

A Modern Dewaxing Technology For Edible Oils Refining

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Abstract

The modern dewaxing process using endogenous wax ester hydrolyses (wax desynthetase) activation in optimal reaction conditions provides an efficient, specific and targeted affinity destructure process orientated towards the wax substrate in order to develop the deparaffinage effect. The lipase W/O interfacial activation was studied in the lipolyse and interesterification process but the endogenous O/S dewax esterase activation was until now non-investigated. The isoparaffins structures formation improves the dewaxing yield at 90.7% reported on the miscella crude oil with 270 ppm waxes content with an almost double-cold dewaxed oil stability.

The present modern dewaxing technology eliminates the low-efficiency cold/low temperature crystallisation and all the negative effects given by the solvent oil recovery from the kieselgur pomace, cooling agent producing and separation by filtration/centrifugation process.

Key words: Oils, refining, dewaxing, biotechnology, dewax esterase

Introduction

Edible oils are commercial products manufactured after refining the crude oil obtained by solvent extraction, mechanical-hydraulically total/partial pressing or combined methods. The industrial oil extraction with solvents (S: n-hexane, benzene, toluene etc.) due to a total botanical oil (O) transfer by molecular diffusion from the oilseeds raw oily material into a miscella phase. The bi-phasic miscella dispersion O/S includes minor compounds solvated from the raw material in lower content than in the pre-pressed oil (Vintilă, 2003). The refining process aims to remove all classes of minor compounds from the crude oil obtained in the solvent extraction, pre-pressing or total pressing process. The chemical classes of minor compounds identified in crude oils are non-acylglycerols-type such as gums, resins, free fatty acids (FFA), water, waxes, heavy metals, colorants and odorant compounds (Vintilă, 2003; Dijkstra, 2012).

Edible oil waxes are usually high molecular weight compounds with high melting point (Dijkstra, 2012), esters of long-chain fatty acids C24-C28 (Carelli, 2002) with long-chain alcohols C16-C36 or steroids. The sunflower oil waxes have usually 36 to 42 atoms of carbon, FFA especially C20-C22 and fatty alcohols especially C24-C26 (Ramos and Rodriguez, 1985); (Mariani and Fedeli, 1989); (Liu et al., 1996).

The oil waxes are minor compound present in crude oils in variable content ranging from 950 to 1090 ppb in industrial sunflower oil (Carelli, 2002) and their presence affects the clarity and cold stability of the commercial oils.

The edible oil waxes were transferred especially from the oilseed hulls and the modern agronomical technologies involve a reducing of the hull content at 5-8% for soya and 20% in case of sunflowers seeds. Also, after the hulling process, the hull content in the oily cake is under the optimal technological limit of 5-7 % which corresponds to a maximum level of 1000 ppb wax content in the crude oil. The oil solvent extraction contributes to the reducing of waxes content in the crude oil and the winterisation in the miscella phase creates an optimal medium for the dewaxing process (Vintilă, 2003).

The conventional dewaxing process is based on the 3 step-deep cooling process of the degummed, neutralised and bleached oils, usually conducted until the oil reach the waxes crystallisation temperature of 5-7⁰ C, maintained 3 hours for waxes crystal maturation (crystal growing & stabilization). The natural or kieselgur-induced waxes cold crystallisation involves an important usage of cooling agent (ethylene glycol, demineralised cooled water) and a specific usage of crystallisation additive such kieselgur.



In the dewaxed complex obtained in the conventional edible oil refining by cold crystallisation with kieselgur seeds, the separated solid matter is composed from crystallised fatty acids and other non-triacylglycerol compounds with negative effects on clarity, brightness and cold stability of commercial edible oils. Other new industrial solutions, such as biological deacidifying, solvent extraction, and membrane separation were imagined in the last decade in order to solve the cost-efficiency problems in the edible oil industry (Subramanian et al. 2004).

The oil industry is well-known as one of the most energy-intensive section of the economic world-wide economy and the thermal central unit from the oil plant must be doubled by a frigorific central unit necessary for the classical winterisation (dewaxing) which affects negatively the economic efficiency of the edible oil processing. Also, an important non-ecological effect could be noted in case of classical dewaxing and chemical refining process.

The edible oil physical refining mean to eliminate the chemical effluents and to realize the deacidifying / neutralization and deodorization process by fractional distillation, usually at 170-190⁰ C and 1-10 mm col Hg. The physical refining involves high pressure steam using and high level of vacuum which due to expensive refining costs. Also, undesirable side-compounds such as free-fatty acids as trans-isomers are reported to be formed (Sengupta and Bhattacharyya, 1992) and other valuable natural bio-protectors, such as tocopherols and carotenoides, are destroyed or thermal denaturated in some edible oils (Ooi et al., 1996).

The miscella refining was a common method applied, in this moment, exclusively for the refining of cottonseed oil (Hodgson, 1996) because of excellent commercial oil quality and good cost-efficiency (Wan et al., 1996). The solvent (85% acetone and 15% hexane) cold winterization was described (Morisson and Robertson, 1975) to have better results at low temperature (under 0⁰ C) and low S/O concentration (20-40%).

A physical refining process conducted with phospholipase A₁ from *Aspergillus oryzae* was proposed (Patent US 20040005399 A1, 2004), followed by a classical low temperature dewaxing. Also, a deacidifying with microbial lipase was investigated in order to increase the final product commercial quality and to make more efficient the technical and economical refining process (Sengupta and Bhattacharyya, 1992).

The esterification process of the free fatty acid with monoglycerides and microbial lipase from *M. Miehei* was investigated (Sengupta and Bhattacharyya, 1996) but with limited results because the final free acidity of the neutralized oil is commercially unacceptable, 2–4% respectively.

An enzymatic degumming process was proposed (De Smet Ballestra Patent) by using DSM's Purifine[®] PLC (phospholipase C) with specificity action on phospholipids (phosphatidylcholine (PC) and phosphatidylethanolamine (PE)).

An efficient pre-treatment followed by an effective physical refining could solve the refining problems in case of rice brain oil (Manjula and Subramanian, 2009). The simultaneous degumming, dewaxing and decolourization/bleaching using conventional chilled rice brain oil (CRBO) associated with NTGS-2200 membrane separation prove a low cost-efficiency industrial processing because due to the waxes separation yield of 39.4% (Table 1).

Table 1. Simultaneous degumming, dewaxing and decolourization of CRBO using NTGS-2200 membrane (Manjula and Subramanian, 2009)

Description	Phosphorus (mg/kg)	Wax (%)	Colour
Feed	249.0 ± 3.5	1.02 ± 0.17	14.2
Permeate	12.0 ± 1.4	0.50 ± 0.03	6.8
PR (%)	95.2	51.0	52.1
Feed	170.6 ± 4.4	0.66 ± 0.04	16.8
Permeate	1.2 ± 0.3	0.40 ± 0.03	7.5
PR (%)	99.3	39.4	55.3



The present paper research will conduct two research objectives:

1. Innovative dewaxing technology describing;
2. The comparative analysis of technological performance (dewaxing yield and dewaxed oil quality evaluated by the cold test results) in conventional cold dewaxing (CD) and innovative dewaxing (ID).

Materials and methods

Miscella Samples. The dewaxing process was analysed in case of high-oleic sunflower crude oil samples with 75% oleic acid content and up to 5% saturated acids in the triacylglycerols native structures. The 25% oil concentration miscella in n-hexane (O/S) has the quality characteristics specific for the product obtained from the continuous belt solvent extraction plant Desmet with a 125 MTPD capacity.

The ID and CD refining. The conventional refining of sunflower edible oil was presented in figure 1 and the cold dewaxing (CD) process in figure 2. The CD involves 3-steps of degummed, neutralized and bleached oil deep-cooling until the waxes crystallisation point of 5-7⁰ C was reached (four hours), in the presence of winterization germs (kieselgur 1%, w/w at oil basis) followed by the waxes maturation step, conducted three hours at 5-7⁰ C.

The oil innovative dewaxing (ID) by using a biotechnological physical refining was presented in figure 3. The oil endogen esterase increased activity optimal range was as followed:

- Temperature: 40-45⁰C;
- pH 7.0-7.5;
- Deparaffinase activation time: 90 minutes.

The solvent, free fatty acids (FFA) and organic alcohols were distillate in the fractioning process, all along with others volatile minor compounds such as residual water, free fatty acids, odorant compounds etc.

The crude oil waxy material is proposed to be enzymatically deconstructed into hydrocarbons, sterols, aldehydes, alcohols, diols, ketones and other volatile compounds, fractional distillation at 190-200⁰C, 10 mm col Hg for 20-30 minutes in an industrial vacuumed distillation column (Fig. 3).

Wax analysis. The wax content of crude and dewaxed edible oil were determined with a GC Clarus 580 equipment using ISO/DTS 23647:2010 (reviewed and confirmed in 2017) gas chromatographic method for determining the wax content of crude vegetable oils coupled at a computer device equipped with PC Software version 6.3.2.7(ISO, 2010). The experimental conditions for standardised waxes content determination were presented in Table 2.

The K response factor was calculated with the equation (1), as followings:

$$k = \frac{A_{HTC} \cdot C_{C44}}{A_{C44} \cdot C_{HTC}} \quad (1)$$

Where A_{HTC} represents the standard (control) peak surface value, A_{C44} the wax experimental peak surface value, C_{HTC} represents the HTC control concentration (mg/ml) and C_{C44} represents the experimental concentration of C44 waxes (mg/ml).

Usually, the K response factor has value between 1.0 and 1.2 for the winterised oil.

The edible oil wax content was calculated according with the equation (2).

$$W = (1 + K) \cdot \frac{(A_{C44} + A_{>C44}) \cdot m_{HTC}}{A_{HTC}} \cdot \frac{K}{m_i} \quad (2)$$

Where W represents the wax content, A_{C44} represents the C44 waxes experimental peak area, $A_{>C44}$ represents the area of waxes with over 44 carbon atoms in molecules peak area, A_{HTC}



represents the control peak area, m_{HTC} is the standard weight (μg) considered 300 μg , m_i is the sample weight (μg) and K the response factor.

Table 2. Experimental conditions for ISO/DTS 23647:2010 waxes content determination.

Technical specification	Characteristics
GC Equipment	GC, Clarus 580
GC column	HP (30 m x 0.32 mm x 0.1 μm)
Flow	2-3x10 ⁻⁶ L
Volume	370°C, FID
Dilution Factor	1.000000

The degree of waxes removal was calculated with the equation (3).

$$R = \frac{C_0 - C_F}{C_0} \cdot 100, [\%] \quad (3)$$

Where R was the dewaxing yield, C_0 represents the wax content of the crude oil (270 ppb), and C_F represents the wax content of the dewaxed high oleic sunflower oil. The cold test for the winterization efficiency evaluation was conducted by constantly maintaining the 10 cL oil samples at 5⁰C and expressed as number of hour in which the oil sample lost the clarity (AOCS, 1989).

Results and Discussions

The conventional oil refining process (Fig.1) implies two refining stages with tree unit operation in each stage: degumming, alkaline neutralization, soap washing/vacuum drying in the first refining stage, bleaching, dewaxing, deodorization in the second refining stage.

The phosphatidic and cephaline gum fractions are removed with water and chemical degumming reagents (10% citric or 85% phosphoric acid solutions) in the chemical refining. In the neutralization with caustic soda solutions, the saponificated FFA were removed as soapstock by centrifugation in two stages, followed by oil washing in order to reduce the residual soap content up to 5ppb.

In the second refining stage, the decoloring, winterization and deodorization processing are conducted until the standardised edible oil characteristics were obtained (Table 3).

Table 3. The quality characteristics of the dewaxed oil

Description	Unit	Standard value, max.	Standard ISO
Waxes	ppb	50	23647:2010
FFA	% oleic acid	0.1	660:2009
Water and volatile compounds	%	0.05	8534:2017
Relative density (%)	g/cm ³	0.918-0.923	6883:2017
Phosphorus	ppb	5	10540-1:2003
Insoluble impurities	ppb	0.05	663:2017

The CD process (Fig. 2) involves three stages of deep oil cooling until the final dewaxing temperature of 5-7⁰C was reached.



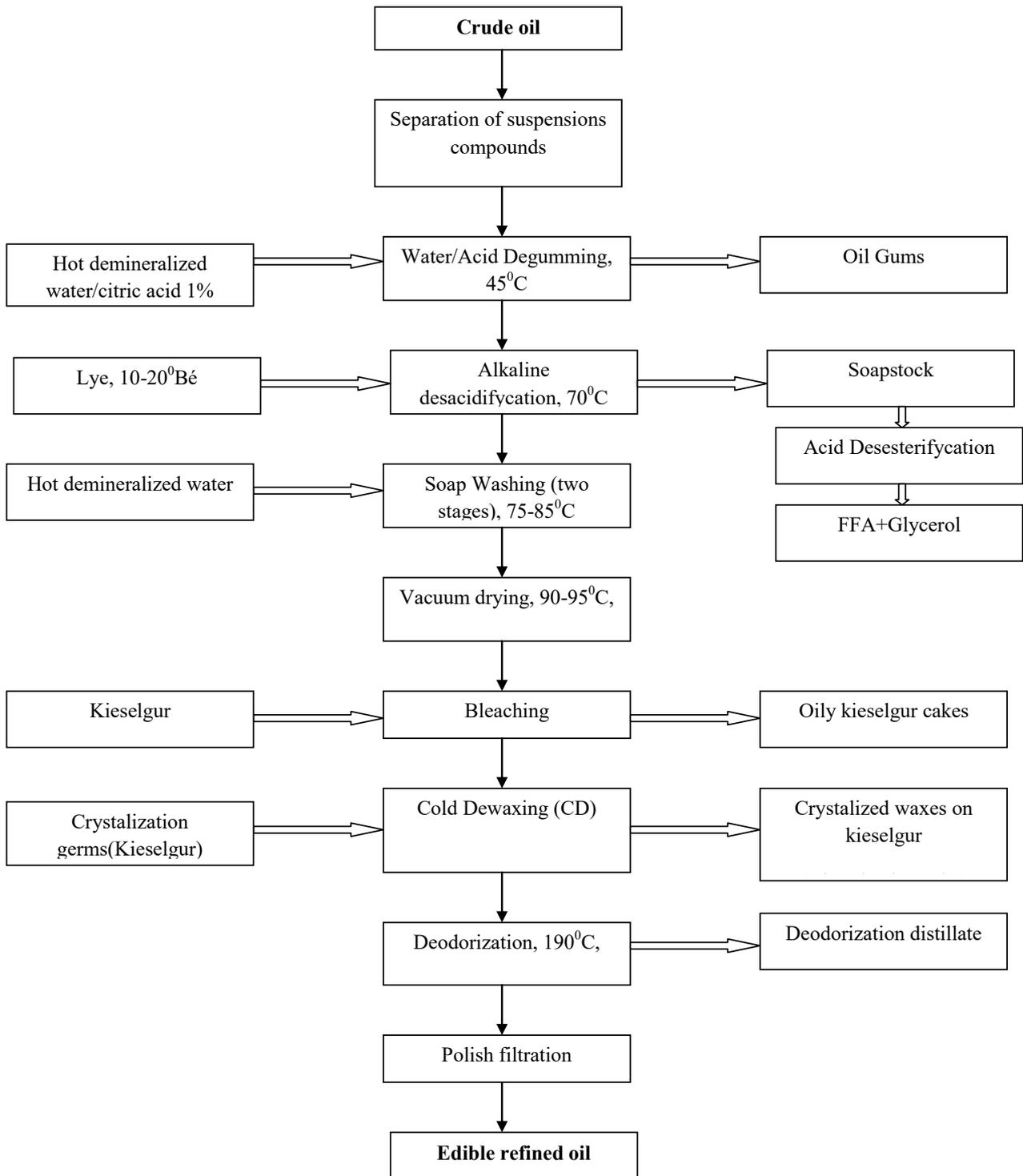


Fig. 1. Conventional edible oil refining process



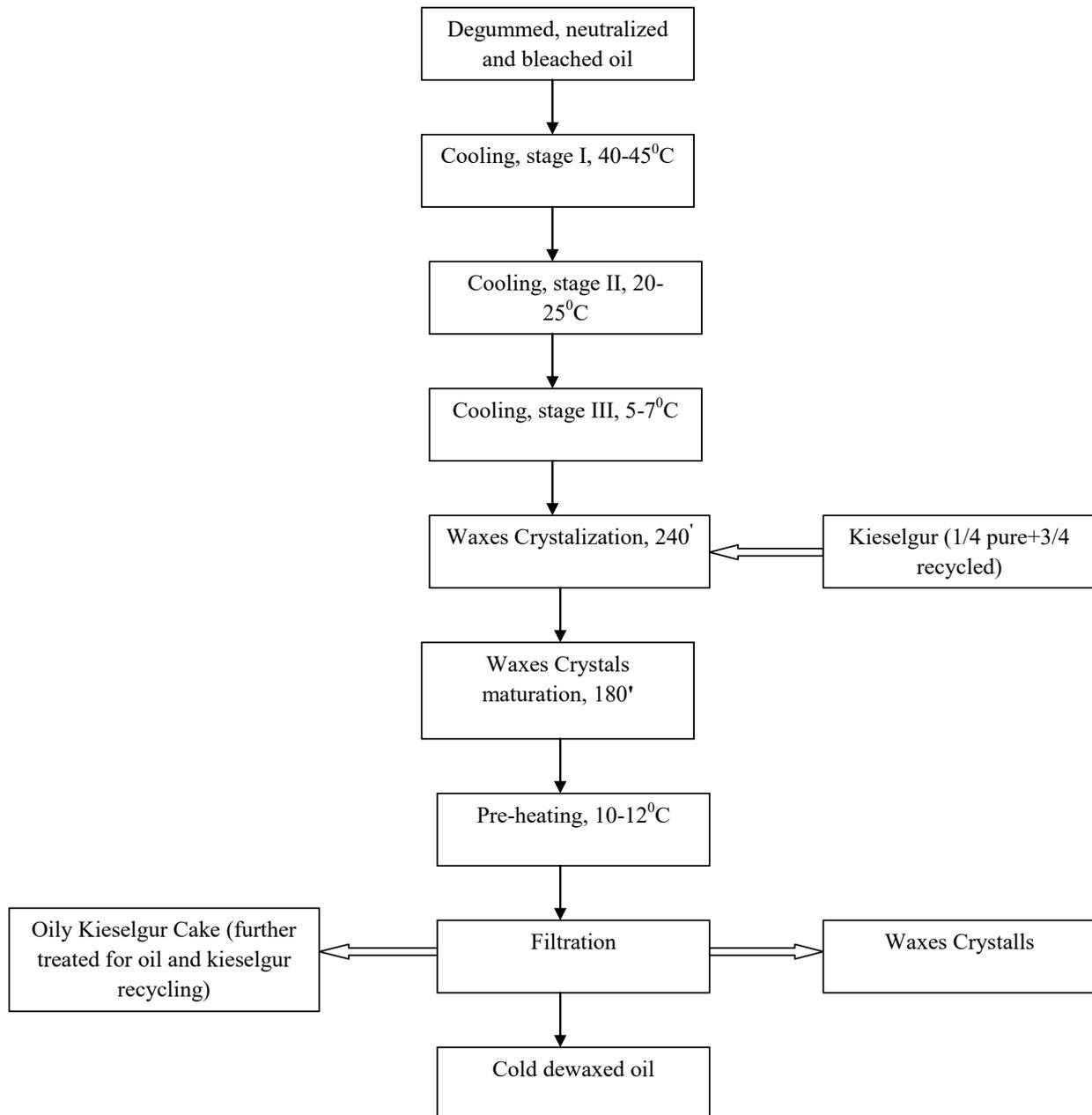


Fig.2. The conventional cold dewaxing (CD)

The aim of cold dewaxing was to realize a separation of the stearin fraction which crystallizes at the dewaxing temperature from the olein phase, as liquid phase at the same temperature.

The stearin phase was formed from all the minor compounds which are solid or reach the solid state in the cold test conditions.

In principal, waxes were separated in the deep cooling process but some other minor compounds and oil fractions could be lost in the process.

The oleine phase was the wax free phase and could be further processed for the odors compounds removal.

The CD process involves a great amount of energy consumed to reduce the primary refined oil from the initial temperature of 90-95°C to the crystallisation temperature of 5-7°C, constantly maintained for crystal formation and crystals maturation for 7 hours.

In addition, kieselgur as crystallisation additive, was used in proportion of 1% w/w, 1/3 fresh powder as oil suspension and 2/3 recycled kieselgur from the winterized cake.

The oil content in the winterized cake was about 50% and should be recovered and recycled by solvent extraction followed by the distillation process.

The CD schema was efficient in terms of waxes removals but the technological costs were substantially higher because of the heavy processing flow diagram and the quantity of energy involved in the conventional dewaxing technology.

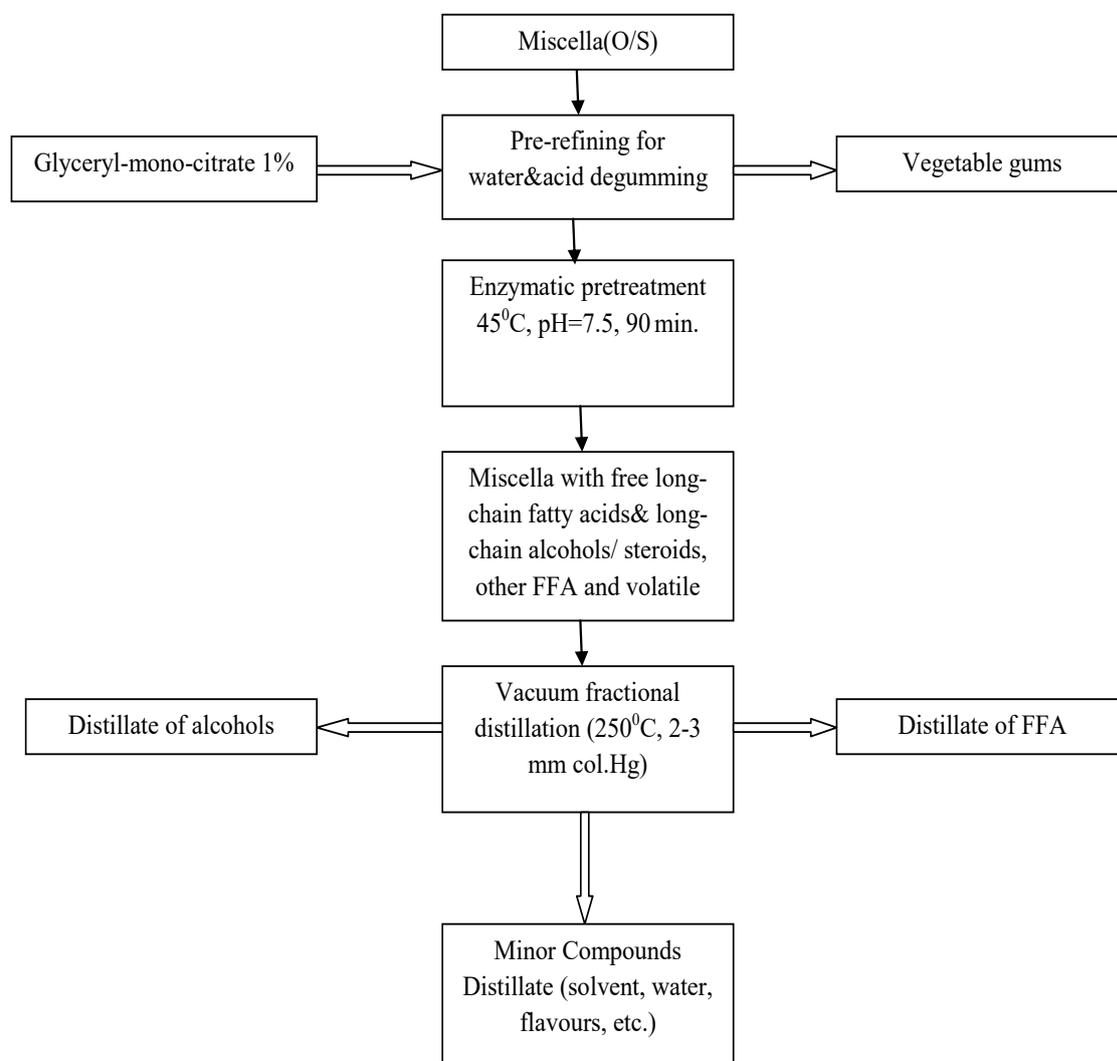


Fig. 3. Innovative oil dewaxing processing

The innovative dewaxing (ID) process using endogenous wax ester hydrolyses (wax desynthetase) activation in optimal reaction conditions of temperature 45°C and pH=7.5 provides an efficient, specific and targeted affinity deconstruction process (Fig. 3, Table 4) oriented towards the wax substrate in order to develop the deparaffinage effect (Fig. 4, Table 5).

Table 4. The dewaxing activity of the O/S endogenous dewax esterase

Substrate	Dewaxing activity (%)
Pre-dewaxed oil	2.5
O/S activated dewax esterase	25
Dewaxed oil	2.0



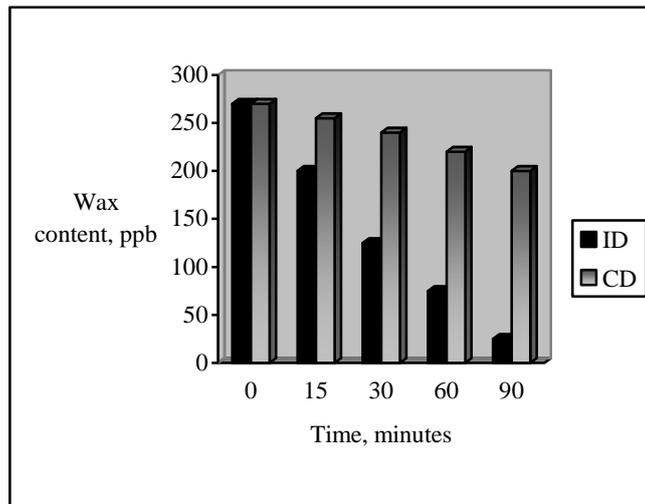


Fig. 4. The dewaxing dynamicity in the innovative dewaxing (ID) and cold dewaxing (CD)

The enhanced endogenous dewax esterase activity in the O/S miscella phase is due to the interfacial induced orientation towards the W/O surfaces. The O/W lipase interfacial activation was demonstrated due to the opening of the β -helical structure from lipase active centres which allow the enzymatic reaction to be conducted with accurate effect, in term of specificity, affinity and rate of substrate conversion (Maruyama T et al. , 2000).

The lipase W/O interfacial activation was studied in the lipolyse and interesterification process but the endogenous O/S dewax esterase activation was until now non-investigated. The isoparaffins structures formation improves the dewaxing yield at 90.7% reported on the miscella crude oil with 270 ppm waxes content with an almost double-cold dewaxed oil stability.

Table 5. The comparative analysis of the high oleic sunflower oil dewaxing yield (R) and cold stability

Dewaxing method	R, (%)	Cold test, h
Solvent dewaxing, 90'	90.7	50
Cold dewaxing, 420'	83.3	26
Cold dewaxing, 90'	25.9	1.5

The dynamicity of minor compounds removal process in the ID refining was mathematically modelled with Freundlich equation (4).

$$\frac{\Delta C}{A} = K C_F^n \quad (4)$$

Where ΔC represents the absolute wax content loss from the crude oil, A represents the enzyme dewaxing dose, K was a constant depending on the enzyme dewaxing activity (0.002-0.02), C_F represents the wax content in the winterized oil and n was an exponent depending on the enzymatic affinity towards the wax structure.

The n-hexane stabilization effect in the interface lipase activation process is the crucial effect persued in the optimal condition of the oil enzymatically dewaxing. Also, a greater thermal stability of the lipase in the organic solvent was reported in the previous studies (Zacs and Klibanov,1984).

Conclusions

The present innovative dewaxing technology eliminates the low-efficiency cold/low temperature crystallisation and all the negative effects given by the solvent oil recovery from the kieselgur pomace, cooling agent producing and separation by filtration/centrifugation process.



A complex refining process was reported because the triade AAA (Aqua –Alcohol- Acid) yields an effective degumming, decoloring and dewaxing process, with the sequestration of the heavy metals and antioxidative oil protection in the fractional distillation conditions.

A pro-dewaxing isomerize reaction of the waxes structures could be developed in the biotechnological induced destructure. Higher yields of wax and other minor compounds removal were reported than in conventional refining process.

The cost-efficiency process was improved considerably because the miscella distillation, caustic neutralization, soap washing followed by vacuum drying and the bleaching are eliminated, as individual refining steps and a flexible 3-steps continuous physical refining (degumming-dewaxing-fractional distillation) were proposed for optimal results.

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