EXTRACTIVE LIMITATIONS OF NATIVE FUNCTIONAL INGREDIENTS: BIOACTIVE LIPIDS BY CHEMICAL MODIFICATIONS

Roche J.[1,2], *Mouloungui Z.*[1,2], *Bouniols A.*[1,2], *Zebib B.*[1,2], *Merah O.*[1,2]

[1]*Université de Toulouse, INP-ENSIACET, LCA (Laboratoire de Chimie Agro-industrielle), F-31030 Toulouse, France*

[2]*INRA, UMR 1010 CAI, F-31030 Toulouse, France*

Abstract:

Phytosterols draw their origin from plants (oilcrops and cereals seeds, vegetables, fruits, nuts). Their chemical structures and their biological functions are similar with those of cholesterol. Efficiency of phytosterols and their saturated homologues (phytostanols) on the reduction of high cholesterol level is proven and confers to them uses as functional ingredients. In nonfood industries, the molecular volume of the triterpenic skeleton of phytosterols, as in the case of cholesterol, is exploited for many uses particularly as a hydrophobic part in production of polydisperse "new surfactants". Due to their physicochemical behaviour to the air/water interfaces, phytosterols are good raw materials for the development of liquid crystals and original biological and pharmaceutical applications. At native state, phytosterols have however low and variable biological levels in seeds. Taking into account the possibilities of chemical modifications and industrial development prospects of phytostérols and their derivatives, selection of varieties associated with the management of crop and wild plants cultural practices represents an interesting way to improve phytosterols content and to modify their component composition to answer to a scheme of vegetable refinery guided by the added value guaranteed by the presence of these minor components.

Key words: lipids, phytosterols, chemical modification, industrial application

INTRODUCTION

The plant sterols, also called phytosterols, are molecules present in all plants in nature. With structures and properties of their own and which determine their biological activity in the living world. For several years, these bioactive molecules, yet little exploited five years ago, experiencing significant growth in several industrial sectors. The major advantage of phytosterols is their natural cholesterol-lowering property [1-5], which allows them to substitute for cholesterol and reduce cardiovascular risks.

Thus, in the native state, they are used in nutrition on the functional food market (in diet) including margarines fortified with plant sterols [6] or modified state, in compound in pharmaceutical manufacturing steroids [7], or in the field of cosmetics as emollient, emulsifier, dispersant, solubilizing [8].

The largest share of phytosterols on the market comes from the crushing industry and refining of oils and fats such as soybean or rapeseed. Phytosterols, considered by-products are then made from recycled water lost deodorization [9].

This source of phytosterols raises the issue of traceability matrices as seeds used are often derived from a mixture whose origin is not always identified. Furthermore, for production from the crushing, extraction of phytosterols sometimes involves methods "at risk" because they involve harmful chemicals or toxic to humans and / or the environment

Thus, in human consumption, the natural phytosterols is an essential criterion. The lipid fraction of oilseeds has the highest percentage in free form or esterified at 1 to 5 g of sterols / kg oil [10].

Regarding non-food industrial uses, other alternatives are designed to chemically modify the structure acquired sterol molecules to impart new useful properties for specific applications unsuspected. However, the extraction of said molecules is sometimes limited due to their low content. Optimization of the phytosterol content of the plant material is an alternative to the use of plant sterols as a byproduct of crushing. Improved levels are available naturally by the implementation of crop management and genotypes adapted and optimized and represent a reliable source of bioactive molecules developed with respect for the environment.

This article proposes to show the state of the art non-exhaustive on the reactivity of phytosterols in food and non food with for example the modification of cholesterol for new applications in high technology industries.

The sterol molecules of plant origin

Plant sterols are all molecules having a structure similar to cholesterol animals (Figure 1). They are derived from oil seeds, cereals, nuts, fruit or vegetables whose contents vary according to species (Table 1). The major phytosterols, sitosterol and campesterol, differ from the structure of cholesterol by methyl and ethyl groups respectively on carbon 24. This structural difference is minimal but nevertheless has a considerable effect on their uptake by the human body. Campesterol, for example, is three times less absorbed than cholesterol, while the absorption of sitosterol, the most common plant sterols in the plant kingdom, reached absorption rate 10 times lower than that of cholesterol (4-5 %) (Table 2). Similarly, stanols, saturated form sterols, have properties that are of their own to further reduce their intestinal absorption (<0.5%) [17]. Thus, a normal diet consumed at optimal doses between 1.5 and 3 g / day, these molecules of plant origin are effective in reducing the intestinal absorption of cholesterol [18-20, 5] and LDL-cholesterol plasma (- 10 to 15%) ("bad cholesterol") without altering the HDL ("good cholesterol") [21, 22]. Such a decrease is associated with the decline in development of cardiovascular disease [23]. Plant stanols are naturally occurring. Their concentration in the native state is very low. The main sources of stanols are cereals such as rye, barley, wheat and oats containing respectively 51, 50, 38 and 25 mg stanols per 100 g fresh seed [10]. However, they may be from a chemical hydrogenation of phytosterols [6]. In addition to their property of inhibiting cholesterol absorption harmful to health, these sterol molecules are also sought for their anti-cancer [24, 25], anti-atherosclerosis [26, 27], anti-inflammation [28] and anti-oxidation [29].

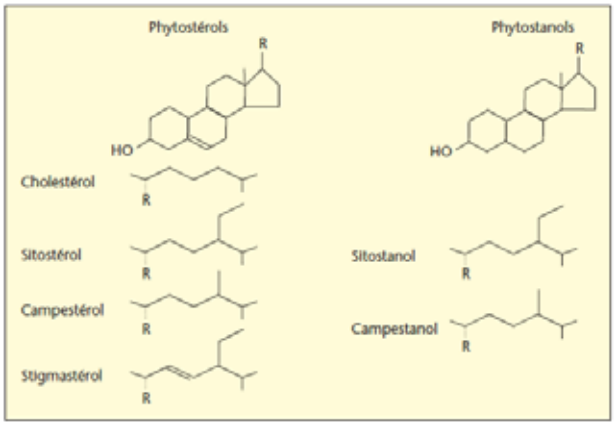

Figure 1. Chemical structures of some phytosterols and their saturated forms.

Table 2. Absorption of sterols by human organisme.

Cholestérol	40-50 %
Campestérol	12-16 %
Sitostérol	4-5 %
Sitostanol	< 0,5 %

Source : Wester, 2000 [16].

Table1. Change in total sterol content according plant species (Oil crops, cereals, fruits, vegetables, nuts).

Graines oléoprotéagineuses (mg/100 g d'huile)																
	Palme	Olive	Colza	Tournesol	Soja	Pois	Haricot	Canola	Maïs	Pépins de raisin	Carthame	Lin	Argan	Pastel	Ricin	Crambe	Tabac
	71-117 (B)[c] 49-61 (R)[c] 66[b] 60-120 [a] 29[g]	144-150[c] 150-162[b] 141[g]	513-979 (B)[c] 250-731 (R)[c] 715-736[b] 540-880 [p] 691 (B)[g]	374-725[c] 210-454[f] 263-340[b] 412-588[d] 325-515 [a] 326 (B)[g]	229-459 (B)[c] 221-328 (R)[c] 161[e] 234-466[f] 203-285[b] 250-418 [a] 550[g]	135[e]	127*	459-807[f] 639-658[b]	809-1557 (B)[c] 715-952 (R)[c] 699-766[b] 830-2250 [a] 1 390[e] 923[g]	259-418 [a] 440 (B)[g] 269 (R)[g]	240-380 [a] 494[e] 380[g]	200-410 [a] 440 (B)[g]	147[g]	522[g]	225 (R)[g]	530[g]	1 100[g]
Moyenne	**67**	**148**	**700**	**405**	**323**	**135**	**127**	**641**	**1 100**	**349**	**395**	**373**	**147**	**522**	**225**	**530**	**1 100**

Céréales (mg/100 g d'huile)						
	Blé	Son de riz	Sorgho	Sarrasin	Coton	Sésame
	2 140[g]	1 325 [e] 3225 (B)[c] 1 055 (R)[c]	178[e]	198[e]	292[b] 431-539 (B)[c] 327-397 (R)[c]	492[b] 714[e] 539-636 [a]
Moyenne	**2 140**	**1 868**	**178**	**198**	**380**	**598**

Légumes/Fruits (mg/kg de matière fraiche)									Noix (mg/100 g d'huile)				
	Orange	Banane	Pomme	Choux[h]	Carotte	Céleri	Avocat	Pomme de terre	Amande	Noix de coco	Cacahouète	Noisette	Noix
	24[c]	16[e]	12[e] 13[c]	39-43[c]	16[c]	6[e]	353[b]	4[c]	143[e] 120-400 [a]	133[e] 69-70[b] 118[g]	167[b] 147[g]	75-195 [a]	170-245 [a]
Moyenne	**24**	**16**	**13**	**41**	**16**	**6**	**353**	**4**	**202**	**107**	**157**	**135**	**208**

a Karleskind, 1992 [11].
b Phillips et al., 2002 [12].
c Piironen et al., 2000 [10].
d Roche, 2005 [13].
e Venketeshewer Rao and Janezic, 1992 [14].
f Vlahakis and Hazebroek, 2000 [15].
g Roche et al. 2006 [52]
h Brussels sprouts, cauliflower, broccoli.
B: Crude oil.
R: Refined commercial oil

Properties and the market for sterol product / market / properties

Depending on their structure, sterols have different properties that determine the type of products used. The hydrophilic / hydrohobe is likely to be modified either by a hydrophilic element natural glycosidic type, or by an element provided by the synthetic hydrophilic oxyethylene units. For their use as surfactants, the physicochemical properties of sterols are significantly altered by the binding of a chemically generated oxyethylene chain. By ethoxylation, the head of the sterol hydroxyl modified acquires hydrophilicity and skeletal triterpene sterols is operated as part of new hydrophobic surfactants polydisperse. This is the physicochemical behavior of these forms ethoxylated to air / water interfaces that determines their chemical reactivity as surfactants. According to the structure of molecules, the surface tension decreases with time and the equilibrium time of the surface tension is very long (Figure 2).

This is explained by the existence of exchange reactions at the surface. Molecules slowly reorient to the air / water interface [8]. Thus, increasing the length of the polyoxyethylene chain is associated with the decrease in micelle critical concentration (CMC) of ethoxylated sterols (Table 3).

Thus, according to the sterol composition structures and the degree of ethoxylation, the reactivity of molecules at the oil / water interface is different and produces a variety of uses in today's market (Table 4).

Another interesting example of bioactive sterols the industrial surfactants next generation is represented by the sterol glucoside which behaves as polar lipids in contrast to sterol esters which are soluble. In this case, the skeleton is a hydrophobic sterol which occupies a large volume with air / water interfaces.

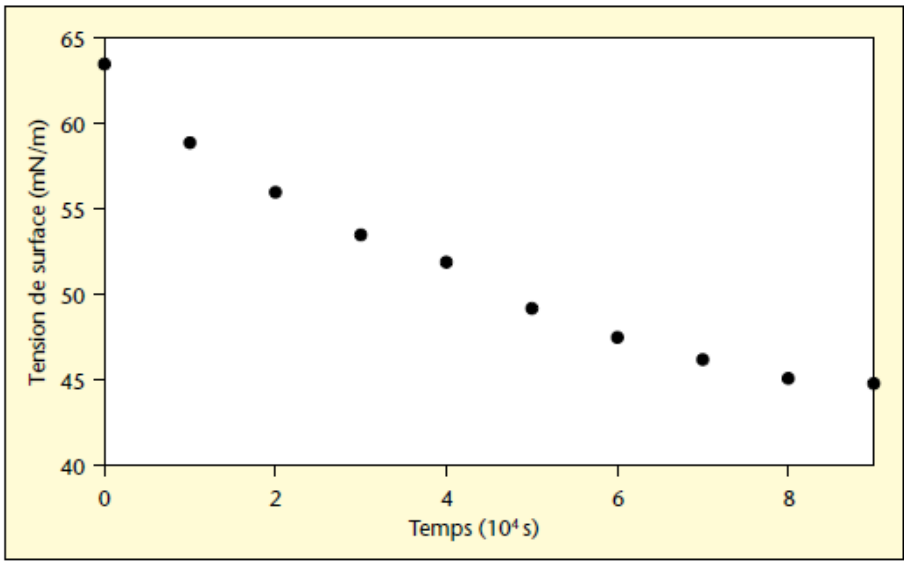

Figure 2. Surface tension for phytosterol with 20 oxyethylene units versus time at a fixed concentration (3.44 × 10 -6 M) (source: Folmer et al., 1999 [30] with permission).

Table 3. Critical micelle concentration in (CMC) and surface tension as a function of oxyethylene units

Unité oxyéthylène	CMC (µM)	Tension de surface à la CMC déterminée (mM/m)
10	10	31
20	7	34
30	3	42

Source : Folmer *et al.*, 1999 [30] (reproduit avec permission).

Table 4. Examples of sterol surfactants commercially available.

Nom du produit	Matériel hydrophobe	Degré d'éthoxylation	Stérol libre	Producteur	Application principale
Generol R E5 Generol R E10	Stérols issus du colza 45 % sitostérol, 25 % campestérol 20 % stigmastérol	5, 10	10-14 % 2-6 % Resp.	Cognis, Allemagne	Coémulsifiant pour produits cosmétiques de type – eau/huile [5] – huile/eau [10]
BPS-5 BPS-10 BPS-20 BPS-30	Phytostérol : 50 % sitostérol Δ5 25 % campestérol 25 % stigmastérol	5, 10, 20, 30		Nikkol, Japon	Émollient [5] Émulsifiant [10-30] Dispersant
BPSH-25	Phystostanol : Phytostérols saturés	25		Nikkol, Japon	Émulsifiant Dispersant

Source : Folmer, 2003 [8] (reproduit avec permission).

Uses cholesterol as a bioactive lipid in chemical industries by chemical modifications

Cholesterol is a sterol troublesome in food and researches performed currently are working to find ways to substitute other molecules of the same structure but less harmful to health. However, in the industrial fields, it can be a source of raw material for the manufacture of synthetic lipids has physical properties, chemical and biological specific.

Based on structural features of cholesterol, two sites of chemical modifications are available and generate cholesterol modified industrial applications. The reactivity of these sites has led to the formation of cholesteric esters or ethers (Figure 3) [31].

The active double bond at C5-C6 of cholesterol is a site of addition reactions called "ene-reactions" to form, for example ester alkenyl succinic acid monocholestérique corresponding to the binding of cholesterol and anhydride alkenylsuccinic (Figure 4) without recourse to the use of organic solvents.

By chemical modification of the rigid sterol structure, the hydroxyl group (OH) at position 3 is a privileged site of acylation reactions and alkylation. Because of their regular and symmetrical molecular conformation, cholesterol derivatives are solid at room temperature. The cholesterol esterification by acyl groups branched or etherification of cholesterol by alkyl groups has reduced the melting point of the cholesteric structures [32]. During the last twenty years, various structures of this type have been generated to identify new uses of cholesterol in the field of cosmetic products [33]. Thus, some cholesteric ethers, such as glyceryl ethers cholesteric, became the active ingredients in the formulation of emulsions very stable oil in water or water in oil.

In any other field, such structures created synthetically sterol [34-38] have also been found to possess particularly advantageous properties for the formation of liquid crystals or molecular aggregates [39] particularly through the acquisition of new properties obtained by non-linear optical hydroxysubstitution azo-benzene derivative with cholesterol esters [40] (Figure 5). The ability of these bioactive molecules to modify their structure has conditioned their use in the photochemical control of phase changes in the formation of optical memory devices or dimensional conductors and photoconductors, etc…[31]. For example, the creation of a structure resulting from a combination of one cholesteric ester unit with a unit of chiral diphenylacetylene has properties that allow other applications to technology optical information storage [41]. These novel molecules derived from cholesterol have mostly very promising uses in industrial non-food. However, the intrinsic ability of cholesterol to naturally adopt another conformation in the phospholipid bilayer of cell membranes has helped to develop applications in the health field. Thus, because of their ability to interact with a polar environment and a nonpolar environment, cholesteric esters were identified as carrier molecules. These bioactive amphiphilic molecules have the ability to insert into the membrane thus facilitating the intracellular delivery of active therapeutic molecules [42-45]. Moreover, unlike conventional artificial liposomal structures to carry drugs or other pharmaceutical molecules, those containing cholesterol have a viable blood one hundred times longer [8], to optimize the delivery of molecules in these structures . Another area of application of cholesterol in the health field is that of gene therapy that develops the use of alternative lipopolyamines safer and more effective strategies for gene transfer. A cholesterol molecule is associated with the field cationic polyamines, conventionally used for their stabilizing properties of specific DNA conformations (Figure 6). Such molecules are a good vehicle for gene transfer because it avoids triggering an immune response because of their privileged interaction with the cell membrane [46, 47].

The ability of cholesterol modified to fit efficiently in cell membranes has developed new applications of cholesterol. Thus, the cholesterol molecule is used as monomers for the construction of synthetic biological structures. This is the case for example of pseudo-membranes artificial constructed from compounds derived from cholesterol polymerized possessing properties similar to liquid crystals that are used in the preparation of dental materials [48].

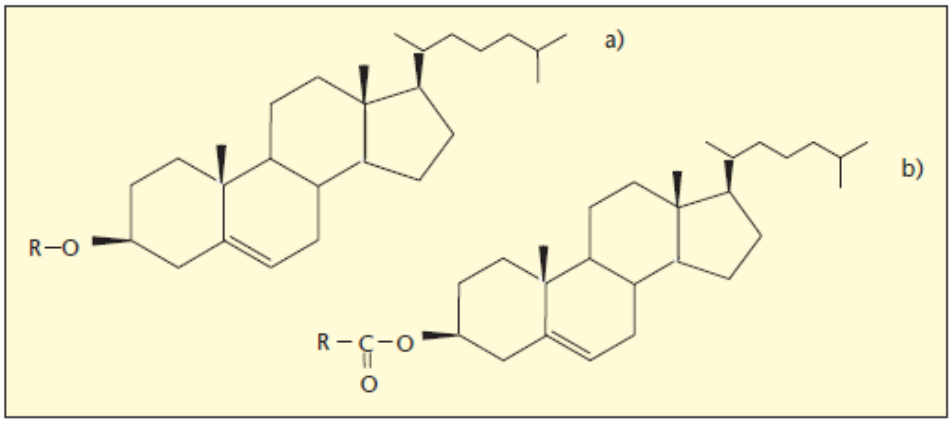

Figure 3. Structure ethers (a) and cholesteric esters (b) (source: Urata and Takaishi, 2001 [31]).

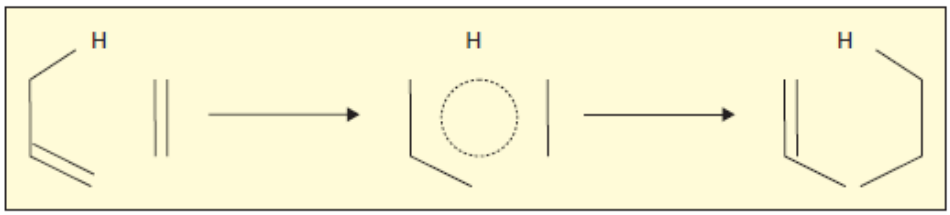

Figure 4. "Ene-reaction" (source: Urata and Takaishi, 2001 [31]).

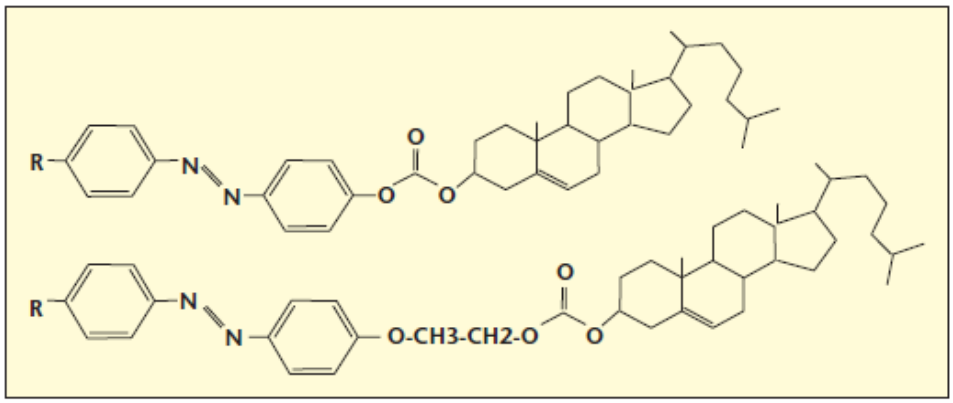

Figure 5. Azo-benzene derivatives from a-hydroxy substitution of cholesteryl ester (Source: Urata and Takaishi, 2001 [31]).

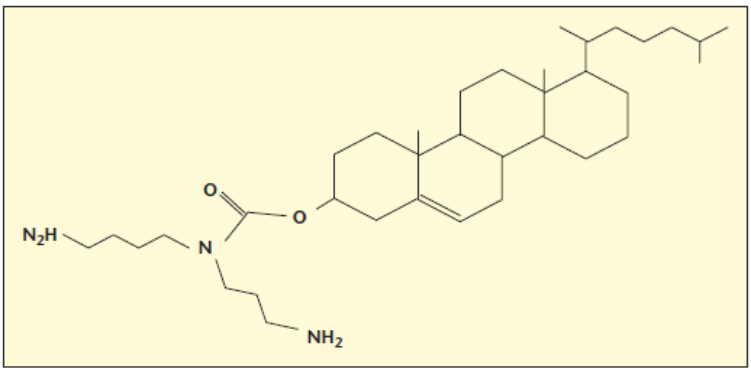

Figure 6. Structure of polyamine-based lipo-ether cholesteric (Source: Urata and Takaishi, 2001 [31]).

In the same vein, cholesterol can enter the polymerization from monomers preorganized which are themselves prepared to form circles to stress or from monomers in confined spaces of organized media [49]. These amphiphilic structures are, for example, micelles in an aqueous medium where the alkyl chains aggregate to form a core while the hydrophilic groups are in contact with water on the outer surface to minimize interfacial energy. Other polymers including amphiphilic structures such as that of cholesterol entering the formation of lipid bilayers, liquid crystals, organic crystals, microporous zeolites, mesoporous materials or inclusion complexes. Such structures are used in biomedical and pharmaceutical industries.

In the case of inclusion complexes for example, crystalline channels cholic acid derivatives (bile acid component) are used as monomer of natural origin [50].

Research perspectives

Although phytosterols have a large capacity to generate various molecules whose applications are probably not yet fully developed or exploited, their low concentration (less than 1% of oil) associated with uncontrolled variability remains an obstacle to their efficient extraction and use of mass in the industrial world. In addition, industrial sources of supply of phytosterols (crushing, refining) are associated with chemical, physical and biological need to control or eliminate. Given the possibilities of chemical changes associated with promising prospects for industrial development, improvement of current biological thresholds is a critical area of research. The agri-genomics can meet their expectations. These tools, developed in our laboratory include the adaptation of management practices associated with those of genomics to characterize the metabolic pathways involved in the biosynthesis of the compounds of the seed. They aim to optimize the directed synthesis of phytosterols in vivo based on varietal selection, through the use of genotypes with optimal potential for expression, and lines of culture adapted and optimized to the accumulation of the desired compounds, order to extend the biological thresholds [51]. Thus, through the use of crop management determined, our previous work on sunflower reveal an increase of sterol content under the effect of severe water stress and high temperatures between the onset of flowering and the end of seed maturation (+ 13% on average while confused genotype) [13, 52]. Furthermore, our study led to the profiling of accumulation of different phytosterols in the maturing seed to define the moments of maximum accumulation depending on the type of phytosterol. The maximum content of total phytosterols in sunflower seeds (230 mg/100 g dry seed) is reached at half the growth cycle of the plant, about 30 days after flowering to physiological maturity corresponding to a content 50% water. This approach allows to consider the early use of immature seeds to implement a method of extracting phytosterols optimized while shortening the growth cycle from seed. Ultimately, our team aims to provide an improved raw material phytosterols, or more generally in bioactive lipids, by exploiting the genetic variability of plant species composition, methods of field production and knowledge on the evolution of these compounds in the seed. Said molecules are to be extracted by gentle methods that we develop in the laboratory where water is the solvent majority [53]. Following preliminary results, it appears that the sterol fraction is extracted along with the lipid fraction in an emulsion rich in fatty acids. Furthermore, changing the hydrophilic / hydrophobic lipid of interest contemplated by the insertion of hydrophilic elements which produces glycerine derivatives of oligocarbonates glycérols'avèrent candidates reliable alternative oxyethylene unit [54 - 56].

Our approach to improve the content of bioactive lipids and modification of their composition is part of an integrated pattern of production / processing of lipids nascent plant refinery designed to meet the thresholds for specific industrial applications. In addition, new applications generated by these molecules represent a breakthrough for the development of sustainable agriculture because they generate alternative uses guided by the gain secured by these minor constituents. The approach rests on the control and optimization of the raw material used for the implementation of extraction facilities of seed lipids oléoprotéagineuses and involves knowledge within the agro-physiological, genomics and lipochemistry.

This is also accompanied by the development of analytical research adapted to low levels, as the determination of levels of different sterols directly on the seed matrix which has the advantage of avoiding an extraction step of oil in contrast to current technology

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