Chapter 3
Biotechnological Potential of Cottonseed, a By-Product of Cotton Production

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Abstract Cotton (*Gossypium hirsutum* L.) is an important fibre crop of global significance. It is grown and harvested in tropical and subtropical regions of more than 80 countries. The state of Chihuahua, in Mexico, is the leader in the production of cotton covering 70% of national production. According to statistics reported in 2016, 488,000 metric tons were obtained and utilized as follows: 93% for textile industry, 2.28% as cattle feed, 1.1% was re-harvested, and the other 3.56% was discharged, and in consequence an environmental impact occurs. That remaining cottonseed constitutes a potential agroindustry residue with biotechnological applications due to its chemical composition: fibre, proteins (as well as essential amino acids such as lysine, methionine, tryptophan, and other amino acids) carbohydrates, and lipids (it is important to highlight gossypol and the fatty acids profile). In this chapter, food and bioenergy applications of cottonseed in terms of bioactive compounds (phenolic content), bioactivity (antioxidant activity), and lipid content (production of biodiesel) are reviewed, as well as the chemical compounds responsible of such applications, different types of extraction methods and analytical protocols for their identification, purification, and quantification.

Keywords Bioactive compounds · Bioactivity · Biotechnological applications · Cottonseed

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Z. A. Zakaria et al. (eds.), *Valorisation of Agro-industrial Residues – Volume II: Non-Biological Approaches*, Applied Environmental Science and Engineering for a Sustainable Future, https://doi.org/10.1007/978-3-030-39208-6_3

3.1 Introduction

Cotton represents one of the most commercially important fibre crops due to its several industrial and agricultural applications (Egbuta et al. 2017; Mendoza et al. 2016; Wegier et al. 2016; McCarty et al. 2018). It has been utilized worldwide for more than 5000 years by different civilizations, being textile fibre the main use (Dugan 2019). About 50 different cotton plant species are found in nature, but only four of them have been domesticated: *Gossypium arboreum*, *Gossypium herbaceum*, *Gossypium hirsutum*, and *Gossypium barbadense* (Egbuta et al. 2017; http://www.cicr.org.in/pdf/cotton_varieties_hybrids.pdf). *G. hirsutum* constitutes approximately 90–97% of the world's cotton production due to its high yields and adaptability to different environmental conditions (Avci et al. 2013; Nix et al. 2017; Rocha-Munive et al. 2018).

Cotton is a semi-tropical or tropical climate crop; however, it is typical of warm areas. Cotton sowing is very sensitive: requires a proper conditionate land, deep soils, constant irrigation, and temperatures of 20–30 °C to grow (SIAP 2017). When cotton crops mature completely, they are processed for obtaining cotton bolls, which represents its main commercial significance (Hernandez 2016). However, important by-products can also be recovered from cotton processing chain, such as cottonseed, which is a value-added component for oil extraction and ruminant feeding due to its chemical composition (Egbuta et al. 2017; He et al. 2013; Bernard 2016; Okonkwo and Okafor 2016). Cottonseed also contains bioactive compounds, namely, antioxidants (flavonoids) or pigments (gossypol), which exhibit potential bioactivities (Nix et al. 2017; Tian et al. 2016).

In cotton ginning several components are present: sticks, stems, burrs, and mainly seeds. According to statistical reports (2018/19) of cotton production, 43.45 million metric tons of cottonseed were obtained, being the third oilseed produced in the world (https://www.statista.com/statistics/267271/worldwide-oilseed-production-since-2008/, 24 April 2019). Then, for an increased world cotton production, higher amounts of cotton residues are generated.

According to the aforementioned, cottonseed is suitable for food and bioenergy applications. Therefore, in the present chapter different types of extraction methods to recover the bioactive compounds present in cottonseed are reviewed as well as the analytical protocols for their identification, purification, and quantification for the diverse applications.

3.2 Cotton Production

World cotton production during 2018/19 was 25.89×10^6 metric tons; corresponding to an average yield of 780.5 kg/ha, for cultivated area of 33.18 million hectares. Compared to the period 2017/18, there was a decrease in world cotton production of 4.2%, because the main producers such as the United States, India,

Table 3.1 Major cotton producing countries during 2018/19

Position	Country	Production (1000 metric tons)
1	China	6042
2	India	5879
3	United States	4004
4	Brazil	2569
5	Pakistan	1676
6	Turkey	806
7	Uzbekistan	718
8	Australia	544
9	México	376
10	Greece	307
11	Mali	294
12	Benin	283
13	Argentina	250
14	Burkina	218
15	Turkmenistan	198

Source: Adapted from the Agricultural forum outlook (USDA 2019)

Pakistan, Central Asia, and Australia were affected by climatic shifts. On the other hand, a recent data (2019/20) forecast refers to an increase in global cotton production of 6.9%, equivalent to 27.54×10^6 metric tons (USDA 2019).

Table 3.1 shows the major cotton producing countries during 2018/19, and it can be seen that Mexico was the ninth cotton producing country, indicating an increase of 9.56% with respect to the period 2017/2018. A continuous annual increment (0.21%) is expected because cotton was included in the Mexican action plan "Planeación Agrícola Nacional 2017–2030", promoted by SAGARPA. According to data reported by SIAP in 2017 (SIAP 2017), 488,000 tons of cotton were produced in Mexico during 2016 and they were used as follows: 93% for textile industry, 2.28% was exported, 1.1% was re-harvested, and the residual 3.56% was considered waste. The latter can be suitable for potential biotechnological applications rather than being disposed of to the environment.

In Mexico, the northern states are leaders in cotton production. That area is arid and temperatures are warm, which is convenient for cotton cultivation. The main producing state is Chihuahua and it contributed with 708,332 tons in 2017. Other important producers are Baja California, Coahuila, Durango, Sonora, and Tamaulipas.

3.3 Chemical Composition of Cottonseed

Cottonseed chemical composition defines its quality and determines its different applications or uses as well as those of its by-products. Different intended or uncontrollable factors as growing location, variety, environmental conditions,

Table 3.2 Proximal composition of cottonseed

Parameter	Okonkwo and Okafor (2016) (% wt.)	USDA ARS (OECD 2015) (% wt.)	Commercial (OECD 2015) (% wt.)
Moisture	9.87	4.7	4.0–8.7
Crude protein	27.27	34.2[a]	21.8–28.2[a]
Crude fibre	22.94	21.4[a]	15.4–28.2[a]
Ash	4.55[a]	4.8[a]	3.8-4.9[a]
Lipid	27.83	36.3[a]	15.4-23.8[a]
Total carbohydrates	30.49	–	–

[a]Dry matter

genetic modifications, or agronomic approaches can affect cottonseed compositions (He et al. 2013; Okonkwo and Okafor 2016). Table 3.2 shows different proximal compositions of cottonseeds reported in studies or found in commercial seeds.

As it is depicted in Table 3.2, the moisture content is not elevated in cottonseed, which allows its preservation and reduces the growth of some microorganisms. On the other hand, protein, fibre, and lipid are the higher contents, which are important for feeding and can provide benefits to the digestive system. Lipids can also be extracted and further employed for the preparation of structured lipids or synthesis of biodiesel. Ash content is the inorganic matter where minerals are found. Cottonseeds are a rich source of magnesium (3.6 g kg^{-1} dry matter [dm]), manganese (16.0 mg kg^{-1} dm), copper (10.0 mg kg^{-1} dm), iron (70.0 mg kg^{-1} dm), phosphorous (5.9 g kg^{-1} dm), zinc (35.0 g kg^{-1} dm), potassium (12.0 g kg^{-1} dm), calcium (1.5 g kg^{-1} dm), and sodium (0.1 g kg^{-1} dm) (Heuzé et al. 2015).

3.3.1 Lipid Content

Vegetable fats and oils are mainly constituted by 98–99.5% of triacylglycerols (TAG), a trisubstituted glycerol (Nadeem et al. 2015). Common fatty acid profile is rich in unsaturated fatty acids, since they represent around 75%, while the remaining are saturated; consequently, showing an unsaturated/saturated ratio of 0.35. The unsaturated fraction is mainly conformed by oleic and linoleic residues, which represent 22 and 52% of the profile, respectively. Palmitic acid, on the other hand, is the major saturated fatty acid corresponding to a 24% content. In that matter, palmitic, oleic, and linoleic acids constitute more than 90% of the fatty acid composition of cottonseed (see Table 3.3). TAG content in cottonseed comprises mainly di-unsaturated (UUS, 43.4%) and tri-unsaturated (UUU 35.7%), and a low concentration of monounsaturated (USS, 12.5%) (Jahaniaval et al. 2000).

On the other hand, Table 3.4 shows the most common distributions of the saturated and unsaturated fatty acids in the different positions (*sn*-1, -2, and -3) of TAG backbone.

Table 3.3 Fatty acid profile of cottonseed

Fatty acid		Typical content (%)	Range (%)
C14:0	Myristic	0.66	–
C16:0	Palmitic	24.55	21.4–26.4
C16:1	Palmitoleic	0.66	–
C17:0	Margaric	0.19	–
C18:0	Stearic	0.21	2.1–3.3
C18:1 n9 cis	Oleic	19.05	14.7–21.7
C18:1 n9 t	Elaidic	2.10	–
C18:2	Linoleic	51.54	46.7–58.2
C18:3	Linolenic	0.19	0–1
C20:0	Arachidic	0.21	–
C20:1	Eicosenoic	0.12	–
C22:0	Behenic	0.17	–
Unknown compound	–	0.34	–
Total SFA	–	26.00	–
Total UFA	–	73.66	–
Total MUFA	–	21.93	–
Total PUFA	–	51.73	–
SFA/UFA	–	0.35	–

SFA Saturated fatty acids, *UFA* Unsaturated fatty acids, *MUFA* Monounsaturated fatty acids, *PUFA* Polyunsaturated fatty acids
Adapted from Mohdaly et al. (2017) and List (2017)

Table 3.4 Estimated composition of triacylglycerols (TAG) present in cottonseed oil

TAG profile	% wt.
PPoP	0.62
POP	3.67
POS	0.54
MLP	1.16
PLP	13.74
PLS	3.91
PPoL	1.93
POL	14.30
SOL	1.31
MLL	1.11
PLL	26.58
SLL	4.75
PoLL	1.30
OLL	10.43
LLL	12.88
Others	1.77

P Palmitic, *Po* Palmitoleic, *M* Myristic, *S* Stearic, *O* oleic, *L* Linoleic, *Ln* Linolenic, *A* Arachidic
Adapted from Ceriani and Meirelles (2004)

Table 3.5 Phytosterol profile in cottonseed oil

Sterol	Codex Alimentarius (1999) (%)	Yücel et al. (2017) (mg/kg)
β-Sitosterols	76.0–87.1	2411 ± 26
Y-Sitosterol	–	–
Campesterol	6.4–14.5	135 ± 4
Stigmasterol	2.1–6.8	27 ± 1
Cholesterol	0.7–2.3	–
Brassicasterol	0.1–0.3	–
Delta-5-avenasterol	1.8–7.3	–
Delta-7-stigmasterol	nd–1.4	–
Delta-7-avenasterol	0.8–3.3	–
Others	nd–1.5	–
Total (mg/kg)	2700–6400	2573

nd not detected

It is worth noting that there exists a trace percentage (0.5%) of two unique fatty acids that have some toxicity properties in the lipid fraction of cottonseed: malvalic and sterculic fatty acids. These residues are characterized by the presence of a cyclopropenoid subgroup in their chemical structure and are better known as cyclopropenoid fatty acids. Nevertheless, when the cottonseed lipids are extracted (oil), the conventional refining process reduces their contents ten times.

Other important lipid family found in cottonseed is phospholipids (PLs). The amount of PLs is very low and is typically measured in 0.7–0.9%, and they are included in the so-called "gums". PLs despite the fact that they generally benefit health properties lowering blood cholesterol, improve muscle performance, and resilience, among others, can affect the oil quality as well as the refined oil yield. PLs acts as an emulsifier, interfering in the neutralization, bleaching, and deodorization steps of the refining process, thus, enhancing oil losses, decreasing shelf-life, and promote that the oil turns dark (Ghazani and Marangoni 2016).

Phytosterols are also present in cottonseed oil (Table 3.5). These chemical compounds exhibit health-promoting effects like the decreasing of cholesterol, as well as antimicrobial, anti-inflammatory, and anticancerogenic properties. Nevertheless, phytosterols do not influence any physicochemical oil property. Between the phytosterols contained: β-sitosterol, campesterol, stigmasterol, and D5-avenesterol, typically β-sitosterol is the most abundant (Codex Alimentarius 1999); however, Mohdaly et al. (2017) reported Y-sitosterol as the most concentrated.

Cottonseed oil is also relevant for its tocopherols content, which reaches ca. 1000 ppm. There are four homologues (α-, β-, γ-, and δ-) and all of them are considered as derivatives of vitamin E, which have powerful antioxidant activities and several nutritional benefits. Moreover, they are a natural oxidative stabilizer of polyunsaturated fatty acids, prolonging the oil operational lifetime, and the shelf-life of foods that contain them. Only α- and γ-tocopherols are usually found in cottonseed oil (Table 3.6) and α-tocopherol is the most reactive of the homologues (Hernandez 2016; List 2017; Lepak et al. 2016).

Table 3.6 Tocopherol profile in cottonseed oil

Tocopherol	Codex Alimentarius (1999) (mg/kg)	Bockisch (1998) (%)	Yamamoto et al. (2018) (mg/kg)[a]
α-Tocopherol	136–674	60–80	360
β-Tocopherol	nd–29	nd	nd
γ-Tocopherol	138–746	20–40	310
δ-Tocopherol	nd–21	nd	nd
Total (mg/kg)	380–1200	900–1100	670

nd not detected
[a]Average values ($n = 3$)

3.3.2 Gossypol

All cotton species present a very particular compound distributed all over the plant, and it is known as gossypol, which is mainly concentrated in seeds (Gadelha et al. 2014). Its concentrations can vary depending on the cotton species and weather, but for *G. hirsutum* a range value of 0.5–1.89% of total gossypol has been reported (Scheffler 2016).

Gossypol is a phenolic terpene aldehyde with antioxidant and toxicity activities produced by pigment glands as defence mechanisms (pests and pathogens), and for the environmental adaptation of the plant (Tian et al. 2016; Scheffler 2016). The pigment glands also produce other 14 phenolic pigments like gossypurpurin (purple) and gossyfulvin (orange), but in depreciative concentrations compared to gossypol (yellow-green) (Gadelha et al. 2014; Cope 2018).

Gossypol consisted of a mixture of two enantiomers: (+)- and (−)-gossypol, which in the case of *G. hirsutum* are approximately present at 60% and 40%, respectively (Alexander et al. 2009). Moreover, most parts of (+)/(−) gossypol enantiomers are bound to proteins (mainly to their lysine and arginine residues), but low amounts remain as free gossypol (Gadelha et al. 2014). Despite both enantiomers can be equally effective against some hazardous factors as insects, (−)-gossypol exhibits higher bioactivity, thus, it is generally more toxic and more difficult to be removed (Alexander et al. 2009; Kakani et al. 2010).

The chemical formula and name of gossypol are $C_{30}H_{30}O_8$ and 1,1′,6,6′,7,7′-hexahydroxy-5,5′-diisopropyl-3,3′-dimethyl-(2,2′-binaphthalene)-8,8′-dicarboxaldehyde, respectively. It contains a polyphenolic structure with three hydroxyl and one aldehyde groups bound in each naphthyl scaffold. The two enantiomers are a consequence of the restricted rotation around the internaphthyl bound (C2–C2′). The modification or removal of the functional groups (hydroxyls and aldehydes) through acid mediums, oxidation, ozonification, methylation, etc., produce a wide variety of gossypol derivatives, namely tautomeric forms, Schiff bases, among others. These derivatives exhibit different important biological activities like contraceptive, antimicrobial, antiviral, antiparasitic, antitumor, and antioxidant (Wang et al. 2009; Lu et al. 2017). Such bioactivities are further described in

Sect. 3.5.3. However, from food perspective, gossypol is the main limiting factor, since their intakes can produce liver damage (hepatotoxicity), poisoning, anaemia, disruption of reproduction (affects fertility and embryogenesis), and immunotoxicity (reduction of leukocytes) (Gadelha et al. 2014; Cope 2018).

Much effort has been devoted for the elimination of gossypol and to enable cottonseed-safe food uses. Some strategies developed to accomplish this, are the processing of glandless cottonseeds and ultra-low gossypol seeds. Glandless cottonseeds can be obtained by removing the glands by mechanical separation, addition of iron salts, or the use of high polar solvents (e.g. anhydrous acetone). The composition between glanded and glandless cottonseeds is similar (Hernandez 2016). The main benefit of glandless cotton is the absence of gossypol, but on the same hand, it also constitutes the main drawback because cotton plant loses its defence mechanisms and is exposed to plagues and diseases, limiting their cultivation to small scales (Tian et al. 2016).

3.3.3 Flavonoids

Flavonoids represent an important control for pest and herbivore, so they guarantee plant health and optimal functioning. They also play an important role in plant growth (Brown et al. 2001) and fibre development (Tan et al. 2013). They also have a potential antioxidant activity (Denev et al. 2013) and contribute to the colouring of the majority of plants flowers, leaves, seeds, among others, exhibiting by themselves a yellow colour (Egbuta et al. 2017). Flavonoids are conformed by several classes, namely, aurones, anthocyanins, biflavonoids, flavanols, flavones, flavonols, flavanones, and isoflavones. According to Nix et al. (2017), 52 flavonoids have been identified in *G. hirsutum*, where eleven are present in the seeds in the form of flavonols (Kaempferol, Kaempferol 3-diglucoside, Kaempferol-3-*O*-neohesperidoside, Isoquercitrin, Quercetin 3-diglucoside, Quercetin-3-*O*-neohesperidoside, Quercetin-3-*O*-robinoside, Rutin and Spiraeoside) and one flavanol.

3.3.4 Cottonseed Proteins

Cottonseeds, as it has been stated before, exhibit a high content of protein. Cottonseed proteins are highly degradable since approximately 70% of total proteins are soluble (albumin and globulin) (Heuzé et al. 2015), where vicilin and legumin families constitute the major components of the protein fraction. Different biological functions of the cottonseed proteins have been recognized which include storage, transcription/translation, synthesis, energy metabolism, antimicrobial activity, and embryogenesis. Moreover, such as oils, some proteins exhibit functional activities (He et al. 2018). Cottonseeds' protein also contains high concentrations of essential amino acids, where lysine and valine are the most abundant.

3.4 Extraction Methods

According to Sect. 3.3, the chemical composition of cottonseed is remarkable for the presence of compounds with important biological and biochemical functions. During so long, agroindustry residues were not considered and they were just disposed to the environment. Nowadays, with the development of novel analytical protocols for the identification, purification, and quantification of chemical compounds, the extraction of the different value-added components present in cottonseed is achievable. Such protocols are described as follows.

3.4.1 Oil–Cottonseed Meal

There exist several oil extraction methods and a single or a combination of them can be conducted. Before proceeding with the extraction, oil seeds need a preconditioning process to efficiently extract and remove impurities. The conditioning consists of the cleaning, delinting, dehulling, separation, flaking, and cooking of cottonseeds. The cottonseed meal is the defatted cake that remains after the kernels oil extraction.

3.4.1.1 Mechanical Extraction

Pure physical separation of the liquid (oil) and solid (cottonseed meal) phases, by the disruption of the lipid cells. Hydraulic or screw presses can be used for the extraction. Their advantages are: (1) chemicals are not applied and (2) low energy and equipment costs, compared to other methods such as solvent extraction. However, this method compromises oil yield, since 85% is generally the maximum yield achieved (Arişanuv and Rus 2017).

3.4.1.2 Solvent Extraction

Solvent extraction can be directly applied or after a pressing step (hydraulic or screw press), and it is a conventional extraction method for the oil industry. The solvent typically utilized for carrying out oil extraction is n-hexane, due to high yields reached and its commercial availability. Other solvents namely, acetone, aqueous ethanol, methylpentane, isohexane, heptane, petroleum ether, trichloroethane, chlorinated hydrocarbons, among others, can be also employed. Besides solvent, other operational parameters including temperature, solvent:oil ratio, particle size, and time, can also affect extraction yield. By means of this method, significant yield can be achieved (ca. 98%), but it requires large investment for equipment, energy demand, cost of solvents, and a further separation step. Saxena et al. (2011)

conducted a comparison between n-hexane and ethanol extraction. They found that both solvents at a temperature of 45 °C, at a ratio of 10:1 (solvent:seed) and a particle size of 0.6 mm, an oil yield of >99% was attained. However, ethanol is safer and eco-friendlier than *n*-hexane. Moreover, cottonseed meal from ethanol extraction contained 50% less gossypol than that of *n*-hexane.

3.4.1.3 Supercritical Fluid

Supercritical fluid is the most promising extraction method due to its simplicity, high efficiency, high oil quality, and short process time, compared to solvent methods; however, the expensive equipment and the impossibility of a continuous extraction are the main drawbacks for its sustainability. To perform the extraction, a supercritical fluid, such as CO_2, is used. Bhattacharjee et al. (2007) optimized a supercritical fluid extraction of cottonseed oil, by monitoring temperature, pressure, and time extraction effects using central composite rotate design and response surface methodology. Optimal conditions were 550 bar, and a temperature and extraction time between the ranges of 70–80 °C and 2–3 h, respectively. Temperature and pressure were the significant parameters for the efficiency of the extraction, and the maximum reported oil yield extraction was 17.26%. The oil yield increased proportionally to the pressure, but no further values were used due to operational limitations.

3.4.1.4 Microwave-Assisted Extraction

Attention has been paid to this novel method. By means of microwave-assisted extraction (MAE), a more efficient oil extraction can be obtained due to the oilseed cells rupture. Temperature, solvent:sample ratio, moisture, and properties of both solvent and sample are the variables to take into account to assay this method. Studies have agreed that the use of MAE enhance yields and require less solvent extraction. Other remarkable advantage is that MAE improves phenolics compounds extraction. Taghvaei et al. (2014) optimized a cottonseed MAE by increasing oil extraction under the optimal extraction times, solvent concentrations and moisture of samples using response surface methodology. Optimal conditions were as follows: 3.57 min of irradiation time, 14% of moisture content, and a ratio of 1:4 (sample: solvent). Following the previous conditions, they achieved an extraction efficiency of 32.6% and a phenolic content of 46 ppm.

3.4.2 Bioactive Compounds

Bioactive compounds (phenols, flavonoids, tocopherols) can be extracted by a wide variety of techniques, which also include the aforecited methods: solvent extraction, microwave- and ultrasound-assisted extraction, supercritical fluid extractions, maceration, pressurized liquid extraction, and subcritical water extraction. Additionally, solvents used are as important as the extraction methods. Polar solvents are the conventional system for the recovery of these compounds. Since the nature of bioactive compounds varies depending on their chemical structures, their affinity for a specific polarity of a solvent varies as well.

Commonly used solvents are ethanol, methanol, acetone, hexane, ethyl acetate mixtures with water (Do et al. 2014). In the case of gossypol, different solvent systems are required, such as aqueous acetone, acetic acid, petroleum ether, anhydrous acetone, acetone–hexane, methylene chloride, and hexane–acetic acid mixtures (Hernandez 2016). Once the bioactive compounds have been extracted, a separation step must be conducted for their identification and quantification. The most used methods for that purpose include gas chromatography (GC), high-performance liquid chromatography (HPLC), capillary liquid chromatography (CLC), thin-layer chromatography (TLC), among others. HPLC is the most sophisticated analytical protocol for the accurate characterization of these types of compounds, due to versatile detectors fitted to the apparatus: photodiode array, ultraviolet, fluorescence, and mass spectrometer.

3.5 Cottonseed By-Products Applications

The chemical composition of cottonseed allows the preparation of high-value products. Cottonseed oil (CSO) and cottonseed meal (CSM) are the main by-products of interest. On the other hand, remarkable bioactivities are exerted by the bioactive compounds also found in cottonseed.

3.5.1 Oil

Frying Oil Due to the amount of SFA and UFA content, high levels of antioxidants (e.g. tocopherols), and the absence of linolenic acid, refined CSO is distinguished as frying oil. SFA (palmitic acid) and tocopherols prevent oil oxidation; because of that, hydrogenation is not necessary, decreasing trans-fatty acids. The high content of linoleic acid (omega-6) favours the texture, mouthfeel, flavour of food, and promotes their shelf life. Arslan et al. (2016) developed different refined CSO and palm olein oil (POO) blend to obtain high-quality oil with improved stability during the frying process. By mixing the vegetable oils, the bioactive lipids, antioxidants (tocopherols and sterols) were increased, and the SFA/UFA ratio was balanced. More SFA was also increased with respect to UFA without compromising the sensorial and nutritional properties of foods, since SFA produces undesirable flavours. The oxidative stability was measured by the detection of polar and polymeric compounds; oxidation products. Their two different blends: A (50% CSO:50% POO) and B (40% CSO:60% POO) showed better quality properties, since the polar and polymeric compounds for blend A and B increased only in 2.27% and 5.40%, respectively, while pure CSO exhibited an increase of 6.30%.

Biodiesel This is one of the most important and promising alternatives against the worldwide depletion of fossil fuel reserves. Crude CSO is a potential feedstock for biodiesel production, since its fatty acid composition confers the proper physicochemical properties required to meet the international quality standards. Biodiesel is obtained via transesterification of a vegetable oil/animal fat and a short-chain alcohol (usually methanol or ethanol) in the presence of a catalyst. Malhotra and Ali (2018) produced biodiesel from virgin CSO utilizing a novel solid catalyst (5-Na/ZnO/SBA-15). They could obtain more than 98% biodiesel yield after 4 h, for a methanol: oil ratio of 24:1, a catalyst concentration of 12% (wt.), and 65 °C. Jamshaid et al. (2018) optimized the biodiesel yield from CSO using the response surface methodology. The optimized variables were oil:alcohol molar ratio, catalyst concentration, temperature, and stirring speed. From their results, a yield of 98.3% was reached for a 6:1 methanol–oil ratio, 0.97% (w/w) of catalyst concentration, 63.8 °C, and 797 rpm. However, there is still a lot of work to do to attain a sustainable production of biodiesel in terms of catalyst (mainly the use of enzymes) and reaction systems.

Edible Oleogel Oleogels have proven to be a potential alternative to products with high lipid content and SFA, such as shortenings; hydrogenated vegetable oils that are solid at room temperature. Moreover, providing the same physicochemical and sensorial properties that are typically conferred by fats. Basically, this is the application consisted of the transformation of a liquid gel to a 3D network, obtaining a gel-like structuring of lipids. This process is conducted by adding a gelator molecule (plant waxes) to the oil of interest and its properly named oleogelation or organogelation (O'Sullivan et al. 2016). Pehlivanoglu et al. (2018) produced different oleogel blends with the aim of reduced fat content of cakes and their SFA as well as by replacing one of their main ingredients: shortenings. The formulation for the different blends included different percentage compositions of high oleic sunflower (HOS), CSO, and blend fat. All the formulations contained CSO due to the fact that it is constituted of >70% of UFA. Once the cake was prepared, their physicochemical and sensory properties were analyzed. Despite some quality properties were significantly affected (e.g. texture), the sensory scores showed that the cakes with oleogels were accepted, being the blend of 50% CSO:50% HOS the most acceptable. Moreover, they reported that all of the formulated oleogels exhibited a less SFA content.

Shortening CSO is a popular ingredient of many shortenings/margarines because of the high value of palmitic acid it presents. As the oil solidifies, it develops tiny fine crystals (β′-crystals) that forms a 3D-network (emulsion) and trap the oils. This confers smooth flavours, good texture and plasticity, good creaming properties, higher temperature stability, and an extended shelf life to foods that contain it. Shortenings are very important for food industry, namely bakery, pastry, chocolate, confectionery, among other industries. However, shortenings are typically hydrogenated to obtain specific textures and functionalities. Consequently, trans-fatty acids are produced in the process, which have harmful effects on health. Interesterification of oils is an alternative that provides similar effects, and CSO has been used for their production. Imran and Nadeem (2015) used different canola oil (CO) and hydrogenated cottonseed oil (HCSO) mixtures for their interesterification. They concluded that the interesterified mixture of 50% CO:50% HCSO exhibited the best physicochemical and sensorial characteristics, and that all the mixtures had a lower trans-fatty acid concentration. Therefore, they stated that their mixtures could be used for the formulation of shortenings with a reduced health detriment.

Pesticide Studies have shown that fatty acids from vegetable oils present toxic activities and could be used for pest control (Bernklau et al. 2016; de Melo et al. 2018). Teodoro et al. (2017) utilized CSO as an insecticide against *Aceria guerreronis* (coconut plantation pest), in order to find alternatives to chemical control, since they are harmful to humans and the environment, besides the fact that pest resistance is a constant issue. Their results showed that CSO can be an effective biopesticide since it was highly lethal and repellent to *A. guerreronis*. Its bioactivity was mainly attributed to the linoleic and oleic acid contained in CSO.

Antibacterial Activity Flavonoids and phenols (tocopherols, sterols, gossypol) are multifunctional compounds that present antioxidant and antimicrobial activities. Vegetable oils are rich in these compounds and CSO is not the exception as it was described previously. In that matter, Xuan et al. (2018) evaluated the antimicrobial and antioxidant activities of commercial CSO and thirteen more, marketed in Japan. The determination of antioxidant activity (DPPH radical scavenging and β-Carotene bleaching method), total phenolic (TPC) and flavonoid (TFC) content and an antimicrobial activity test were conducted for the evaluation. CSO exhibited the second highest lipid peroxidation inhibition value. Middle TPC in comparison with the other oils were reported and the TFC were very similar among the oils. The identified flavonoid and phenolic compounds were benzoic acid, esculetin, and isoquercetin. Even when CSO did not show a high TPC, it exerted one of the highest antimicrobial activities against *Staphylococcus aureus* and *Escherichia coli*.

3.5.2 Proteins

Feeding CSM is extensively used for beef production since they contain high nutrition value and up to around 40% of protein (Świątkiewicz et al. 2016). It is known that CSM can partially replace soybean meal (major protein source of farm animals) obtaining, in general, the same nutritional values. Broderick et al. (2013) evaluated the effects of partial replacement (50%) of soybean meal with cottonseeds (*G. hirsutum* and *G. barbadense*) and CSM (*G. barbadense*) on the production of lactating dairy cows. The results indicated that all the diets were comparable to soybean meal (100%) on the production and composition of milk. However, gossypol content is the limiting factor of true CSM potential as a protein source because monogastric animals are much more susceptible and tolerate only small quantities. Świątkiewicz et al. (2016*)* concluded, based on their research and analysis of different studies, that a partial replacement between the range of 10–15% of soybean meal with CSM for poultry feeding is safe and even cheaper.

Such as CSM, cottonseed hulls (CSH) are destined for livestock feeding. Contrary to CSM, CSH presents lower protein content, but are an important roughage source, being quite useful for high-fibre diets. Moreover, they present a low cost, thus, decrease feeding costs. Eiras et al. (2016) analyzed the effect of a nutritional regimen based in CSH on the sensory attributes of young bull meat. They discovered that none of the three high-fibre diets (210, 270, or 330 g kg^{-1} cottonseed hull on dry matter) had an undesired effect in the sensorial properties (visual appraisal, flavour, tenderness) and all of them were accepted by the consumers.

Packaging Food packaging is critical for the protection of food quality and its safe distribution. However, in the last years, consumers have been increasingly looking for healthier and more sustainable products. In that matter, the developing of innovative packaging technologies was necessary to satisfy consumers' petitions. Active packaging goes beyond protecting food from external factors (dust, toxins, microorganisms, moisture) and facilitating the handling of food through the supply chain. In addition, active packaging can confer bioactive components such as antimicrobials and antioxidants to foods contained, or reduce the absorption of undesired components, improving the conditions and quality of packaged food (Yildirim et al. 2017). de Oliveira Filho et al. (2019) incorporated protein hydrolysates from CSM into alginate films, due to their high antioxidant properties. Besides the antioxidant properties, it showed antimicrobial and antifungal effects against *S. aureus*, *Colletotrichum gloeosporioides,* and *Rhizopus oligosporus*. Thus, their active film showed potential use as an active packaging. This strengthens the exploitation of unutilized cottonseed meal.

Wood Adhesive Industries of wood furniture are extremely important, and their demand rises as the world population increases. For the production of wood furniture, wood adhesives have become a key factor that determines the quality and production rate of the products. Most of the wood adhesives are synthetic adhesives derived from non-renewable products, and as they are mostly formaldehyde-base are toxic for people. An eco-friendly, renewable and non-toxic alternative are the vegetable protein adhesives. Pradyawong et al. (2018) used different blends of water washed CSM supplemented with cottonseed protein isolated or cottonseed protein residues after extraction. All the mixtures present protein values between 34.9% and 94.8%, and their physicochemical properties and adhesive strength were measured. Mixtures with a protein content of 65–70% exhibited comparable physicochemical properties and adhesive strength to their product with highest protein value, full cottonseed protein isolated (94.8%). Therefore, using mixes with water washed cottonseed meal can be more cost effective.

3.5.3 Gossypol

Gossypol is a multifunctional bioactive polyphenol that has a promising future in biotechnology applications. Nevertheless, more research is required to fully understand gossypol action mechanisms to potentiate its bioactive activities without compromising human and animal health.

Antibacterial, Antiviral, and Fungicidal Effects It has been proven the antibacterial effects against *S. aureus*, *E. coli*, *Saccharomyces cerevisiae*, among others. Due to the toxicity of gossypol in humans and animals, novel application methods for its safe use are in development. Studies have demonstrated that gossypol has been used safely in cutaneous gels. Based on these studies, Clément and Tang (2018) prepared two cutaneous formulations of gossypol and used them in ex vivo to analyze skin penetration. The results suggest that both formulations were safe since they were distributed in the outer layer of the skin. In that matter, they indicated the potential use of their gossypol formulas against skin bacteria including *Staphylococcus epidermidis*. However, for more reliable results, studies in vivo or clinical are needed to confirm the results.

Antiviral and fungicidal activities are other biological functions of gossypol. Li et al. (2015) obtained thirty-three gossypol Schiff derivatives, containing alkylimine, oxime or hydrazine moiety, and they were tested for their antimicrobial, antifungal, and antiviral activities. For the antiviral tests, all derivatives exhibited higher antiviral activities against *Tobacco mosaic virus*, where five of them exert an even higher inhibition than their controls; gossypol and ribavirin (commercial antiviral). On the same hand, high antibacterial activity in all derivative components was observed. In these assays, one particular component ([pyridin-3-yl] methanamine Schiff base) showed a complete inhibition (100%) of *Culex pipiens pallens.* The other included microbes in the study were *Mythimna separata*, *Helicoverta armigera*, and *Ostrinia nubilalis*. Lastly, in the fungicidal tests, *Physalospora piricola* was highly inhibited by all the compounds. Other fungi where the components showed good fungicidal activity were *Alternaria solani*, *Fusarium graminearum*, *Phytophthora capsici,* and *Cercospora arachidicola Hori*.

Contraceptive The contraceptive activity of gossypol has been studied in humans since the 1970s. It was first applied in men with the purpose of developing the first contraceptive method for men. Even though it was effective, serious side effects such as hypokalaemia and sterility finished the tests. Further studies showed that gossypol affects both male and female reproduction, inhibiting spermatogenesis, and affecting the oestrous cycle, pregnancy, and embryogenesis. In an effort to achieve a gossypol men's safe contraceptive, Wen et al. (2018) using a controlled drug release carrier (zero-order release), attained major progress. They could maintain the gossypol contraceptive activity using a 50-fold lower dose compared to conventional oral dose, without the hypokalaemia antifertility effects. For the complete restoration of fertility a recovery period is required.

Anticarcinogenic Gossypol has proven strong anticancer effects. Xilong et al. (2017) strongly inhibited the expression of two breast cancerous cells (MDM2 and VEGF) using gossypol. This resulted in cancer cell apoptosis and suppression of tumour angiogenesis. Besides confirming the anticarcinogenic effect of gossypol, a better understanding of its action mechanism was elucidated, promoting the development of novel anticarcinogenic drugs. Xie et al. (2017) synthesized a novel Schiff base drug constituted by gossypol and L-arginine. The drug presents excellent antitumor effects against lung cancer cells (A549) and human gastric cancer cells (SGC-7901).

3.6 Future Work

For our research centre, it is important to focus on the integral use of agroindustry wastes, in particular because our centre is located in the most important cotton producing zone. As it has already been mentioned, the main use of cotton is textiles and large amounts of cottonseed are disposed in the environment. Then, relevant biotechnological projects are being conducted in our centre for the extraction of oil and produce biodiesel by means of a novel supported biocatalyst and on the other hand, the recovery of bioactive compounds is being assayed for their bioactivities. It is worth noting that the projects are also evaluated for their technical and economic feasibility and sustainability.

3.7 Conclusions

In this chapter, the bioactivities and energy applications of the bioactive compounds present in cottonseed have been reviewed, as well as the different types of extraction methods and analytical protocols for their identification, purification, and quantification.

Even when several efforts have been reported, new and novel applications are needed and not only for cottonseed but also for other agroindustry wastes. The chemical composition of cottonseed is suitable for the recovery of the bioactive compounds present in order to achieve a wider characterization and the development of new applications and analytical methods for their extractions.

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