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Allergens and Toxins from Oleaginous Plants: Problems and Solutions

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1. Introduction

Population growth, industrialisation worldwide and the consequent increase in the use of fossil fuels such as petroleum contribute to the advancement of environmental damage. Burning fossil fuels releases carbon dioxide (CO_2) into the atmosphere, which contributes to an increase in the earth's greenhouse effect and climatic change (Balat & Balat, 2010). Factors such as increased demand for energy, price hikes in crude oil, global warming due to emission of green house gases, environmental pollution, and a fast diminishing supply of fossil fuels contribute to the search for alternative sources of energy (Atadashi et al., 2010).
Petroleum is a finite fuel resource that is rapidly becoming scarcer and more expensive. Petroleum-based products are one of the main causes of anthropogenic carbon dioxide (CO_2) emissions to the atmosphere (Balat & Balat, 2010). The current scenario of the world, confronted with the twin crises of fossil fuel depletion and environmental degradation, encourage research programs to reduce reliance on fossil fuels by the use of alternative and sustainable fuel sources, such as solar energy, wind energy, geothermal energy, tidal energy, ocean thermal energy, hydropower, biofuels and others (Atadashi et al., 2010; Sharma & Singh, 2009).
Biodiesel are one a biofuel that can be compound for fatty acid alkyl esters (methyl esters) that are produced from renewable natural sources such as vegetable oils, animal fats and microalgal oil by a new technology, the transesterification reaction (Atadashi et al., 2010). Biodiesel is considered a biodegradable, sustainable and clean energy because the oleaginous plants used to produce the biofuel absorb carbon dioxide during growth to a greater extent than that which is released to the atmosphere when used as a fuel in diesel engines (Sharma & Singh, 2009).

1.1 Sources of biodiesel

Various raw materials and technologies have been used for biodiesel production; however, to be profitable, biofuels need provide a net gain of energy, be environmentally sustainable, be cost-competitive and be produced in sufficient quantities without reducing the food supply (Nass et al., 2007). Biofuels are produced from renewable natural sources such as vegetable oils, animal fats and microalgal oil, and at present, many natural sources have been researched as prospective renewable fuels. With advances regarding the search for new sources of energy show, there are well-established raw materials for the processing and synthesis of biofuels.

Among the oilseeds used for biodiesel production are soybean (*Glycine max* L.), sunflower (*Helianthus annus* L.), cottonseed (Gossypium spp.), rapeseed (*Brassica napus* L.), castor bean (*Ricinus communis* L.), physic nut (*Jatropha curcas* L.) and other plants (Singh & Singh, 2010).

The source of biodiesel usually depends on the crops amenable to the regional climate. Countries such as the U.S.A. and those belonging to the European community are self-dependent in the production of edible oils and even have a surplus amount to export (Sharma & Singh, 2009).

Different countries are looking for different types of vegetable oils as substitutes for diesel fuels, depending upon the climate and soil conditions. Biodiesel has been in use in countries such as the U.S.A., Malaysia, Indonesia, Brazil, Germany, France, Italy and other European nations. However, the potential for its production and application is much more. Malaysia is far ahead of the rest in production terms. The feedstock available for the development of biodiesel in these nations is 28% for soybean oil, 22% for palm oil, 20% for animal fats and 11% for coconut oil, while rapeseed, sunflower and olive oils constitute 5% each (Sharma & Singh, 2009).

The source material for biodiesel production in Brazil varies widely among regions. Soybean, *Helianthus annuus* (sunfl ower), *Gossypium hirsutum* (cotton), *Ricinus communis* (castor bean), and *Brassica* spp. (colza) are grown in the south, southeast, and central regions; *Elaeis guineensis* (African palm), *Attalea speciosa* (babassu), soybean, and castor bean are found in the northeast and north regions (Nass et al., 2007).

This review discusses the major products obtained from oil plants that are used in biodiesel synthesis and their allergenic and toxic by-product compounds and describes the research already carried out with castor bean (*Ricinus communis* L.) oleagionous widely used for biodiesel production and other oilseeds used in the synthesis of biodiesel such as physic nut (*Jatropha curcas* L) species under investigation in the world and rapeseed (*Brassica napus* L.) widely used culture in European countries.

2. Products obtained after biodiesel synthesis

During biodiesel production, several residues such as press cakes, husks and glycerol are generated. The integral utilisation, according to the biorefinery concept, of all the fractions generated in biodiesel production is a requirement for the economy and the sustainability of the process, and for the rational exploitation of the raw materials.

2.1 Press cakes

Press cakes, the residues remaining after mechanical or solvent extraction of oils from seed kernels, can be utilised as raw materials in different bioprocesses for the production of chemicals and value-added products such as amino acids, enzymes, vitamins, antibiotics and biopesticides (Martín et al., 2010). However, those uses are restricted to edible oil cakes, which are recognised to have a high nutritional value, due to their high protein content. Non-edible oil cakes have been less investigated, and their uses are limited to organic fertilisers and biogas production (Ramachandran et al., 2007).

Possible non-food applications of cake proteins are in the field of adhesives, coatings, chemicals, fertiliser, such as seed press cake fertiliser and amino acid chelated micronutrient fertiliser. Protein research is of the high interest to many research groups with various industrial applications (Lestari et al., 2010).

Many oilseeds are used for the synthesis of biodiesel and with the growing worldwide interest in biodiesel production, is expected that the planting of oilseeds will grow exponentially. Among the candidates for oil biofuel synthesis are the castor bean, the physic nut and the rapeseed.

2.1.1 Castor bean cake

The castor bean plant (*Ricinus communis* L.) originated in Ethiopia and gradually dispersed towards South Africa, the Mediterranean region and warm areas of Asia, until finally establishing itself as a natural species in the majority of warm climate regions of the world. This plant is distributed throughout the tropics and subtropics, and is well adapted to temperate regions (Garcia-Gonzalez et al., 1999). This seed contains 45-48% oil and is important as a source of vegetable and medicinal oil with numerous benefits to humanity. As for industrial uses, dehydrated castor oil is used in the paint and varnish industry, in the manufacture of a wide range of sophisticated products like nylon fibers, jet engine lubricants, hydraulic fluids, plastics, artificial leather, fibre optics, bulletproof glass and bone prostheses and as an antifreeze for fuels and lubricants utilised in aircraft and space rockets (Ogunniyi, 2006; Conceição et al., 2005).

After oil extraction by pressing the seeds of *R. communis* L., organic matter known as castor cake is retained in the filters (Gandhi et al., 1994). The castor cake, once considered a byproduct of oil extraction, is today a product of castor bean that arouses considerable economic interest (Morais & Silva, 2008). This organic mass has constituents similar to those found in the endosperm of the seeds, such as proteins, tannins, etc. Many of these constituents are toxic or have allergenic activity (Felix et al., 2008).

Castor cake has a high protein content (~43%) and is often used as an organic fertiliser as an excellent nitrogen source and presenting insecticide and nematicide properties (Directorate of Oilseeds Research, 2004). As constituents of the high protein content of castor cake, 60% of the proteins are globulins (only soluble in salt solutions), 20% are glutelins (soluble in dilute acids and alkalis), 16% are albumins (soluble in water and dilute neutral pH buffer) and 4% are proteases (Silva Jr. et al., 1996). The protein content is not recommended for use as an animal feed because it is toxic due to the presence of the proteins ricin (toxoalbumin) and ricinin and the allergen complex, CB-1A (castor bean allergen) that is a mixture of proteins of low molecular weight (Felix et al., 2008; Silva JR. et al., 1996). Martín et al. (2010) have proposed that the high protein and carbohydrate content in castor press cake can be used as a potential feedstock following some fermentation processes.

2.1.2 Physic nut cake

Jatropha curcas L. is a tropical plant belonging also to the family of Euphorbiaceae,. It is cultivated mainly as a hedge in many Latin American, Asian and African countries and it is an oilseed crop, grown mainly for oil production. Besides oil, the jatropha seed kernel contains approximately 25–30% protein (Openshaw, 2000). After oil removal, the proteins remain in the jatropha cake. Jatropha seed protein may have similarities with other well-known oilseed proteins such as soy, canola or sunflower protein. In contrast to soy and sunflower, jatropha seed contains toxic compounds such as curcin (Lin et al., 2003) and phorbol esters (Devappa et al., 2010; Li et al., 2010; Martinez-Herrera et al., 2006) which make jatropha protein unsuitable for food applications. In addition to the several toxic or antinutritional compounds previously cited, trypsin inhibitors, lectins, saponins and phytate

also might cause or at least aggravate the adverse effects, but the short-term toxicity of the kernels has been ascribed mainly to phorbol esters (Makkar et al., 2009).

2.1.3 Rapeseed cake

Rapeseed *(Brassica napus L.)* is mainly produced for its high oil content (45-50%). It is the most commonly grown oilseed crop in Europe. *Brassica napus* (rape) has as the main components of it seeds lipids (about 35% of the dry weight of the seed) and proteins (about 20–25%) (Pantoja-Uceda et al., 2004; Schmidt et al., 2004). The main storage proteins of *Brassica napus* (oilseed rape) are the 2S albumins (napins) and the 12S globulin cruciferin (Barciszewski et al., 2000).

Rapeseed cake is a high-protein product (30-40%) from industrial oil extraction, obtained from the mechanical pressing of seeds (Swiatkiewicz et al., 2010). Originally, its use was limited to animal feed because of the presence of undesirable substances (glucosinolates, erucic acid) (Swiatkiewicz et al., 2010; Schmidt et al., 2004).

Rapeseed cake contains a considerable amount of protein, rich in sulphur amino acids, and, because of its higher crude fat level and low fibre content, rapeseed cake is a richer source of metabolisable energy for monogastric animals as compared to solvent-extracted rapeseed meal (Swiatkiewicz et al., 2010).

In the processing of rapeseed oil seeds for biodiesel production, 65% of the feedstock is converted into a lignocellulosic cake residue. This product, which is rich in hemicelluloses and has a high content in hydroxyl groups, is currently used as cattle feed or for energy production. Nevertheless, the upgrading of this byproduct through its conversion to low-cost polyols by oxypropylation and their incorporation into polymer formulations could entail a considerable valorization of the residue and, thus, economic and environmental improvements for the process.

2.2 Husks

Husks, generated during dehusking of the seeds for obtaining the kernels, generally are of low economic value, and they are mainly disposed of or burnt. In some cases, the husks are used as solid fuel or as raw materials for activated charcoal production (Martín et al., 2010).

Singh et al. (2008) have proposed that all parts of the *J. curcas* fruit can be utilised efficiently for energy purposes. That paper showed how a holistic approach was been taken to utilise all the components, including the husks, that can be used for gasification. Jatropha seed husk could be used successfully as feedstock for an open core down-draft gasifier, either as a feedstock or in briquetted form.

Pollution of the environment by heavy metal ions is a serious problem because of their toxic effects on humans and other living organisms. The use of hazelnut husks for the removal of copper and lead ions from aqueous solution has been described by Imamoglu and Tekir (2008). Ngah and Hanafiah (2008) have presented a review that describes the use of husks for the removal heavy metal ions from wastewater by the use of chemically modified plant wastes as adsorbents. In this paper, they described a number of plant wastes as adsorbents, including rice husks.

The investigation carried out by Martín et al. (2010) revealed that the husks of neem (*Azadirachta indica*) and moringa (*Moringa oleifera*) can be considered potential substrates for ethanol production due to their high cellulose content (approximately 30%).

Today, a higher level of utilisation of all parts of a raw material is shown as a promising economic alternative. The production of biofuels generates many products which may have high value and be used in various industrial applications.

2.3 Glycerol

Increased biodiesel production has been driven by rapidly depleting fossil fuels, plus increasing concerns about global warming and the environment. For each gallon of biodiesel produced, 1 lb of glycerol is also produced as a by-product. One mole of glycerol is produced for every 3 mol of methyl esters, which is equivalent to approximately 10 wt.% of the total product (Karinen & Krause, 2006). This increase in glycerol production has depressed the price of refined glycerol.

Glycerol is a trivalent alcohol widely used in the pharmaceutical, food, cosmetic and chemical industries. It is produced from a diversity of procedures, among them the transesterification of vegetable oils and animal fats. During biodiesel production from vegetable oil and animal fats, two phases are produced after transesterification and distillation of the excess alcohol: one upper ester phase (EP) that contains the main product, biodiesel and the lower glycerol phase (GP) that consists of glycerol and many other chemical substances such as water, organic and inorganic salts, a small amount of esters and alcohols, traces of glycerides and vegetable colours (Hájek & Skopal, 2010).

3. Toxic and allergenic compounds

Many oilseed plant candidates and those currently used for the synthesis of biodiesel present toxic or allergenic compounds that are constituents of the seeds, which, as a consequence, can also be found in some products obtained after extracting the oil. Other problem is that some of these compounds are also found in others parts of the plant such as the 2S albumin from *R. communis* (an allergen) present in the pollen of this oilseed. The presence of these compounds limits the economic applications of the press cake and is a risk to the workers and the population living nearby.

3.1 Toxins

3.1.1 *Ricinus communis*

Castor bean is an oleaginous candidate for oil production (Singh & Singh, 2010), which will contribute to enhancing the cultivation of this plant. Castor bean seeds, however, contain a strong toxin (ricin), a toxic volatile alkaloid ricinine (1,2-dihydro-4-methoxy-1-methyl-2-oxo-3-piridinocarbonitrila-$C_8H_8N_2O_2$) and an allergenic protein fraction (CB-1A or 2S albumin isoforms), which severely limits the usefulness of the castor meal after oil extraction (Godoy et al., 2009; Audi et al., 2005; Garcia-Gonzalez et al., 1999; Thorpe et al., 1988).

The castor bean is an oilseed member to the Euphorbiaceae family and yields an oil that is used for biodiesel production. Furthermore, the residual cake is very useful for fertilisation and it is rich in proteins, opening the possibility of its use as animal feed. However, this second application addresses the problem of the presence of ricin, an extremely toxic protein.

Ricin is a protein found exclusively in the endosperm of castor bean seeds and has not been detected in other plant parts such as the roots, leaves or stems. It represents 1.5 to 2% of the total weight of the seed (Anandan et al. 2005; Cook et al., 2006). It is primarily responsible

for the toxicity of castor oil and is among the most toxic proteins known to man (Moskin, 1986). The ricin toxin is a 62–66 kDa protein produced by castor beans (*Ricinus communis*). This holotoxin consists of two polypeptide chains, approximately 32 kDa and 34 kDa in size, linked by a disulphide bond (Figure 1). The A chain (RTA) is a potent ribotoxin, inhibiting protein synthesis in mammalian cells at doses as low as a single RTA molecule per cell. The B chain (RTB) is a lectin, which binds to galactose residues on the cell surface. (Audi et al., 2005; Rao et al., 2005; Brandt et al., 2005).

Sehgal et al., 2010 demonstrated the presence of three isoforms of ricin in castor seeds. The isoforms were sub fractionated into ricin I, II and III by chromatography. Their molecular weights lie between 60–65 kDa. Ricin I, II and III were highly cytotoxic against Vero cell line with IC50 values of 60, 30 and 8 ng/ml respectively. Difference in cytotoxicity of isoforms was confirmed through hemagglutination assay and ricin III caused higher degree of hemolysis.

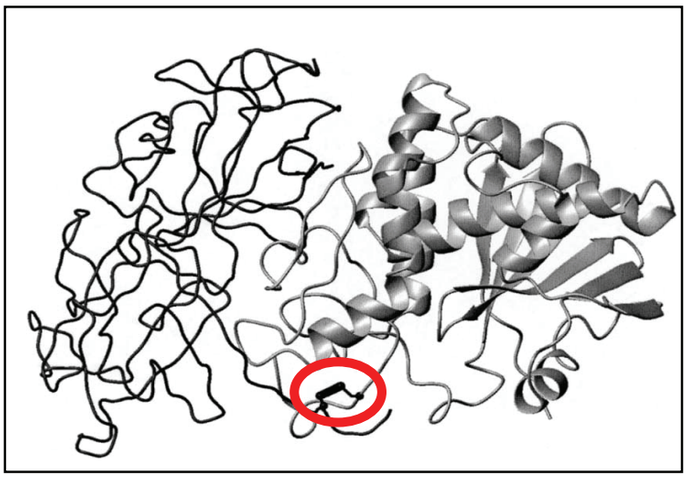

Fig. 1. Structure of the ricin molecule. The B chain is located on the left and the chain A is on the right. The red circle indicates the disulfide bridge linking the A and B chains (Rutenber & Robertus, 1991)

Ricin is a potent toxin that kills eukaryotic cells by inhibiting protein synthesis. Therefore, it is a protein of the class of toxins known as ribosome inactivating proteins, RIPs (Cook et al., 2006; Olsnes et al., 1999).

RIPs can be either type 1 (monomer) or type 2 (dimeric) (Stirpe & Bartelli, 2006). Type 1 RIPs present only the A chain, which is a glycosidase that removes an adenine residue from 28S ribosomal RNA. The RNA, after depurination, is susceptible to hydrolysis in alkaline pH and to acids in the presence of aniline. The region of the modified rRNA is essential for elongation factor binding and modified ribosomes cannot support protein synthesis (Olsnes, 2004).

The B chain is required for binding to the target cell and intracellular direction of the A chain (Olsnes, 2004; Day et al., 1996). When there are A and B chains, the toxin is classified as a type 2 RIP, which is the case for ricin (Cook et al., 2006). The ricin A chain is very efficient inside the cell, since a single molecule inactivates thousands of ribosomes per minute. Thus, one molecule can inactivate ribosomes faster than the cells can synthesize new ribosomes and, therefore, only one molecule kills the cell (Olsnes & Kozlov, 2001). The

value of the oral LD50 for rats and mice is between 20 and 30 mg/kg body weight, while in humans the toxic oral dose is 1–20 mg/kg of body weight (Alexander et al., 2008; Audi et al., 2005; Rao et al., 2005).

Despite its high toxicity, it is possible to develop immunity against ricin, as demonstrated in the studies of Tokarnia and Döbereiner (1997) in which cattle that received small doses of ricin (by ingestion) developed some immunity and later supported a higher dose with symptoms of intoxication, but stayed alive, while animals that received the higher dose directly were not resistant.

In the medical area, ricin has been prominent among a group of toxic proteins that have been used as immunotoxins and therapeutic agents used in the treatment of cancer and autoimmune diseases (Brandt et al. 2005). This toxin has also drawn attention due to its criminal use in the murder of Bulgarian journalist Georgi Markov in 1978 in London (Olsnes, 2004).

- Solutions:

Ricin is a major impediment to the use of castor cake for animal food (Na et al., 2004). The transformation of castor cake into a non-toxic product that can be used for animal feed already has long drawn the interest of many researchers around the world, and some satisfactory results have been obtained. A number of methods have been employed to detoxify castor oil seed meal, some of which appear to be more effective than others (Puttaraj et al., 1994).

In recent years, several methodologies have emerged to detoxify castor bean cake and use it as animal feed. Anandan et al. (2005) reported that physical processes based on heat (boiling, autoclaving, hot air oven) and alkali-based chemical processes (sodium hydroxide, calcium hydroxide and ammonia) could detoxify castor cake. The efficacy of the treatments was assessed based on the qualitative and quantitative changes in ricin content. Of all the methods employed, autoclaving (15 psi., 60 min) and lime treatment (40 g/kg) completely destroyed the toxin as observed by electrophoresis, however, toxicologic assays were not done.

Godoy et al. in 2009 used solid-state fermentation (SSF) of castor bean waste to achieve ricin detoxification, to reduce allergenic potential and to stimulate lipase production. The fungus, *Penicillium simplicissimum*, an excellent lipase producer, was able to grow and produce the lipase enzyme in castor bean waste. The biodetoxification process described could extend the use of fermented castor bean waste and potentially be used as an animal feed or fertiliser, without causing damage to the environment.

The SSF processes used by Godoy et al. (2009) permitted the total detoxification as observed by electrophoresis and toxicological analysis. This process offers potential advantages in bioremediation and biological detoxification of toxic compounds second Pandey et al., 2000.

3.1.2 *Jatropha curcas*

Jatropha curcas is another member of the Euphorbiaceae family and is known for its toxicity. It is grown in Central America, South America, Southeast Asia, India and Africa. The kernels have about 50% oil and the seeds contain curcin, a toxic glycoprotein with a 54% homology with the ricin A chain and with a similar mode of action (Alexander et al., 2008; Kumar & Sharma, 2008), as well as phorbol esters, which are polycyclic compounds

(Devappa et al., 2010; Martinez-Herrera et al., 2006) that can induce skin tumours when administered to mice (Chen et al., 1988).

Curcin, a kind of type I RIP, was first isolated from the seeds of *Jatropha curcas* by Stirpe et al. (1976). It was found to inhibit the growth of some tumour cells (Lin et al., 2003). Curcin is a similar protein to ricin, a toxic protein isolated from castor beans (*Ricinus communis*), which has two chains, one a functional lectin and the other capable of inhibiting protein synthesis (Rakshit et al., 2008; Stirpe et al., 1976). The absence of a lectin portion of this protein prevents binding to cells and impairs internalisation, thus becoming much less toxic than the type II RIPs such as ricin present in the seeds of castor bean. Recently, Lin et al, 2010 have purified a curcin molecule that was a glycoprotein with 4,91% of the total neutral-surge content. It strongly inhibits the protein synthesis of rabbit reticulocyte lysate, with an IC50 of 0.42 nM. The isolated curcin had a hemagglutinating activity, when its concentration was more than 7.8 mg=L. The secondary structure of curcin was analyzed by Circular Dichroism (CD) spectrum. The results of acute toxicity in mice show that mice oral Semi-lethal dose LD(50) was 104.737 +/- 29.447 mg=kg; mice parenteral semi-lethal dose LD(50) was 67.20 +/- 10.445 mg=kg.

Due to the toxic compounds found in physic nut seeds, the press cake cannot be used for animal feed, despite its high protein content. Experiments have shown the toxicity of the seeds of *J. curcas* in mice, rats, sheep, calves and chicks (El-Badwi et al., 1995). In contrast to this, Panigrahi et al. (1984) found no dramatic effects of poisoning in mice and rats fed on seeds of Mexican origin (edible varieties) that naturally occur in Mexico (King et al., 2009).

Beyond the concern about the presence of curcin in physic nut cake, there is another concern to be addressed: the presence of phorbol esters. The term phorbol ester is used today to describe a naturally occurring family of compounds widely distributed in plant species of the Euphorbiaceae and Thymelaeceae families (Rakshit et al., 2008). They are defined as polycyclic compounds in which two hydroxyl groups on neighbouring carbons are esterified into fatty acids. These compounds are present in many plants, including the physic nut. The structure of phorbol esters is dependent on a tetracyclic diterpene carbon skeleton known as tigliano, the main portion of alcohol in the phorbol esters (Goel et al., 2007).

Phorbol esters and their various derivatives are said to promote tumours. In addition to this effect, they induce significant biological effects, even at low concentrations. The primary action of phorbol esters occurs in biological membranes. This toxin tends to bind to receptors of membrane phospholipids (Weinstein et al., 1979). The phorbol esters are analogues of diacylglycerol, an activator of many isoforms of protein kinase C (PKC). The most investigated activity of these esters is their binding and activation of protein kinase C (PKC), which plays a critical role in signal transduction pathways and regulates cell growth and differentiation (Goel et al., 2007). Contradictory to their tumour-promoting ability, there are reports on the pro-apoptosis capacity of phorbol ester on tumour cells (Brodie & Blumberg, 2003; Gonzalez-Guerrico & Kazanitez, 2005). Some phorbol esters are inhibitors of HIV replication and have antileukemic activity (Goel et al., 2007).

The phorbol esters are acutely toxic, and oils containing phorbol esters are known purgatives (Gandhi et al., 1995). Adoption of varieties lacking phorbol esters, in addition to providing a potential source of income from animal feed, would also eliminate any potential risks associated with prolonged exposure to phorbol esters.

- Solutions:

A range of methods have been used to try detoxify defatted seed meal. Hass and Mittelbach (2000) suggest a method for detoxification of the seed oil using traditional oil refining processes to examine the effect processing on the content of phorbol esters. That paper, almost no effect could be observed with degumming and deodorisation, whereas the steps of deacidification and bleaching could reduce the content of phorbol esters by up to 55%.

Extraction with polar organic solvents and combined heat/$NaHCO_3$ treatments using a combination of both solvent extraction and heat/$NaHCO_3$ treatment, have been shown to promote a 48-fold reduction in phorbol ester content in the seed meal of the physic nut (Martinez-Herrera et al., 2006).

Heat treatments, such as autoclaving for example, usually inactivate the curcin, allowing the use of this as food for ruminants. It is known that heat treatment alone is not able to decrease the concentration of phorbol esters. Then, in 2008, Rakshit et al. described satisfactory results in toxicity studies with rats using alkali (2% NaOH or 2% $Ca(OH)_2$) and heat treatments (autoclaved at 121ºC) to deactivate phorbol esters as well as the lectin content of the physic nut meal. After these treatments, the phorbol ester content was reduced up to 89% in whole and dehulled seed meal. The rats fed with treated meals exhibited delayed mortality compared to untreated meal-fed rats.

The phorbol ester content was analysed in fractions obtained at different stages of oil pre-treatment and biodiesel production from the physic nut by Makkar et al. (2009). Makkar et al. observed that the phorbol esters were destroyed by the stripping process during biodiesel production. In physical refining (degumming, silica/bleaching, deodorisation/stripping at 240–260ºC and under vacuum) the deodorisation conditions were much more severe, leading to phorbol ester degradation.

3.1.3 Brassica napus

Rapeseed (*Brassica napus* L.) is an important crop for the production of vegetable oil for human consumption, and more recently for the biodiesel. *B. napus* is member of the Brassicaceae family and the crop is mainly grown for its biodegradable oils which can be used for the production of cooking oil, machine oil, diesel substitutes and as a base oil for the plastics industry. The high protein seed residue following oil extraction provides a good source of animal feed (Welch et al., 2000).

After oil extraction, a residue with high protein content is obtained that can be used as a valuable animal feed. However, anti-nutritive factors, such as the glucosinolates or erucic acid in rapeseed may cause various specific physiological effects in humans and in animals (Fahey et al., 2001). Glucosinolates are considered anti-nutritive factors for animal production but, on the other hand, they have an important role in plant protection against pests, diseases and also weeds (Rahmanpour et al., 2010; Haramoto and Gallandt, 2004).

Originally, the rapeseed cake uses for animal feed were limited because of the presence of undesirable substances (glucosinolates, erucic acid) (Swiatkiewicz et al., 2010; Schmidt et al., 2004). Major deleterious effects of glucosinolate ingestion in animals are reduced palatability, decreased growth and production. Ruminants are less sensitive to dietary glucosinolates. Among the monogastric animals, pigs are more severely affected by dietary glucosinolate compared to rabbits, poultry and fish (Tripathi & Mishra, 2007).

- Solutions:

The oil meal of Brassica origin is a good source of protein for animal feed but the glucosinolate content limits its efficient utilisation. Various processing techniques have been applied to remove glucosinolates in order to minimise their deleterious effects on animals. Tripathi and Mishra (2007) presented in their review some techniques, described by other authors, to remove glucosinolates; water extraction, heat and $CuSO_4$ treatments were found to be suitable for rapeseed meal quality improvement.

The work presented by Petisco et al. in 2010 measured the quality parameters of intact seeds of Brassica species using visible and near-infrared spectroscopy (NIRS). Petisco t al, 2010 demonstrated that NIRS technology is viable for the quantification of oil, protein and total glucosinolates in seed samples of *B. napus* and/or *B. carinata* without sample preparation. The accurate predictions provided by the NIR equations confirmed that NIR technology could be very useful for the rapid quality evaluation of intact rapeseeds, thus avoiding the need for grinding and thereby saving time. The speed of analysis and the non-destruction of the seed make this technique well-adapted for breeding purposes as well as for quality control in oil factories and in feed manufacturing. The problem of erucic acid has been solved by conventional breeding technology of rapeseed. The term canola (**CAN**adian **O**il **L**ow **A**cid) refers to strains of *B. napus* and *B. campestris* containing less than 2% of total fatty acids as erucic acid.

Despite the problems regarding the presence of glucosinolates in rapeseed cake for use in animal feed, it can also be used as a biopesticide. The utilisation of the meal as a biopesticide requires seed meal storage prior to field application. Morra and Borek (2010) studied the effect of a storage period to maintain glucosinolate stability in *B. napus, B. juncea* and *S. alba* seed meals. Glucosinolate concentrations measured every six months using HPLC-MS decreased only in meal samples stored at 4ºC, and to the greatest extent in samples stored within paper bags. This procedure can be used for maintaining glucosinolate stability and facilitating the utilisation of rapeseed cake as a biopesticide.

3.2 Allergens

The term allergen is used to identify substances that have the ability to promote two or three distinct molecular properties: i) the property to raise awareness (i.e., induce the production of antibodies of high affinity, particularly IgE, by the immune system), ii) ability to bind to IgE antibodies and also iii) the property to enable an allergic reaction (i.e., trigger allergic symptoms in a sensitised person) (Aalbers, 2000).

IgE-mediated reactions are believed to be responsible for most induced allergic reactions of the immediate hypersensitivity type (type 1), and the diagnosis relies on specific biological and clinical features. Such allergic reactions involve activation of effector cells, mainly mast cells and basophils, leading to an inflammatory response and specific clinical manifestations (Aalbers, 2000).

The pathogenesis in allergy has two phases: (i) usually, the primary contact with an allergen involves awareness of the naïve immune system to produce an IgE response and (ii) later repetitive exposure to the same allergen results in elicitation of an allergic reaction and the clinical manifestations (Moreno, 2007). The body's cells, having been previously sensitised, upon contact with the allergen are attracted to the place of antigen inoculation, and then try to orchestrate cellular mechanisms to eliminate and/or protect the body from further

damage, thus helping to exacerbate symptoms in allergic individuals (Sichere & Leung, 2008) such as sneezing, difficulty breathing, cramps, hives, itching, etc.

3.2.1 Plant allergens

The plant seed is not only an organ of propagation and dispersal but also the major plant tissue harvested by humankind, a major source of dietary protein. Although the vast majority of the individual proteins present in mature seeds have either metabolic or structural roles, a seeds also contain one or more groups of proteins that are present in high amounts and that serve to provide a store of amino acids for use during germination and seedling growth (Shewry et al., 1995).

Because of their abundance and economic importance, proteins are characterized into groups on the basis of their extraction and solubility in water (albumins), dilute saline (globulins), alcohol/water mixtures (prolamins) and dilute acid or alkali (glutelins). The major seed storage proteins include albumins, globulins and prolamins (Breiteneder & Radauer, 2004; Shewry et al., 1995).

Plant allergens can be also proteins that act as defense, enabling the plant to defend itself against biotic and abiotic stresses. Many plant tissues which are consumed by humans contain thousands of these allergenic proteins. Approximately 0.5% of the U.S. population is affected by various stages of IgE-mediated food allergy (El-Agamy, 2007, Breiteneder & Radauer, 2004).

Plant allergens are classified into families and superfamilies, based on their structure and function. Proteins are clustered into families if they have residue identities of 30% or greater or if they have lower sequence identities but their functions and structures are very similar. Families whose members have low sequence identities but whose structures and functional features are placed together in superfamilies (Breiteneder & Radauer, 2004). All storage protein fractions are mixtures of components that exhibit polymorphism both within single genotypes and among genotypes of the same species. This polymorphism arises from the presence of multigene families and, in some cases, proteolytic processing and glycosylation (Shewry et al., 1995). Additionally, it has become evident that the level of exposure and the properties of the allergen itself are important factors for determining its allergenic potential (Breiteneder & Mills, 2005).

Plant food allergens belong to the most abundant cupin and prolamin superfamilies and the protein families of the plant defence system. In the cupin superfamily are grouped the 7S and 11S seed storage proteins and transfer proteins [nsLTPs], α-amylase/trypsin inhibitors; the prolamin storage proteins of cereals are grouped into the prolamin superfamily (Breiteneder & Radauer, 2004).

According to many studies, it is known that the oilseeds described in this review (castor bean, physic nut and rapeseed) have allergens from the 2S albumin family. So, the study of the structure/function of these allergens will be addressed.

3.2.2 2S albumin

The 2S albumins are a major group of storage proteins present in many dicotyledonous plant species (Shewry et al., 1995). These proteins belong to the prolamin superfamily that includes the nsLTPs and cereal seed inhibitors of α-amylase/trypsin or both. All the proteins of this superfamily are of low molecular weight, are rich in cysteine and present similar three-dimensional structures rich in α-helix (Breiteneder & Radauer, 2004).

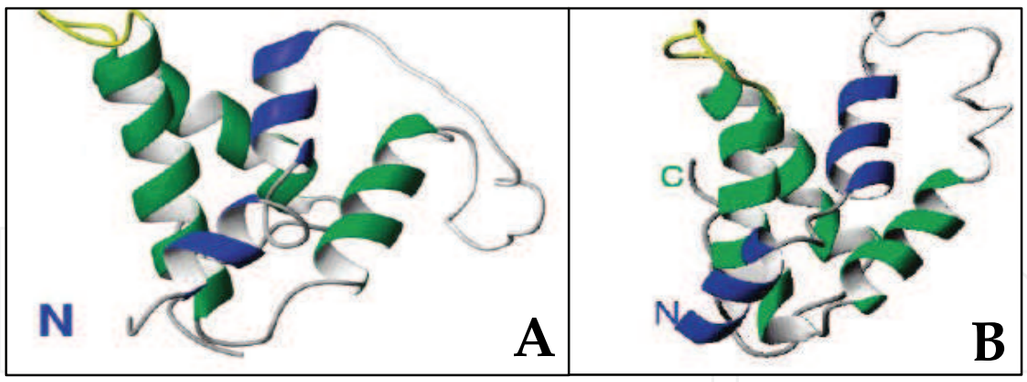

Fig. 2. (A) Ribbon representation of the solution structure of rRicC3, showing helices (blue and green) and loops (gray, but the hypervariable loop in yellow) (Pantoja-Uceda et al., 2003). (B) Ribbon representation of the solution structure of rproBnIb (Pantoja-Uceda et al., 2004)

The Figure 2 shows two 2S albumins; in (A) the three dimensional structure of recombinant RicC3 determined by NMR methods (Pantoja-Uceda et al., 2003) and in (B) the structure of the precursor form of the recombinant napin BnIb, rproBnIb (Pantoja-Uceda et al., 2004). Both 2S albumins show similar three-dimensional structures rich in α-helix.

- Ric c 1 and Ric c 3:

The 2S albumins from castor bean are synthesized at specific times during seed development and deposited within vacuoles (corpuscle protein) during seed development, then can be degraded during germination, supporting the growth of the seed (Ahn & Chen, 2007; Regente & La Canal, 2001). They are synthesized in the endoplasmic reticulum as a precursor protein of high molecular weight, Figure 3. Later, this precursor is proteolytically cleaved, generating a peptide ligand and other small peptides (Jolliffe et al. 2004; Shewry et al., 1995). Glycosylation of proteins may occur during protein synthesis when carbohydrates are incorporated, mostly mannose and glucosamine (Jolliffe et al. 2004; Bewley & Black, 1994).

It was believed that the 2S albumins were metabolically inactive, but currently, due to their ability to inhibit proteinases, alpha amylase (Nascimento, 2011) as well as their allergenic (Machado & Silva, 1992) and antifungal (Aggizio et al. 2003) properties, it is believed they are involved in defence functions in plants (Regente& La Canal, 2001). The allergenic properties of 2S albumins are resistant to thermal and chemical denaturation, possibly even detoxification treatment, and the allergy may be triggered by contact and inhalation (Machado & Silva, 1992; Silva Jr. et al., 1996). The 2S albumins are also able to reach the gut immune system intact so as to induce sensitization and elicitation of allergic reactions at the gut mucosa (Pantoja-Uceda et al., 2004).

Historically, in 1943, Spies and Coulson described one protein fraction of low molecular weight, heat stable protein from castor bean seeds, which was designated CB-1A (Castor Bean allergen). In 1947, hypersensitivity triggered by castor bean was first described, and in 1977, Li and co-workers isolated and characterized a protein from the seeds of *Ricinus communis* L. with low molecular weight and high glutamine content, which showed properties similar to those proteins previously isolated from castor beans. Later, in 1978,

Youle and Huang showed that CB-1A was the same storage protein characterized by Li et al. in 1977. In 1982, Sharief and Li isolated and sequenced a protein from the seeds of *Ricinus communis* L. (Ric c 1), with coefficient 2S sedimentation, consisting of two subunits linked by sulphur bridges. The smallest contained 34 amino acids (Ric c 1 small chain) with an apparent molecular mass of 4 kDa and the larger subunit contained 61 amino acids (Ric c 1 large chain) with a molecular mass of 7 kDa.

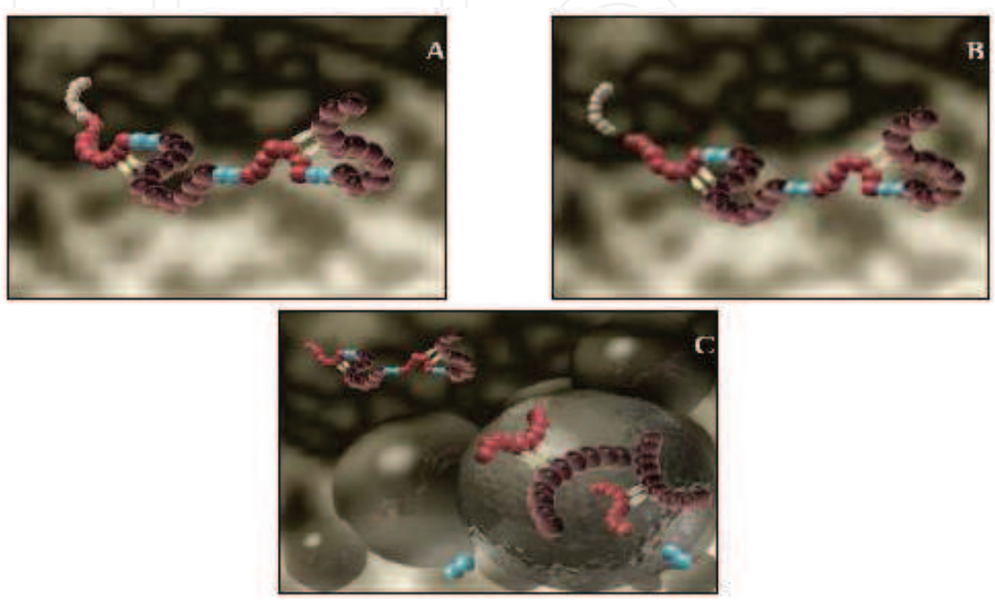

Fig. 3. Schematic of the processing of the precursor isoforms Ric c 3 and Ric c 1. A) Precursor signal peptide intact with beige, yellow sulphur bridges, Ric c 3 and Ric c 1 respectively in red (light chain) and brown (heavy chains), peptide binding in blue, B) Loss of signal peptide, C) loss of peptide connection with subsequent separation of the two isoforms

In 1992, Machado and Silva isolated and sequenced one second allergen of the castor bean seeds, named Ric c 3, with molecular weight around 11 kDa, present in the same precursor of Ric c 1 with 29 kDa. The primary structure of the allergen was fully elucidated in 1996. Since 2003, many other allergenic proteins belonging to the 2S albumin class have been identified in castor bean seeds by Machado and co-workers (Felix et al. 2008).

Currently, it is known that the allergen complex CB-1A represents about 12.5% by weight of the cake, as determined by the precipitation test with the antigen diluted. This complex consists of approximately 20 isoforms, with molecular mass between 10 and 14 kDa (Machado et al, 2003, Machado & Silva, 1992).

It is known that allergic diseases have increased in recent years and that over 30% of the population suffers from allergic diseases. The main causative agents are pollen, fungal spores, dust mites, animal epithelia. (Prueksakorn & Gheewala, 2008; Robotham et al., 2002). Medical problems such as conjunctivitis, rhinitis and urticaria have been associated with castor bean seeds, as well as the pollen (Garcia-Gonzalez et al., 1999).

The allergy triggered by the 2S albumin of castor bean is mainly caused by the inhalation of cake dust, representing a problem for the workers in extraction plants and for the population that inhabits the area around of these extraction plants (Garcia-Gonzalez, et al.,

1999). Another factor to be considered is the risk of allergic reactions of field workers using the castor cake as a fertilizer and who are subject to the dust.

There are few reports regarding the role of allergens in their pollen. In India, a study conducted by Singh and co-workers in 1992 demonstrated that there is variation in the protein profile of extracts of castor bean pollen in different years and places in this country. In 1997, the cross-reaction and the presence of common epitopes between seed and pollen extracts of castor beans were confirmed (Singh et al., 1997). That same year, some studies demonstrated a cross-reaction of castor bean pollen with pollen from other plant species, *Mercurialis annua* (Vallverdú et al., 1997) and *Putranjiva roxburghii* (Singh et al., 1997). In 1999, studies performed by Garcia-Gonzalez et al. demonstrated that the castor bean pollen causes symptoms of respiratory allergy. Accordingly, Paru and co-workers in 1999 proposed a new approach for identification and partial characterisation of allergenic proteins from the pollen of *Ricinus communis* L. In 2002, Palosuo et al. demonstrated the cross-reactivity between allergens from castor beans and other vegetables of the Euphorbiaceae family, confirming the importance of studies of cross-reactivity in diagnostic research.

Singh & Kumar in 2003 demonstrated, quantitatively and qualitatively, the prevalence of pollens in the region of India, noting that, among other aeroallergens, there is a significant distribution of castor bean pollen in this area. Knowing also that air pollution has been described as an important factor for the recent increase in the incidence of respiratory diseases and that the air carries many grains of pollen, the work done by Bist et al. in 2004 observed a variability of castor bean pollen protein before and after exposure to air pollutants.

- Jat c 1:

Seeds and pollen in general present allergenic proteins with additional defense properties such as proteases, amylase inhibitors or antifungal factors. Though protective for the plant, these antinutritional and toxic factors may have deleterious effects or even be toxic to animals and humans. Nothing was known about the presence of allergens in *J. curcas* seeds until the work of Maciel et al. (2009) which provided further information on the presence of allergenic proteins in this oilseed.

Maciel and co-workers, in 2009, described the presence of an allergenic 2S albumin (12 kDa), called a Jat c 1 (Figure 4), isolated from seeds of *Jatropha curcas* L. These N-terminal sequences presented similarities with 2S albumin from *Ricinus communis*, *Cucurbita maxima*, *Sesamum indicum*, *Solanum lycopersicum* and *Helianthus annus*. Sequence analysis revealed an important common feature: the conservation of four cysteine residues that are important for 2S albumin folding.

Jat c 1

(Small chain): VRDK**C**GEEAERRTLXG**C**ENYISQRR

(Large chain): PREQVPRQ**CC**NQALE

Fig. 4. Partial sequence data of *Jatropha curcas* 2S albumin. Data sequencing was performed by Edman degradation (Maciel et al., 2009)

Maciel et al. in 2009 also demonstrated the ability of this allergenic protein binding to IgE attached to rat mast cells, inducing histamine release from these cells. Its allergenic properties were demonstrated by the PCA test, a type I allergic reaction in vivo. Another feature shown by Maciel was that 2S albumin isolated from physic nut also showed strong crossreactivity with the major allergens from castor bean, Ric c 1 and Ric c 3. These data indicated that an individual sensitized to allergens from the castor bean (Ric c 1 and Ric c 3) could become sensitive to 2S albumin from *J. curcas* (Jat c 1) and that the inverse condition may also be possible, suggesting that Jat c 1 has potential intrinsic allergenicity.

Since allergy to oleaginous seeds has emerged as an important clinical condition following an increase in the use of biodiesel, and given the risk due to cross-reactive allergens (as observed for allergens from *J. curcas* and *R. communis*), advances in the identification and characterization of common aeroallergens and allergens from oleaginous seeds are necessary for the establishment of a specific therapy.

- Napins:

The oilseed rape (*B. napus*) ranks as the most commonly grown oilseed crop in Europe (Krzyzaniak, et al., 1998). Rapeseed (*Brassica napus* L.) is mainly produced due its high oil content (45-50%). After oil extraction, a meal is obtained containing most of the proteins (30-40%) (Boucher et al., 2007; Pantoja-Uceda et al., 2004).

Rapeseed protein meal contains two predominant classes of seed storage proteins: 12S globulin (cruciferin) which represents 25–65% of its protein content (Raab et al., 1992) and 2S albumin (napin). Napins belong to the 2S albumin class of proteins and hence are water soluble, stable at high temperature (up to 88±C) (Krzyzaniak, et al., 1998) and represent 15-45% of the total rape seed protein content depending on the variety (Raab et al., 1992). These proteins belong to the albumin storage proteins; in the seeds of recent varieties, they are present in lower quantities than cruciferins.

Various forms of napins (2S albumin) are also found in seeds of other Brassicaceae. They can be classified into three classes according to molecular weight 12.5, 14.5 and 15 kDa (Monsalve & Rodrigues, 1990).

Mature napins exhibit molecular weights between 12,500 and 14,500 Da (Raab et al., 1992). They are encoded by a multigenic family, initially synthesized as a precursor which is proteolytically cleaved to generate mature napin chains. Napins are expressed during seed development as precursors of 21 kDa. They comprise two polypeptide chains held together by two disulphide bonds: a small (4500 Da) and a large one (10,000 Da) (Krzyzaniak et al., 1998). The large chain includes two additional intrachain disulphide bonds, which reinforce the stability of the proteins (Byczynska & Barciszewski, 1999; Monsalve & Rodriguez, 1990). Napins are characterised by their strong basicity (isoelectric point, pI ~ 11) mainly due to a high amidation of amino acids (Raab et al., 1992).

Napins are polymorphic proteins due to their origin from multigene families. As a result, their isolation from the seeds renders a microheterogeneous material unsuitable for three-dimensional structure determination, by either X-ray diffraction or NMR (Rico et al., 1996).

Many isoforms of napin exist because of the large number of napin genes and differences in proteolytic cleavages. Five isoforms were first identified according to their molecular weights (Monsalve et al., 1991). One of them (isoform BnIb, called 2SSI-_BRANA in the Swiss-prot databank nomenclature) has been totally sequenced and its three-dimensional structure determined by NMR (Pantoja-Uceda et al., 2004; Rico et al., 1996). BnIb (12.7 kDa)

is a representative member of a distinct group of rapeseed 2S albumins, referred to as "low molecular weight napins" (LMW-napins) to distinguish them from the more common and abundant group of "high molecular weight napins" (14.0-14.7 kDa) (Monsalve et al., 1991).
The 2S albumin class of proteins constitutes the major seed storage protein group in *Brassica napus*, representing about 20% of the total protein content in mature rape seeds. 2S Albumins from several species such as mustard, castor bean, Brazil nut, English walnut, sunflower and peanut have been shown to be type I allergy inducers of remarkable incidence, suggesting that this family of storage proteins is intrinsically allergenic (Pantoja-Uceda et al., 2004).
Coincidental with the expansion of rapeseed cultivation, there have been increases in the number of reported cases of asthma and other conditions related to allergenicity and irritancy, but it is not clear evidence that rapeseed has adverse effects on human health (Murphy, 1999). The work conducted by Murphy (1999) described that the allergens present in rapeseed pollen have only a minimal impact on public health.
The distinction between oilseed rape and grass pollen was described by Welch and co-workers in 2000. They showed that these pollens are immunologically distinct and there is no evidence of cross-reactivity between them. Individuals allergic to grass pollen will not necessarily develop a specific nasal or airway response to inhaled oilseed rape pollens.
Chardin et al. 2008 aimed to characterize the IgE specificity of various patients suffering from pollen polysensitization to identify both peptidic and carbohydrate cross-reactive determinants. They showed the rapeseed, grass and Arabidopsis proteins were separated by isoelectric focusing, followed by SDS-PAGE, and transferred to a nitrocellulose sheet. They showed that multiple pollen sensitizations could result from multiple sensitizations to specific proteins or from a cross-sensitization to a wide range of glycoproteins. That paper also allowed for improving the diagnosis of allergy and its medical treatment.
Knowing that the oilseed rape production is widespread in cereal growing areas and that many patients who attend the clinic (district general hospital, UK) for seasonal allergies claim that they are allergic to it, the aim of the work in development by Trinidade et al. (2010) is to determine the prevalence of oilseed rape allergy in this population. They observed that oilseed rape hypersensitivity was relatively uncommon, comprising only 2% of the population tested (n = 28). Oilseed rape does not cause significant allergy, even in areas of high production. It is likely that those patients exhibiting oilseed rape allergy may in fact be symptomatic due to the effect of other allergens, acting synergistically with the oilseed rape allergen (Trinidade et al., 2010).

3.2.3 Solutions

Several methodological solutions for reducing or eliminating allergens can be used to obtain positive results. Heat processing induces, in most cases, irreversible denaturation of proteins, leading to aggregation, and such structural changes do not always correlate with decreased allergenicity. Depending on the system, heating may have no effect or it may decrease or increase allergenicity. This occurs because of the existence of sequential and/or conformational epitopes in allergen structure.
The knowledge of the protein's primary structure is essential for initial strategies for protein modification of its epitopes. Many studies have shown positive results with various experiments performed with unmodified and chemically modified proteins. In 2002, Cai and

co-workers identified the amino acid residues of allergenic proteins (trichosanthin, a Chinese herb) with an important role in the IgE response. Using an assay with these proteins mutated at their residues important for IgE binding, they showed that the protein specifically lost its binding activity and exhibited reduced IgE induction in the immunized mice. Kamal et al. (2005) described that the tryptophan residue is essential for immunoreactivity of a diagnostically relevant peptide epitope of *A. fumigatus.* The loss of specific IgG and IgE antibody binding of the modified protein by ELISA confirmed the critical role of tryptophan (Trp17) in the immunoreactivity of this protein. With the same objective, allergen modification and a better understanding of the functional role of castor bean allergens is fundamental to preventing allergy induced by *R. communis* (Ric c 1 and Ric c 3). Accordingly, Felix and co-workers (2008) showed the mapping of IgE binding epitopes of Ric c 1 and Ric c 3, the allergens from castor bean, by a mast cell degranulation assay. They identified four continuous epitopes in Ric c 3 and two in Ric c 1. This knowledge may allow the induction of protective antibody responses to antagonise the IgE recognition. All the data showed that the IgE epitope of these proteins were determined and shown to play a critical role in induction of IgE, and modification of the IgE epitope may be a useful strategy to reduce the allergenicity of an allergen. Deus-de-Oliveira evaluated the possibility of use of compounds of calcium in order to inactivate allergenicity of isolated 2Salbumin and castor cake. The samples were incubated with a solution of calcium hydroxide, calcium carbonate or calcium oxide, 4 and 8% in the ratio of 1:1 (v/v), during 12 hours, at the room temperature. The calcium treatments modified the allergen of castor bean and all they are effectives as was valued by reducing the allergenicity as observed by quantification of mast cells degranulation. Simultaneously, castor meal detoxification was also obtained using treatments with $CaCO_3$, $Ca(OH)_2$ and CaO. The results obtained in by Deus-de- Oliveira contribute to get of a safer product for manipulation of the workers and with the possibility of expanding the economical applicability, for example, in animal feed.

4. Conclusion

Oilseeds are renewable sources of oil, protein and carbohydrate for edible and industrial applications. Traditionally, the commodity value for oilseeds has been the meal (or cake) produced after mechanical pressing or solvent extraction oil from the seed. The press cake obtained after oil production could be used for animal feed but each of these cakes may have in its constitution toxic or allergenic compounds (Thelen, 2009).

The study of these structures, allergens and toxins allows better choices on the oilseed crop being planted extensively in order to allow better worker and population health. In addition, an understanding of the allergens and/or toxic compounds present in oilseeds allows us to propose methodological strategies to eliminate or reduce such compounds.

The challenge is huge in this direction because there is a large expansion in the application of other oilseeds for biofuel synthesis, and new allergens and toxic compounds need to be unravelled.

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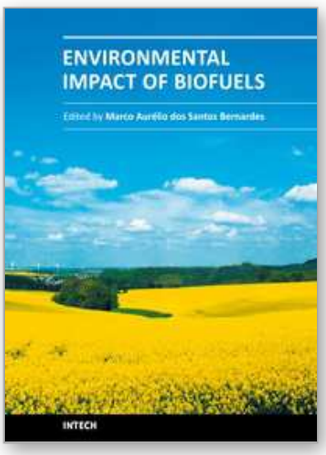

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This book aspires to be a comprehensive summary of current biofuels issues and thereby contribute to the understanding of this important topic. Readers will find themes including biofuels development efforts, their implications for the food industry, current and future biofuels crops, the successful Brazilian ethanol program, insights of the first, second, third and fourth biofuel generations, advanced biofuel production techniques, related waste treatment, emissions and environmental impacts, water consumption, produced allergens and toxins. Additionally, the biofuel policy discussion is expected to be continuing in the foreseeable future and the reading of the biofuels features dealt with in this book, are recommended for anyone interested in understanding this diverse and developing theme.

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