COSMOS

ADDING VALUE TO CAMELINA AND CRAMBE OIL

Camelina & crambe **O**il crops as **S**ources for **M**edium-chain **O**ils for **S**pecialty oleochemicals

(Grant Agreement No. 635405)

D 4.1 C18-C22 monounsaturated FA samples

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1 Introduction

Long- and medium-chain monounsaturated fatty acids (MUFA) are important raw materials for lubricants, plasticisers, surfactants and detergents industries. An important objective of the COSMOS project is to produce oils rich in certain MUFA using as starting material refined camelina and crambe oils obtained from seeds. One of the approaches to further increase the level of certain MUFA in the oils is to use physical fractionation processes. As a 'side stream' of these 'enrichment processes', oil fractions enriched in polyunsaturated fatty acids (PUFA) are also obtained.

Although native camelina oil is a 'drying oil' rich in PUFA (particularly linoleic and linoleic acid), we are interested in obtaining enriched gondoic acid (C20:1n9) oil. This less common MUFA has potential as a feedstock for certain oleochemical applications.

For crambe oil we focus on its main MUFA, erucic acid (C22:1n9), which is already present in high levels of about 55–60% of total fatty acids. In COSMOS, we develop processes to obtain oil fractions with even higher levels of erucic acid.

In order to obtain the enriched oils, two approaches are being followed in COSMOS: the first one is using enzymatic methods and the second one, described in this report, is using physical processes to reduce the level of non-desired fatty acids, as well as fractionation techniques that result in oil fractions enriched in either MUFA or PUFA.

All the necessary processes to perform a physical separation have been developed at Solutex. Vegetable oils are predominantly composed of triglycerides (TG). Since different fatty acids are attached to the glycerol backbone of TG, the first step necessary is to detach the fatty acid chains from the glycerol backbone. The method chosen to achieve this is transesterification with ethanol, resulting in fatty acid ethyl esters (FAEE). Once the fatty acids are released as FAEE, different fractionation/extraction techniques can be applied to obtain enriched fractions.

For certain downstream processes, free fatty acids (FFA) are preferred over ethyl esters. Therefore, the obtained MUFA-enriched FAEE fractions were also processed into FFA through enzymatic hydrolysis.

2 Materials and Methods

All the chemical reagents used in the technical processes are approved by Solutex Quality Control Department. The analytical methods used for the oil analysis are part of the European Pharmacopoeia (current version).

The oils "camelina refined oil" and "crambe refined oil" were extracted from the seeds and purified (refined, bleached and deodorised) by the subcontracted party OLEAD as part of COSMOS project work package 4 (Task 4.1), and used as starting material to obtain the deliverable samples (D4.1). The main physicochemical parameters of both oils were determined to establish whether any further refining operations were necessary. The analysis results are shown in Table 1, which includes oligomers, partial glycerides profile and oxidation parameters (Anisidine and Peroxide values and TOTOX), humidity and acid value. Table 2 includes fatty acid profiles of the two refined oils.

Table 1: analytical results of camelina and crambe refined oil (glycerides profile, water Karl-Fischer, unsaponifiable matter, oxidation parameters and acid value)

	CAMELINA REFINED OIL	CRAMBE REFINED OIL
Oligomers (%Area)	0.26	0.19
Triglycerides (%Area)	98.17	98.06
Diglycerides (%Area)	1.00	0.72
Monoglycerides (%Area)	<0,1	0.28
Total glycerides (%Area)	99.17	99.06
Ethyl Esters (%Area)	0.57	0.75
Water K-F (mg/kg)	411	252
Unsaponifiables (%)	0.94	0.78
Peroxide value (meq O_2/Kg)	1.5	0.75
Anisidine value	8.3	2.2
TOTOX	11.3	3.7
Acid value (mg KOH/g)	0.54	0.26

Table 2: analytical results of camelina and crambe refined oil (Fatty acids profile)

		CAMELINA REFINED OIL	**CRAMBE REFINED OIL**
Name	Fatty acid	Composition (%Area)	Composition (%Area)
Lauric	C12:0	0.00	0.00
Myristic	C14:0	0.05	0.06
Palmitic	C16:0	5.21	1.77
Palmitoleic	C16:1 n7	0.08	0.11
Hexadecaenoic	C16:4n1	0.00	0.00
Stearic	C18:0	2.38	0.67
Oleic	C18:1n9	14.17	14.93
	C18:1n7	0.98	0.58
Linoleic	C18:2n6	14.97	7.83
Linolenic	C18:3n3	35.45	5.15
Stearidonic	C18:4 n3	0.00	0.00
Arachidic	C20:0	1.41	0.81
Eicosenoic	C20:1 n9	15.77	2.25
Arachidonic	C20:4 n6	1.37	0.00
ETA	C20:4 n3	0.00	0.00
EPA	C20:5n3	0.00	0.00
Behenic	C22:0	0.29	1.80
	C22:1n11	3.37	2.04
Erucic	C22:1n9	0.00	58.62
	C21:5n3	0.00	0.00
	C22:5n6	0.46	0.15
DPA	C22:5n3	0.00	0.00
DHA	C22:6n3	0.00	0.00
Lignoceric	C24:0	0.20	0.54
Nervonic	C24:1n9	0.64	1.11

3 Transesterification and fractionation

3.1 Transesterification of triglyceride-rich camelina and crambe oils to the respective fatty acid ethyl esters.

In order to separate the different fatty acids and carry out the subsequent purification or fractionation, it is necessary to break the glyceryl-ester bonds of the TG. For this purpose, a chemical transesterification was performed using ethanol and a basic catalyst to obtain three FAEE and a glycerol from each triglyceride of the refined oil (Figure 1).

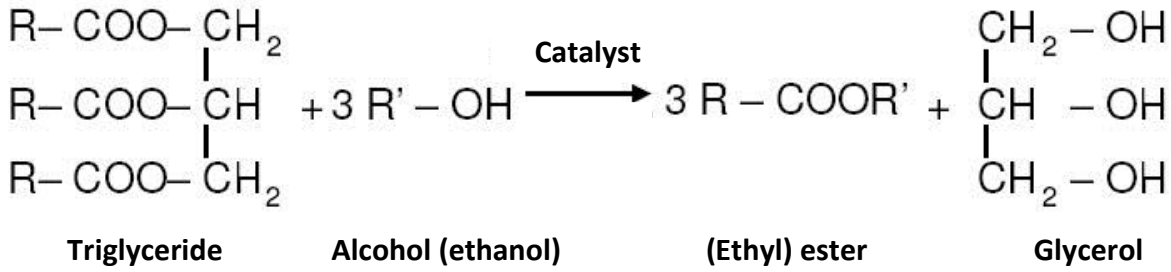

Figure 1: general transesterification reaction. In parenthesis, specific case of obtaining ethyl esters.

An optimization and scale-up of the process was carried out in the laboratory, using camelina and crambe refined oils. The biggest batch consisted of 36 kg of camelina oil and 40 kg of crambe oil.

According to the analysis results, no preceding refining operations were considered necessary. Reaction conversions (% of FAEE) and yields (expressed as kg FAEE/kg TG*100) were very high, as shown in Table 3, which describes the overall result of the esterifications performed during the scale-up:

Table 3: global results for camelina and crambe refined oil esterifications.

	Conversion (%FAEE)	Yield (%weight)
Camelina	97.85	100
Crambe	97.90	97

3.2 Processing routes for obtaining oils enriched in gondoic or erucic acid.

Using as starting material camelina or crambe oil as FAEE, different series of trials were developed to determine the most suitable processing routes to obtain oil enriched in gondoic/erucic acid and other oil fractions.

The available technologies tested were the following ones:

- *Short-path molecular distillation*: it constitutes a separation process for a liquid mixture, using selective evaporation and condensation. In this case, distillation takes place inside the evaporator, making shorter the distance between the evaporator and the chiller. This aspect improves molecular interaction -and therefore distillation-, and allows very short contact times. The pilot plant works under high vacuum conditions, which reduces boiling point and allows working with lower temperatures that minimize the degradation risk in the oils.
- *High vacuum continuous fractional extraction* (HVCFE): it constitutes a form of distillation in which the degree of separation is higher than in a simple distillation. In this process, the product undergoes a series of distillation steps in a column to match the desired fractionation.
- *Urea complexation*: it constitutes a selective complexation process of most of the saturated and monounsaturated fatty acids that take part from the starting oils, through a formation of adducts with urea in ethanol solution. In solution with ethanol, urea can form inclusion complexes with linear aliphatic compounds, but not with branched compounds. These inclusion compounds are combinations of two molecules, and one contains in its crystalline structure to another.

  The stability of the complexes increases when the chain length is larger, and is less effective when the number of double bonds is higher. For a certain chain length, urea forms complexes preferably with saturated, then with monounsaturated, and finally with polyunsaturated fatty acids or esters. The effectivity of urea complexation depends on the temperature, the urea/oil ratio, and the position and number of double bonds.

Another available technology is *supercritical fluid extraction* (SFE) using carbon dioxide as a solvent. This solvent becomes supercritical above 31°C and 74 bar, when it shows properties of both a liquid and a gas. The extraction selectivity depends on the polarity and volatility of the compounds. SFE has not been used, for reasons explained in the sections below.

The main oil parameters have been analysed throughout the process to obtain the target samples. The analytical methods used are those collected in the European Pharmacopoeia.

The results on how a specific delivered sample was obtained are explained below. When the process is repeated, the yields, conversions and concentrations can undergo small variations.

Isolation of gondoic acid enriched oil samples

The starting camelina oil contains 15.77±2.36% of gondoic acid (C20:1n9). After subjecting the corresponding FAEE to urea complexation, short path distillation and high vacuum continuous fractional extraction techniques, it was found that the best choice for concentrating this fatty acid was the last one (i.e. HVCFE). SFE was not considered for various reasons: the short path distillation results, the composition of the starting material and the previous experience of the company showed that the distillation results were not going to be improved with SFE technique. Using HVCFE, a concentration of 45.10±6.76% of gondoic acid was achieved in one fraction and the other is rich in PUFA. The process yield for the concentrated gondoic acid fraction is 46%.

The gondoic acid rich fraction obtained had a dark colour and the oxidation parameters were high. A short path molecular distillation was applied in order to improve the appearance of the sample. The distillate obtained with 72% yield (wt%) was yellowish and had the same gondoic acid composition (45.39±6.81%), but the oxidation parameters were still high.

A bleaching process using active earths was performed in order to reduce the anisidine and peroxide values and therefore improving the sample quality. The yield of this step was 90%. The concentration of gondoic acid of the product (Camelina oil EE gondoic) was 46.00±6.90%. The analytical results can be found in section 3.3.

The free fatty acid (FFA) form of the gondoic-enriched oil was preferred in some applications. Therefore, a final step was necessary to transform the FAEE to FFA, for which an enzymatic hydrolysis was used. This reaction was performed with *Candida antarctica* lipase and demineralized water. The conversion to FFA was 82% and the process yield 70%. The concentration of gondoic acid in this final sample was 50.46±7.57% (see section 3.3 for analytical results)

All the intermediates obtained during the process were analysed, controlling the main parameters of each process (fatty acid profile, glyceride profile, oxidation, acid value, water content). During the optimization experiments and before the final analysis performed by the QC laboratory, the fatty acid profile was analysed; the conditions modulated –if required; and the goal achievement was duly checked.

Figure 2 shows the process roadmap to obtain the deliverable samples rich in gondoic acid and denominated as follows:

- Gondoic-enriched camelina FAEE
- Gondoic-enriched camelina FFA

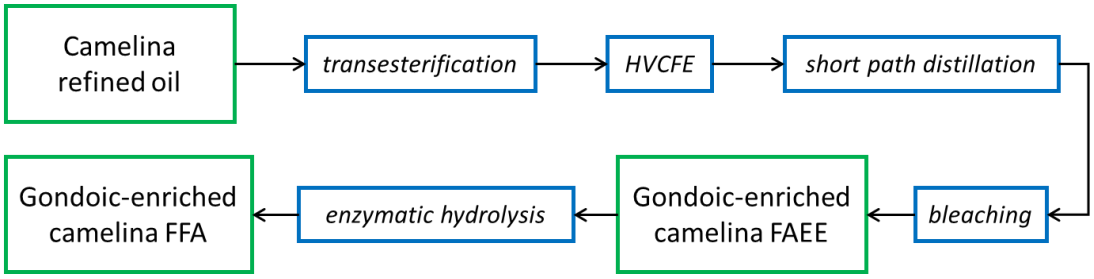

Figure 2: process roadmap to obtain the deliverable samples of oil concentrated in gondoic acid using as starting material camelina refined oil.

The general description and analytical results of the final samples are given in section 3.3.

Isolation of erucic acid enriched oil samples

The crambe oil starting material contains 58.62±8.79% of erucic acid (C22:1n9). Three technologies were tested to concentrate the targeted erucic acid ethyl ester: urea complexation, SFE and short path molecular distillation. The third one showed good results by obtaining an erucic acid EE concentration of 77.33±11.60% in the residue. The process yield for this fraction is 49% (see section 3.3 for analytical results). SFE can match this enrichment in technical terms, but the short path molecular distillation is more convenient in economics terms. HVCFE can also match the enrichment but not the productivity, oxidation parameters and appearance of the oil obtained by this distillation.

In this case, the oxidation parameters had acceptable values and a bleaching was not necessary.

An enzymatic hydrolysis was carried out under the same conditions as for gondoic acid EE to convert the FAEE oil to FFA oil. The conversion to FFA was 79% and the process yield 72%.

All products obtained during the process were analysed, controlling the main parameters for each process. The general description and analytical results of the final samples are given under section 3.3.

Figure 3 shows the process roadmap of erucic enriched products. These products are named as follows:

- Erucic-enriched crambe FAEE
- Erucic-enriched crambe FFA

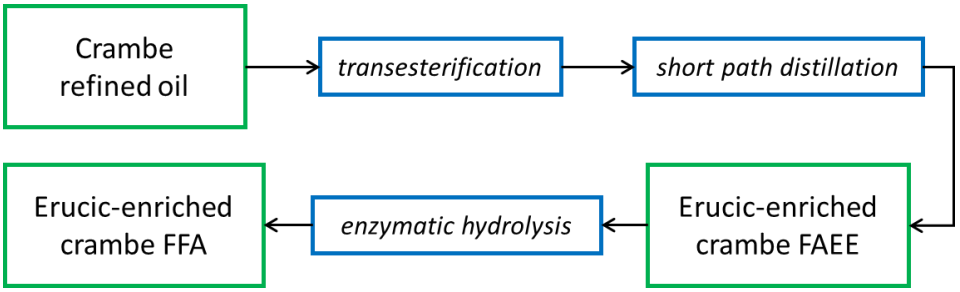

Figure 3: process roadmap to obtain the deliverable samples of oils concentrated in erucic acid using crambe refined oil as starting material.

3.3 Delivered samples

Gondoic acid enriched oil samples: *Camelina oil EE Gondoic* and *Camelina oil FFA Gondoic*

The sample "Camelina oil EE Gondoic" (gondoic-enriched camelina FAEE) is slightly yellow to brownish yellow and liquid at room temperature. "Camelina oil FFA Gondoic" (gondoic-enriched camelina FFA) has a similar colour but is solid at room temperature. Both are almost insoluble in water and miscible with acetone, ethanol, heptane and methanol. In Figure 4 both samples are shown.

Figure 4: pictures of "Camelina EE Gondoic" on the left and of "Camelina FFA Gondoic" on the right.

The samples were analysed to ensure their quality and the chemical composition and main parameters of oil stability evaluation were checked. In the case of the FFA sample, the water content and the acid value were also analysed (the last one in order to check the conversion to the acid form). These data are shown under Tables 4 and 5:

Table 4: analytical results of the delivered samples “camelina oil EE gondoic” and “camelina oil FFA gondoic”. (glycerides profile, water Karl-Fischer, oxidation parameters and acid value)

	CAMELINA OIL EE GONDOIC	CAMELINA OIL FFA GONDOIC
Oligomers (%Area)	<0,1	<0.1
Triglycerides (%Area)	<0.1	1.29
Diglycerides (%Area)	1.36	3.35
Monoglycerides (%Area)	3.05	<0.1
Total glycerides (%Area)	4.41	4.64
Ethyl Esters (%Area)	95.59	95.36*
Water K-F (mg/kg)	N.A.	669
Peroxide value (meq O_2/Kg)	1.16	0.36
Anisidine value	28.7	28.6
TOTOX	31.02	29.32
Acid value (mg KOH/g)	N.A.	164.50

N.A.=not analysed, *FAEE + FFA (%Area)

Table 5: fatty acids profile of the delivered samples "camelina oil EE gondoic" and "camelina oil FFA gondoic" (measurement uncertainty = 15%)

		CAMELINA OIL EE GONDOIC	**CAMELINA OIL FFA GONDOIC**
Name	Fatty acid	Composition (%Area)	Composition (%Area)
Lauric	C12:0	0.00	0.00
Myristic	C14:0	0.00	0.00
Palmitic	C16:0	0.00	0.00
Palmitoleic	C16:1 n7	0.00	0.00
Hexadecaenoic	C16:4n1	0.00	0.00
Stearic	C18:0	4.60	4.28
Oleic	C18:1n9	4.66	4.25
	C18:1n7	0.46	0.57
Linoleic	C18:2n6	2.08	1.61
Linolenic	C18:3n3	8.27	6.12
Stearidonic	C18:4 n3	0.09	1.03
Arachidic	C20:0	4.45	4.12
Eicosenoic	C20:1 n9	46.00	50.46
Arachidonic	C20:4 n6	1.60	0.00
ETA	C20:4 n3	0.03	0.19
EPA	C20:5n3	0.00	0.00
Behenic	C22:0	1.03	0.87
	C22:1n11	10.76	11.90
Erucic	C22:1n9	0.08	0.00
	C21:5n3	0.95	0.00
	C22:5n6	0.95	0.06
DPA	C22:5n3	0.72	0.00
DHA	C22:6n3	0.00	0.00
Lignoceric	C24:0	0.00	0.57
Nervonic	C24:1n9	0.00	0.00
% saturated		10.08	9.84
% monounsaturated		61.96	67.18
% polyunsaturated		14.69	9.01

Erucic acid enriched oil samples: *Crambe oil EE erucic* and *Crambe oil FFA erucic*

The sample "Crambe oil EE Erucic" (erucic-enriched crambe FAEE) is slightly yellow to brownish yellow and liquid at room temperature. "Crambe oil FFA Erucic" (erucic-enriched crambe FFA) has similar colour but is solid at room temperature. Both are almost insoluble in water and miscible with acetone, ethanol, heptane and methanol. Figure 5 shows both samples:

Figure 5: pictures of "Crambe oil EE Erucic" on the left and of "Crambe oil FFA Erucic" on the right

The samples were analyzed to ensure their quality and their chemical composition and the main parameters of oil stability evaluation were checked. In the case of the FFA sample, the water content and the acid value were also determined (the last one in order to check the conversion to the acid form). These data are shown in Tables 6 and 7:

Table 6: analytical results of the delivered samples "crambe oil EE erucic" and "crambe oil FFA erucic". (glycerides profile, water Karl-Fischer, oxidation parameters and acid value)

	CRAMBE OIL EE ERUCIC	**CRAMBE OIL FFA ERUCIC**
Oligomers (%Area)	<0.1	<0,1
Triglycerides (%Area)	1.01	0.69
Diglycerides (%Area)	2.96	<0,1
Monoglycerides (%Area)	1.62	0.44
Total glycerides (%Area)	5.59	1.13
Ethyl Esters (%Area)	94.41	98.87*
Water K-F (mg/kg)	N.A.	362
Peroxide value (meq O_2/Kg)	3.16	0.56
Anisidine value	0.3	5.7
TOTOX	6.62	6.82
Acid value (mg KOH/g)	N.A.	157.7

N.A.=not analysed, *FAEE + FFA (%Area)

Table 7: fatty acids profile of the delivered samples "crambe oil EE erucic" and "crambe oil FFA erucic" (measurement uncertainty = 15%)

		CRAMBE OIL EE ERUCIC	**CRAMBE OIL FFA ERUCIC**
Name	Fatty acid	Composition (%Area)	Composition (%Area)
Lauric	C12:0	0.00	0.00
Myristic	C14:0	0.00	0.00
Palmitic	C16:0	0.33	0.24
Palmitoleic	C16:1 n7	0.00	0.05
Hexadecaenoic	C16:4n1	0.00	0.06
Stearic	C18:0	0.30	0.23
Oleic	C18:1n9	5.58	3.25
	C18:1n7	0.14	0.10
Linoleic	C18:2n6	2.90	1.67
Linolenic	C18:3n3	2.06	1.10
Stearidonic	C18:4 n3	0.00	1.89
Arachidic	C20:0	0.81	0.77
Eicosenoic	C20:1 n9	1.97	1.29
Arachidonic	C20:4 n6	0.15	0.13
ETA	C20:4 n3	0.00	0.00
EPA	C20:5n3	0.00	0.54
Behenic	C22:0	2.72	3.43
	C22:1n11	0.00	0.00
Erucic	C22:1n9	77.33	78.58
	C21:5n3	0.00	0.08
	C22:5n6	0.23	0.00
DPA	C22:5n3	0.04	0.00
DHA	C22:6n3	0.00	0.00
Lignoceric	C24:0	0.00	0.00
Nervonic	C24:1n9	0.00	0.00
% saturated		4.16	4.67
% monounsaturated		85.02	83.27
% polyunsaturated		5.38	5.47