab] | 9037.3[a] |
| SC-$CO_2$ Runs 1&2 High & Low Fractions | 944.2[ab] | 3334.0[ab] | 0.0 | 4964.6[a] | 9242.7[a] |
| SC-$CO_2$ Runs 3&4 High & Low Fractions | 943.0[ab] | 3284.9[ab] | 0.0 | 4924.4[ab] | 9152.3[a] |
| SC-$CO_2$ Runs 5&6 High & Low Fractions | 962.2[ab] | 3310.9[ab] | 0.0 | 4948.9[ab] | 9222.0[a] |
| SC-$CO_2$ Runs 7&8 High & Low Fractions | 932.4[ab] | 3350.9[ab] | 0.0 | 4946.1[ab] | 9229.4[a] |

[a-d] Means within a column followed by the same letter are not significantly different ($p>0.05$).

to those of the conventional refined oil. This was expected since these oils were not refined. Up to 40% of sterols are removed during the refining process, particularly during the deodorizing step [14]. However, only the brassicasterol levels of the $CO_2$-assisted pressed oils were different from the conventional refined oil.

Literature sterol values [14] indicate that normal levels found in crude canola oil are between 4500 and 11300 mg of total sterols/kg; for individual sterols, levels in crude canola oil are as follows: brassicasterol 225-1469 mg/kg, campesterol 1111-4361.8 mg/kg, stigmasterol 9-113 mg/kg and β-sitosterol 2029.5-6542.7 mg/kg. Sterol levels of all oil samples analysed in this study were within these ranges.

In the conventional and SC-$CO_2$-extracted oils, most volatile compounds were found to be aldehydes. Compounds that were found in both of these oils included pentanal, hexanal, heptanal, octanal and nonanal. Additionally, 2-heptenal was identified in the SC-$CO_2$-extracted oil. For the cold pressed oil however, few aldehydes were found and long chain n-alkanes (C21-C29) were identified.

*Sensory Evaluation by Trained Panel*

Figure 1 presents a spider plot depicting the trained panel results for all the attributes assessed. Significant differences ($p \leq 0.05$) were found among the three evaluated oils for all attributes. Compared to conventional refined canola oil, SC-$CO_2$-extracted canola oil was more intense in all attributes except buttery aroma and flavour. Compared to cold pressed canola oil, SC-$CO_2$-extracted oil was less nutty and rubbery, but more intense in pickle aroma, mustard aroma and mustard flavour. In general, SC-$CO_2$ extracted oil was distinguished from the other two oils by sharp, distinct attributes such as pickle, pine and mustard aromas, and mustard and pine flavour. Although the cold-pressed canola oil was rated as having the strongest flavour and aroma intensities, SC-$CO_2$ extracted oil was also rated significantly more intense than conventional refined canola oil in these attributes. For appearance attributes, conventional refined canola oil was rated as light and yellow, SC-$CO_2$-extracted oil was darker and orange, and cold pressed oil was darkest and orange-brown.

The higher amounts of free fatty acids (Table 2) of SC-$CO_2$-extracted canola oil may account for the stronger intensity of pickle and mustard attributes of this oil compared to cold pressed and conventional refined oils as evaluated by the trained panel. Based on the trained panel data, it was expected that cold pressed canola oil would have higher amounts of low molecular weight volatile compounds since it was evaluated to have very intense attributes. However, that was not the case based on the chemical composition analysis, which also showed the presence of long chain alkanes and further work is needed to establish the correlations between sensory and chemical analysis.

**Conclusions**

Chemical composition analysis and sensory evaluation of canola oils obtained by conventional pressing, hexane extraction and refining were compared to those obtained by SC-$CO_2$ extraction and $CO_2$-assisted pressing. As expected, characteristics of the SC-$CO_2$-extracted oil were in between those of cold pressed and refined oils. $CO_2$-assisted pressed oil had higher free fatty acid and peroxide values but similar total sterol content compared to the cold pressed oil. Further work is needed to better characterize these oils and assess their consumer acceptability.

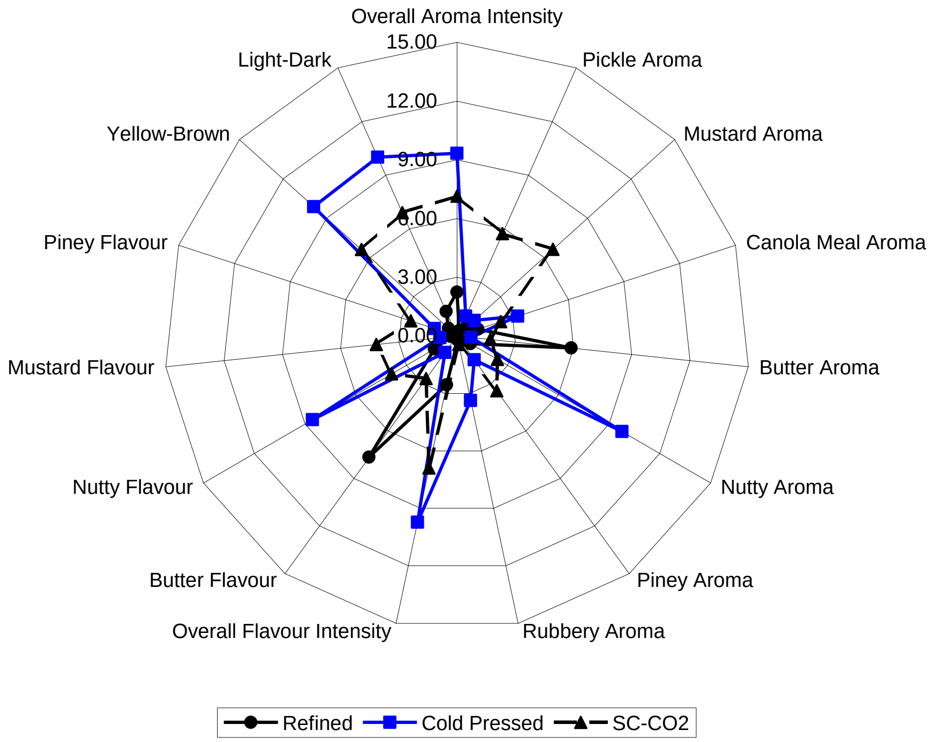


Figure 1. Spider plot of trained panel evaluations of canola oil extracted by three methods

## References


[1] Temelli, F. and Guclu-Ustundag, O. 2005. Supercritical fluid technology for fats and oils processing. In "Bailey's Industrial Oil and Fat Products", 6th Edition. F. Shahidi (ed.), John Wiley & Sons, Inc., New Jersey. Ch. 10, Vol. 5, pp. 397-432.

[2] Bulley, N.R., Fattori, M., Meisen, A., and Moyls, L.. Supercritical Fluid Extraction of Vegetable Oil Seeds (1984). J. Am. Oil Chem. Soc. 61(8):1362-1365.

[3] Fattori, M., Bulley, R.N., and Meisen, A. Fatty Acid and Phosphorous Contents of Canola Seed Extracts Obtained with Supercritical Carbon Dioxide (1987). J. Agric. Food Chem. 35:739-743.

[4] Fattori, M., Bulley, R.N., and Meisen, A. Carbon Dioxide Extraction of Canola Seed: Oil Solubility and Solubility and Effect of Seed Treatment (1988). J. Am. Oil Chem. Soc. 65(6):968-974.

[5] Lee, A.K.K., Bulley, N.R., Fattori, M., and Meisen, A. Modeling of Supercritical Carbon Dioxide Extraction of Canola Oilseed in Fixed Beds (1986). J. Am. Oil Chem. Soc. 63(7):921-925.

[6] Temelli, F. 1992. Extraction of triglycerides and phospholipids from canola with supercritical carbon dioxide and ethanol. J. Food Sci. 57(2):440-442, 457.

[7] Dunford, N. and Temelli, F. 1995. Extraction of phospholipids from canola with supercritical carbon dioxide and ethanol. J. Am. Oil Chem. Soc. 72(9): 1009-1015.

[8] Dunford, N.T. and Temelli, F. 1996. Effect of supercritical $CO_2$ on myrosinase activity and glucosinolate degradation in canola. J. Agr. Food Chem. 44(8): 2372-2376.

[9] Dunford, N.T. and Temelli, F. 1997. Extraction conditions and moisture content of canola flakes as related to lipid composition of supercritical $CO_2$ extracts. J. Food Sci. 62(1): 155-159.

[10] Chordia, L. 2000. Proceedings of the 5th International Symposium on Supercritical Fluids. Paper 5416516.

[11] AOCS, Official Methods and Recommended Practices of the American Oil Chemists Society, Fourth Ed., Vol. 1, 1989.

[12] Mounts TL, Abidi SL, Rennick KA. 1996. Effect of genetic modification on the content and composition of bioactive constituents in soybean oil. J Am. Oil Chem. Soc. 73: 581-586.

[13] Canola Council of Canada. 2005. Canola standards and regulations. http://www.canola-council.org/PDF/Standards1-2.pdf#zoom=100. (Accessed 09/06/05).

[14] Ratnayake WMN, Daun JK. 2004. Chemical composition of canola and rapeseed oils *in* Rapeseed and canola oil. Ed. Gunstone FD. Blackwell Publishing and CRC Press.