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Review article

PROCESSING TECHNIQUES OF REMOVING ANTINUTRIENTS FROM OILSEED CAKES AS BY-PRODUCTS INTENDED FOR ANIMAL FEEDING

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Abstract: Contemporary animal feed production increasingly embraces zero-waste strategies, focusing on the use of food waste and cost-effective by-products to promote environmental sustainability. Oilseed cakes, by-products from oil extraction, have emerged as valuable resources due to their nutritional benefits and diverse applications. However, their use in animal feed is challenged by various antinutritional factors (ANFs) such as glucosinolates, cyanogenic glycosides, phytic acid, saponins, sinapine, tannins, and enzyme inhibitors, which can negatively impact nutrient absorption, digestibility, and feed palatability. The chemical properties of these ANFs differ considerably, and their concentrations vary significantly depending on the plant type. This variability makes it difficult to pinpoint a universally effective method for their reduction and removal. This paper aims to provide an overview of the different ANFs present in oilseeds used for animal feed, their concentrations, and their adverse effects on animal health and feed intake. It also reviews traditional methods for ANF removal, including heating, autoclaving, soaking, and chemical treatments, and explores novel techniques such as fermentation and enzymatic methods based on previous studies.

Key words: *antinutrients, oilseed cake, food and feed diet, proteins.*

INTRODUCTION

Contemporary animal feed production is increasingly adopting "zero waste" strategies, incorporating food waste and cost-effective by-products from food production to mitigate environmental pollution. Factors like the growing global population, economic crises, and climate change have intensified the focus on circular economy principles and sustainable production in animal feed manufacturing. Within this framework, agro-industrial and food waste represent valuable resources with significant potential for feed production. The food sector's by-products are now finding diverse applications, aiming for inexpensive raw materials and subsequently affordable products. Among these, oilseed cakes have gained prominence due to their economic significance, cost-effectiveness, and nutritional value, including protein, fibre, carbohydrates, and antioxidants. They are increasingly valued as ingredients in livestock (Rakita et al., 2023). Globally, one of the most widespread produced waste is from the oil industry, where after the oil extraction, oilseed cakes remain as a valuable by-product. In 2023, global production of oilseeds reached 659 million tons (USDA, 2023). Because the oilcake accounts for appro-

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ximately 50% of the original seeds' total weight, this further indicates the generation of a large waste amount (Mirpoor, Giosafatto & Porta, 2021). In addition, the application of oilcake has wide potential in various industries, such as animal feed, biogas and biodiesel production, as well as in packaging material and as a substrate for surfactants, antibiotics, vitamins and enzymes in the food and cosmetic industries (Petraru & Amariei, 2023; Šuput et al., 2023). Oilcakes are a valuable source of protein (30-35%) and fats (6-10%) which represent the main nutrients in animal nutrition.

Oilseed cakes are also rich in dietary fibres, which can contribute to animal and human health (Serrapica et al., 2019). While oilseed cakes are generally known for their protein content, certain varieties, like flaxseed, hempseed, camelina seed, and rapeseed cakes, can also serve as substrates for n-3 polyunsaturated fatty acids (PUFA) (Spasevski et al., 2019; Ilić et al., 2022).

Since human and animal nutrition is deficient in PUFA, these oilseed cakes are very beneficial for human and animal health. However, the widespread use of oilseed cakes may be limited by the presence of certain undesirable antinutritional factors (ANFs). These factors can affect organoleptic properties, protein digestibility, and the absorption of macro- and micronutrients.

The effects of these factors vary depending on the digestive processes of animals; for instance, trypsin inhibitors can negatively impact monogastric animals but are not a concern for ruminants as they degrade during digestion. Phenolic compounds, such as sinapine, tannins, and chlorogenic acid, can form bonds with proteins.

This interaction results in decreased solubility and altered digestibility and can impact the sensory characteristics of animal feed, contributing to attributes such as bitterness and dark colouration (Tan, Mailer, Blanchard & Agboola, 2011).

Phytic acid, acting as a highly negatively charged anion, functions as a potent chelating agent. Consequently, it precipitates essential minerals like zinc, iron, calcium, and magnesium, making them inaccessible for metabolic processes. Glucosinolates, serving as precursors to potentially harmful compounds, can compromise animal health and reduce feed palatability. Cyanogenic glycosides have the potential to degrade into hydrogen cyanide, a highly toxic substance (Bekhit et al., 2018). Furthermore, trypsin inhibitors contribute to reduced nitrogen retention, leading to an increase in metabolic nitrogen extraction by animals. Therefore, before incorporating oilseed cakes into animal diets, ANFs should be removed or reduced to prevent any negative effects on the animals. To improve the quality, safety and economic utilisation of oilseed cake in animal feed, various methods can be employed to eliminate or inactivate ANFs.

These methods include enzymatic methods (using specific enzymes to break down antinutrients), fermentative methods, chemical methods (pH modification, extraction of proteins, ammonification or the addition of sodium carbonate, choline, methionine or ferrous sulfate) and physical methods (heat-cooking, soaking, dehulling, toasting, autoclaving) (Ancuta & Sonia, 2020). This paper critically reviews the available literature on ANFs and provides potential methods for their removal from by-products derived from the oil industry, aiming to enhance their usability in animal nutrition.

Antinutrients in oilcakes

ANFs found in edible crops are naturally occurring compounds that may serve as plant defenses against pests and pathogens and they are key factors that interfere with the digestion, absorption, and utilization of nutrients in humans and animals.

The most prevalent ANFs in oilseeds are glucosinolates, cyanogenic glycosides, phytic acid, protein inhibitors, tannins, sinapines, and others. ANFs in feedstuffs are classified based on several criteria.

These include their chemical nature, which determines their composition and properties; their activity in animals, which refers to the biological effects and interactions they have within animal systems; and their thermal stability, indicating how they respond to heat treatment processes. Understanding these classifications helps to develop strategies to mitigate their adverse effects and improve the nutritional quality of feedstuffs (Kumar et al., 2012; Mohammadi, Abdolkhani & Fakhari, 2024). The classification of ANFs is shown in Table 1.

Table 1.
The classification of antinutritional factors

ANFs classified based on their chemical properties

Proteins	Glycosides	Phenols	Miscellaneous
Protease inhibitor	Saponins	Gossypol	Anti-minerals
Lectins (hemagglutinins)	Cyanogens	Tannins	Anti-vitamins
	Glucosinolates (goitrogens) or Thioglucosides		Anti-enzymes

ANFs classified based on their effect on utilization of nutrients

Protein utilization inhibitors	Mineral utilization inhibitors	Carbohydrates utilization inhibitors	Lipid utilization inhibitors	Vitamin utilization inhibitors
Protease inhibitor (trypsin and chymotrypsin inhibitor)	Phytic acid	Lectins	Saponins	Saponins
Lectins (hemagglutinins)	Oxalic acid	Amylase inhibitors	Phytates	Tannins
Saponins	Glucosinolates (Thioglucosides)	Phenolic compounds		
Tannins	Gossypol			

ANFs classified based on their thermal stability

Heat-Labile ANFs	Heat-Stable ANFs
Protease inhibitors	Phytic Acid
Lectins	Saponins
Cyanogenic glycoside	Tannins
Amylase inhibitors	Gossypol

Sources: EFSA (2008); Kumar, Sinha, Makkar & Becker (2010); Kumar et al., 2012; Mohammadi et al., 2024; Samtiya, Aluko & Dhewa, 2020; Shi, Mu, Arntfield & Nickerson, (2017a); Thakur, Sharma & Thakur (2019).

The quantity of ANFs varies depending on the type of plant and the parts of the plant used as feeds. According to Tripathi & Mishra (2007), during the 1980s, rapeseed meal contained a glucosinolate content ranging from 125 to 207 µmol/g of dry oil-free meal, with an average value of 166 µmol/g.

However, the development of oilseed varieties with low levels of ANFs in seeds was a milestone in oilseed breeding, resulting in an average total glucosinolate content of 38 µmol/g, ranging from 9 to 69 µmol/g of dry oil-free meal in France (Bourdon & Aumaitre, 1990; Zhang, Wen & Chang, 2020). Today, very low-glucosinolate rapeseed varieties are available; with glucosinolate content of less than 25/g of dry oil-free meal (Tripathi & Mishra, 2007).

The main cyanogenic glycosides present in linseed are linustatin, neolinustatin, and linamarin. Their concentrations in the seed ranged from 213–352 mg/100 g, 91–203 mg/100 g, and less than 32 mg/100 g, respectively (Huang et al., 2023). Gupta, Gangoliya & Singh (2015) reported that the content of phytic acid varies from approximately 1.0 to 5.4% (dry weight) in oilseeds such as soybeans, sesame seeds, sunflower kernels, flaxseeds, and rapeseeds. Wang, Oomah, McGregor & Downey (1998) stated that the variation in sinapine content in seeds was much greater than the variation in sinapine concentration in oil-free meals. The same authors reported that the sinapine concentration in the oil-free meal of strains and cultivars of the five Brassica species and *S. alba* ranged from 16 to 24.3 mg/g of the oil-free meal.

The total phenolic content of flaxseed meal (wet weight) ranges from 162 to 362 mg/100 g (El-Beltagi, Salama & El-Hariri, 2007), while in soybean seeds it ranges from 87.2 to 216.3 mg/100 g, expressed as gallic acid equivalents (Mujić et al., 2011). Trypsin inhibitor activity is associated with two protein families, the Kunitz trypsin inhibitor and the Bowman-Birk trypsin inhibitor families, which represent 6% of the total protease inhibitors found in soybean seeds (Okedigba et al., 2023).

Table 2.
Antinutritional factors in oilseed cake

Types	Oilcakes	Effect on animals	Reference
Glucosinolates	camelina, rapeseed, hempseed	goiter and hypothyroidism, negative effects on reproductive performance, reduced feed intake, liver damage	Lomascolo, Uzan-Boukhris, Sigoillot & Fine (2012); Tangendjaja (2015); Pojić et al. (2015)
Cyanogenic glycosides	flaxseed, hempseed	impaired growth and reproduction, reduced feed intake	Tangendjaja (2015); Teh & Bekhit, (2015); Łopusiewicz et al. (2019)
Phytic acid	camelina, soybean, sunflower, rapeseed, hempseed	decreased absorption and digestion of nutrients	Tangendjaja (2015); Jannathulla, Dayal, Ambasankar & Muralidhar (2018); Pojić, Hadnađev Dapčević, Hadnađev, Rakita & Brlek (2015)
Saponins	soybean, sunflower	highly toxic to fish, inhibition of active transport of nutrients, bitter taste	Kumar et al. (2012); Thakur et al. (2019)
Sinapine	camelina, rapeseed	bitter taste, dark coloration	Tan et al. (2011); Juodka et al. (2022)
Tannins	rapeseed, soybean, sunflower, hempseed	decreased solubility of proteins, astringency	Tangendjaja (2015); Jannathulla et al. (2018); Pojić et al. (2015)
Trypsin inhibitors	soybean, sunflower, flaxseed, hempseed	decreased protein digestibility	Tangendjaja et al. (2015); Jannathulla et al. (2018); Pojić et al. (2015); Dong, Hardy & Higgs (2000)

High concentrations of ANFs or their products can become toxic, therefore, reducing their levels in edible crops is necessary due to their adverse effects (Gemede & Ratta, 2014). Most oilseed crops, which are used as a source of vegetable oils, contain ANFs which also lag in their by-product oilcakes. The major groups of ANFs in oilcakes are discussed below.

Glucosinolates

Glucosinolates (GLS) are a group of anti-nutritional compounds present in abundance in Cruciferae or Brassicaceae family including many important species such as *Camelina sativa* and *Brassica napus* (rapeseed/canola) (Di Gioia, Pinela, Bailón, Fereira & Petropoulos, 2020). Russo & Reggiani (2012) stated that the use of camelina is limited by the presence of GLS. Within species, there is considerable variability in the profile and composition of GLS. A single plant may contain up to four distinct GLS in significant amounts, whereas as many as fifteen different GLS can coexist within the same plant. The negative impacts of GLS on animal health and production include inhibition of growth and productivity, diminished feed palatability and intake, as well as health issues such as hypothyroidism, and other related disorders.

Regarding the group of animals sensitive to GLS, non-ruminants are more sensitive to dietary GLS than ruminants, and young animals, in particular, are significantly affected by their negative effects (Tripathi & Mishra, 2007). When animals consume plants containing GLS, these compounds undergo a series of metabolic transformations that can variously affect the animal's health and nutrition. GLS are located in all plant parts but remain physically separated from myrosinase. When damage occurs to the plant tissue during processing or ingestion, myrosinase is released, enabling it to interact with GLS (Tripathi & Mishra, 2007). Myrosinase catalyzes the breakdown of GLS into various products, including isothiocyanates, nitriles, oxazolidine-2-thiones and indole-3-carbinols (Barba et al., 2016). This reaction can also occur in the digestive tract of animals due to microbial myrosinase-like activity. However, our understanding of specific bacterial myrosinases remains limited, with only a few studies addressing this topic (Sikorska-Zimny & Beneduce, 2020). Factors such as the pH of the digestive tract, the presence of metal ions, and other dietary components can influence the activity of myrosinase and the types of hydrolysis products that are formed. The hydrolysis products (isothiocyanates, nitriles, and thiocyanates) are absorbed through the gastrointestinal tract into the bloodstream, after which they are distributed to various tissues and organs throughout the body (Tripathi & Mishra, 2007). Isothiocyanates hinder the thyroid gland's ability to absorb iodine. Iodine is crucial for thyroid hormone synthesis, tiiodothyronine (T3) and thyroxine (T4). Thyroid hormones are essential in modulating a range of physiological processes, including growth, development, metabolic regulation, and reproductive functions (Lisco et al., 2023). Reduced iodine uptake can lead to decreased production of thyroid hormones. Goitrogens, which include certain isothiocyanates and other breakdown products of glucosinolates, can induce goitre-a condition in which the thyroid gland enlarges as it tries to compensate for reduced iodine uptake. This enlargement can negatively affect thyroid function (Lisco et al., 2023). Tan et al. (2022) observed that goitrin, which is formed by the action of myrosinase on glucosinolates, inhibits the oxidation of trimethylamine (TMA) to the odourless trimethylamine-N-oxide by competing for the active site of flavin-containing monooxygenase 3. Therefore, the GLS content in rapeseed meals represents another factor influencing TMA deposition in yolks, causing the fishy odour in eggs (Tan et al., 2022). Some glucosinolate breakdown products can inhibit thyroid peroxidase, an enzyme essential for the synthesis of thyroid hormones. Without adequate thyroid peroxidase activity, the thyroid gland's ability to produce T3 and T4 is impaired (Galanty, Grudzińska, Paździora, Służały & Paśko, 2024). GLS and their metabolites can alter the metabolism and clearance of thyroid hormones in the body, potentially leading to imbalances in thyroid hormone levels. The effects of glucosinolates on thyroid function can vary depending on the species and the amount consumed.

Cyanogenic glycosides

Cyanogenic glycosides are nitrogenous secondary plant metabolites derived from various L-amino acids (Vetter, 2000). They protect the plant from insects and other animals and serve as nitrogen reserves. Flaxseed contains a significant amount of cyanogenic glycosides. Elevated concentrations of cyanogenic compounds in flaxseed significantly limit its inclusion in animal feed formulations. These compounds are toxic due to their ability to undergo a reaction with the enzyme β-glucosidase, leading to the hydrolysis of glycosides and the formation of acetone cyanohydrin and sugars. Cyanohydrins further decompose either spontaneously (pH >5 and temperature >35 °C) or enzymatically (α-hydroxynitrile lyase) into hydrogen cyanide (HCN) and aldehydes or ketones, which is the main reason for the limited use of flaxseed in animal nutrition (Xu et al., 2022; Wu et al., 2008). Once in the bloodstream, HCN can be detoxified by the liver by conversion into less toxic thiocyanate, which is then excreted in the urine (Cressey & Reeve, 2019). Chronic exposure to lower levels of cyanide can lead to thyroid dysfunction and neurological disorders. Poisoning with hydrogen cyanide leads to oxygen deficiency in cells and affects the nervous system, endocrine system, and cardiovascular system (Wu et al., 2008).

Phytic acid

Phytic acid, also known as inositol hexaphosphate (IP6), is a naturally occurring compound occurring in many plant seeds, primarily found in the outer layers or bran of seeds. Since phytic acid can chelate micronutrients in feed, making them unabsorbable and limiting their bioavailability, it is commonly referred to as an anti-nutrient. Juodka et al. (2022) observed that the content of phytic acid in camelina is similar to that in sunflower, but 1.5 times higher than in rapeseed. Phytic acid is a metabolite negatively charged under various pH conditions (Maenz, 2001). Consequently, it can bind positively charged molecules in the diet and endogenous gastrointestinal tract secretions including digestive enzymes and mucins, across all pH conditions. This binding action leads to a decrease in nutrient digestibility and an increase in the endogenous secretion of nutrients (Woyengo & Nyachoti, 2013). Because of its enduring chemical stability, phytic acid cannot be broken down through physical treatments like heating, and no animal enzyme system is capable of digesting it. The current widespread methodology employs the enzyme phytase to degrade phytic acid (Prajapati & Shah, 2022). Besides the negative properties of phytic acid and its influence on nutrient digestibility, additional research has been conducted to assess any potential indirect effects on meat products obtained from animals given diets high in phytic acid. The results demonstrated that the inclusion of dietary corn germ containing phytic acid at levels of 0%, 10%, 20%, and 40% did not affect feed conversion, weight gain, or carcass characteristics (Harbach et al., 2021).

Saponins

Saponins are steroidal or triterpenoidal glycolsides distinguished by their bitter taste, their capacity to produce foam upon interaction with various solutions, and their potential to induce hemolysis of red blood cells (Thakur et al., 2019). These compounds are present in various plant-derived feed ingredients for animals, including pulses and oil seeds such as kidney beans, chickpeas, soybeans, groundnut, lupin and sunflower. According to Kumar et al. (2012), the saponin content in different legume seeds ranges from 18 to 41 mg/kg, while in defatted roasted soybean flour it is approximately 67 mg/kg. Saponins have been identified as ANFs because they adversely affect growth and decrease feed intake due to their bitter taste and irritating effect on the throat (Thakur et al., 2019). Saponins have both positive and negative roles in animal nutrition. In general, they are considered less significant in the context of monogastric animal nutrition due to their low levels in most common feed ingredients. According to Kholif (2023), saponins disrupt protein digestion in the intestines, interact with cholesterol in cell membranes, cause cell rupture, and selectively remove protozoa in the rumen, thereby improving nitrogen utilization efficiency and potentially enhancing ruminant performance.

Sinapine

Most of the oilseed cakes contain several non-protein ANFs, such as phenolic compounds, which impair the biological utilization of their nutrients. Rapeseed contains polyphenols at a concentration 10 to 30 times higher than in other types of oilseeds, with sinapine content making up 70-80% and free sinapic acid 6-14% of the total polyphenols (Li, Li, Cao & Liu, 2020). Sinapic acid and its corresponding esters (sinapine, sinapoyl malate, sinapoyl glucose) are secondary metabolites present in various parts of plants and are involved in multiple biological processes (Nguyen, Stewart, Ioannou & Allais, 2021). Sinapine has several undesirable properties as a constituent in animal feeds. Its bitter taste diminishes the palatability of animal feed, and when present in the diet of brown egg-laying hens at levels exceeding 1 g/kg, it induces a fishy odour or taste in the eggs (Russo & Reggiani, 2012). During the hydrolysis of sinapine, sinapic acid is generated. This compound can further negatively impact protein digestibility and plasma thyroxine (T4) levels in poultry diets (Qiao & Classen, 2003). The detrimental effects of sinapine on animal health have rarely been demonstrated in experiments, except for issues like fishy egg taint. Most concerns are observed in pig diets, as pigs have a more developed sense of taste and smell unlike poultry. Landero, Beltranena & Zijlstra (2012) showed that the bitterness of cake influenced the feed intake of pigs, thereby directly impacting the average daily feed intake and weight gain. Furthermore, research on how sinapine affects the physiology and nutritional digestion of ruminant and non-ruminant animals is lacking, making further investigation highly desirable.

Tannins

Tannins are a diverse group of naturally occurring compounds primarily found in plants and they belong to a class of polyphenolic substances. Beside their antinutritional nature, tannins may impart off-flavours and undesirable dark colour. Tannins are known to interact with proteins forming complexes which, in turn, decrease the solubility of proteins and make protein complexes less susceptible to proteolytic attack than the same proteins alone (Beszterda & Nogala-Kałucka, 2019). Tannins can have either positive or negative effects on ruminants, contingent upon the quantity consumed, the compound's structure on the physiology of the consuming species. A reduction in palatability could be caused through a reaction between the tannins and the salivary mucoproteins, or a direct reaction with the taste receptors, provoking an astringent sensation (Frutos, Hervás, Giráldez & Mantecón, 2004). Therefore, careful and calculated inclusion of the various oilseed cakes into animal diets is necessary and required.

Enzyme inhibitor

Protease inhibitors are ANFs which suppress the activity of proteolytic enzymes in the gastrointestinal tract of animals. Their significance in research has grown due to their effective ability to reduce enzyme activity through the formation of protein-protein interactions. These inhibitors operate by employing a catalytic mode of action, blocking the active site of enzymes and preventing their function. Two prominent types, trypsin inhibitor and chymotrypsin inhibitor, are predominantly found in raw grain legume plants. Specifically, trypsin inhibitors impede the function of trypsin and chymotrypsin enzymes in the gut, leading to the inhibition of protein digestion and thus limiting the intake of amino acids needed to construct new proteins (Manzoor et al., 2021). Legumes contain high amounts of protease inhibitors, α-amylase inhibitors and lectins, which could lead to low mineral bioavailability as well as reduced nutrients absorption and digestibility (Samtiya et al., 2020).

METHODS OF REMOVING ANTINUTRIENTS

Various methods that have been employed to reduce ANFs from oil cakes by-products are summarized in Table 3.

Heating and autoclaving

Numerous studies suggest that autoclaving is one of the most efficient methods for reducing various ANFs compared to other processing methods (Manzoor et al., 2021). It was reported that boiling at 121 °C for 10 min led to a decrease in the content of ANFs, and an improvement in protein and starch digestibility (Rehman & Shah, 2005). Previous studies showed that boiling or cooking improved nutritional value by reducing ANFs like tannins and trypsin inhibitors (Patterson, Curran & Der, 2017). According to Embaby (2011), roasting brown and white sesame seeds partially reduced tannins, phytic acid, and trypsin inhibitor activity, with decreases ranging from 16.6% to 61.2%. An additional study reported significant enhancements in the nutritional quality of legumes following the cooking process, primarily attributed to reductions in lectin and saponin levels (Maphosa & Jideani, 2017). Simultaneously, there was an elevation in the concentration of free minerals. This phenomenon can be attributed to the chelating properties of phytic acid, whereby reducing its presence facilitates the liberation of minerals. Heat treatments can remove or lessen cyanogenic glycolsides, proteases, trypsin inhibitors, glucosinolates, and phytic acid. Jensen, Liu & Eggum (1995) showed that it is possible to decrease the total content of glucosinolates in rapeseed meal by up to 94% through toasting at 100 °C for 120 minutes. Extrusion can also serve as a method for removing ANFs. Extrusion is a process that utilizes high temperature and pressure to shape the material and transform it into a unique texture. Extrusion is often used in animal feed processing to improve the nutritional value and digestibility of the feed. Imran, Anjum, Butt, Siddiq & Sheikh (2011) showed the efficacy of the extrusion process in removing cyanogenic glycosides from flaxseed meal. Over 90% of HCN from flaxseed was removed by optimizing parameters during the extrusion process (Wu et al., 2008).

Studies have shown that optimizing extruding parameters, such as using high screw speeds, specific barrel temperatures, and appropriate moisture content, can lead to significant reductions in ANFs while preserving the nutritional quality of the product (Moraru & Kokini, 2003; Rathod & Annapure, 2016).

In addition, higher temperatures and moisture content can lead to more effective reduction of certain ANFs like phytate and tannins. Extrusion pressure significantly impacts the rheological, nutritional, and anti-nutritional properties of the extruded products, and is influenced by factors such as die type, shape, and mechanical force. However, the drawback of using heat treatments to detoxify oilcakes from ANFs is the loss of nutritional constituents such as proteins, fatty acids and other bioactive compounds (Yang, Huang & Chang, 2022).

The losses of nutritional constituents during heating and autoclaving can vary depending on several factors, including the specific food matrix, processing parameters, and duration of treatment. Thus, optimizing these conditions is important to remove ANFs while preserving essential compounds.

Soaking

Soaking is a simple physical process that facilitates the removal of soluble ANFs compounds and contributes to cooking time reduction. Furthermore, this treatment releases enzymes, such as endogenous phytases occuring in plant foods such as almonds, nuts, grains, and other edible seeds. Additionally, soaking reduces the number of enzyme inhibitors, enhancing both digestibility and nutritional value (Manzoor et al. 2021). In a prior investigation, it was reported that soaking *Mucuna flagellipes* seeds for 6 hours resulted in a 27.9% reduction in phytic acid, while soaking for 24 hours led to a 36.0% reduction at room temperature (Udensi, Arisa & Maduka, 2008). Furthermore, another study reported a reduction in phytic acid amount from 47.45 to 55.71% with an increase in soaking time from 2 to 12 hours (Ertaş & Türker, 2014).

Table 3.
Methods of removing antinutrient ingredients from oil cake by-products, with their effects and disadvantages

Method	Main effect	Disadvantages
Heating, autoclaving	Best method for removing cyanogenes, proteases, trypsin inhibitors, glucosinolates, and phytic acid. It was efficient for removing glucosinolates and improving flavour and palatability.	Heat could affect nutritional constituents such as proteins, fatty acids and other bioactive compounds.
Soaking	This treatment can reduce the number of enzyme inhibitors, thereby improving both the digestibility and nutritional value of cakes.	It caused disappearance of nutritional constituents such as water-soluble protein and minerals.
Chemical methods	Appropriate methods for removing glucosinolates, phenolic compounds and tannins, which contribute to specific flavours and odour. Organic solvents such as ethanol, methanol, acetone, hexane are most often used for these purposes.	Organic solvents are not appropriate due to their toxic effect. Implies the application of an additional step of their removal due to the further application of the raw material.
Fermentation	This bioprocessing technique could convert agricultural by-products and crops into more valuable, nutrient-rich, and sustainable products. For this purpose, bacteria, yeasts and fungi cultures are used, depending on the specific raw material being treated.	Fermentation processes can often take a considerable amount of time to complete, which may not be suitable for industries requiring quick turnover.
Enzymatic methods	Enzyme-assisted methods are very attractive because of their high efficiency, sustainability, and ecological friendliness.	These methods are highly specific and rely on the characteristics of enzymes.

Therefore, soaking for a specified period (12 to 24 hours) is recommended for effectively lowering tannin and phytate levels. Greiner and Konietzny (2006) demonstrated that soaking in a temperature range between 45 and 65 °C and a pH value between 5 and 6 hydrolysed a significant amount of phytate. The soaking treatment positively influenced the crude protein and crude lipid content of cottonseed and groundnut meals (Duodu et al., 2018). However, the disadvantage of soaking treatment is that it results in the loss of water-soluble proteins and minerals.

Chemical methods

Chemical methods utilize organic solvents, such as ethanol, methanol, acetone, and hexane, to remove ANFs. The effectiveness of these methods varies based on the concentration of the solvents, as well as the extraction time and temperature. Subas et al. (2020) reported that the crude protein content of sunflower protein isolate increased from 80% to 87% following the removal of phenols from sunflower oil cake using methanol as an extraction solvent. Furthermore, applying ethanol, methanol, and acetone was highly effective in removing over 79% of glucosinolates from rapeseed oil cake (Carré, 2021). Purkayastha et al. (2013) used different organic solvents for removing antinutrients from rapeseed press cake and showed that the 0.2% $HClO_4$-containing Me/Ac mixture (1:1 v/v) was the most effective solvent for extraction of the major antinutrients (polyphenols, phytates, allylisothiocyanate). However, organic solvents can be toxic and pose harmful effects on human and animal health. Their use can lead to feed contamination and residues in the final product. Once organic solvents are applied, removing them becomes necessary, which can increase production costs and require additional processing steps. If not properly disposed of or treated, waste materials generated during the removal process can have adverse environmental consequences. Furthermore, using organic solvents can cause the loss of nutritional components from the raw material, reducing the nutritional value of the final product. Ideal alternative solvents for eco-friendly extraction should exhibit strong solvency and high flash points, along with low toxicity and a minimal environmental footprint. These solvents should readily biodegrade, be derived from renewable sources, and be easily recyclable without harming the environment. Chemat et al. (2019) demonstrated that finding a solvent that meets all the aforementioned requirements is a challenging task.

Fermentation

Solid-state fermentation is a bioprocessing technique with the potential to convert agricultural by-products and crops into more valuable, nutrient-rich, and sustainable products. This method plays a crucial role in solving challenges related to food security and agricultural waste management. Bacteria, yeasts and fungi cultures are used for this purpose, depending on the specific raw material being treated (Manzoor et al., 2021). *Rhizopus oligosporus* DSM 1964 and ATCC 64063 were used for reducing phytate and enhancing the bioavailability of calcium, magnesium, and phosphor from flaxseed press-cake (Duliński et al., 2017). Furthermore, the beneficial properties in the reduction of tannins and improvement of chemical properties in groundnut oil cake by using *Pichia kudriavzevii,* were described by Ghosh and Mandal (2015). Short-term fermentation was the most efficient means of removing gossypol, achieving a 45% reduction in groundnut meal and 67% in groundnut husk. Long-term fermentation was found to be the most effective method for decreasing phytic acid, with reductions of 72% in cottonseed meal and 69% in groundnut meal (Duodu et al., 2018). A dual solid-state fermentation process was conducted on soybean meal, with an inoculum mixture of *Aspergillus* spp. and *Bacillus* spp. The results demonstrated a noteworthy decrease in trypsin inhibitors, reducing from 2.56 mg/g in soybean meal to 0.97 mg/g in fermented soybean meal (Ruiz, Robles-Montes & van Eys, 2020). The quality of solid-state fermented products is influenced by factors such as initial moisture, particle size, pH, temperature, media composition, operational system, mixing, sterilization, water activity, inoculum density, agitation, aeration, extraction of product and downstream processing. Fermentation has the potential to increase the concentration or bioaccessibility of bioactive compounds. These compounds possess certain beneficial properties related to the increase of necessary nutritional and sensory characteristics. Moreover, fermentation can contribute to the enrichment of the available amino acids, vitamins, and minerals of feed. In the study conducted by Das and Ghosh (2015), it was shown that phytate and tannin levels in sesame oil cake decreased through fermentation with *Lactobacillus* *acidophilus,* emphasizing its potential use in aquaculture. Shi, Zhang, Lu and Wang (2017b) stated that fermented feed usually contains >9 log cfu/g LAB (*Lactobacillus*) and a high concentration of lactic acid (>150 mmol/l), which can prevent the proliferation of microorganisms in the gastrointestinal tract of animals. Ashayerizadeh, Dastar, Shargh, Sadeghi Mahoonak & Zerehdaran (2018) showed that fermented rapeseed meal reduced the colonization of *Salmonella enterica* serovar Typhimurium in gut and internal organs, decreased stress and improved growth performance in broiler chicks challenged by *S.* Typhimurium.

Enzymatic methods

Enzyme-assisted methods for removing ANFs have attracted considerable attention because of their high efficiency, sustainability, and ecological friendliness. These methods are highly specific and rely on the characteristics of enzymes. They exhibit regional selectivity and react under mild conditions while preserving the biological activity of compounds. Additionally, enzymes can improve flavour, enhance the d-egree of hydrolysis and peptide yield of proteins, reduce bitterness and increase palatability. Enzymes used for this purpose include alkaline, neutral, and acidic proteases, trypsin, papain, enzymes that mediate the production of flavour compounds, laccase, oxidative polyhydrogenase, and cell wall polysaccharide-degrading enzymes (Samtiya et al., 2020). Jensen, Olsen & Sorensen (1990) reported that enzymes like pectinase, protease, and hemicellulase reduced glucosinolate content in canola meal by approximately 30-50%.

CONCLUSIONS

This review provides crucial insights into the antinutritional compounds found in oilseed cake by-products. Prevalent antinutrients in oilseeds include glucosinolates, cyanogenic glycosides, phytate, saponins, sinapins, tannins, and protease inhibitors. These elements impede the nutritional quality of oilseed cake as food, diminishing mineral absorption, and protein digestibility, and potentially causing toxicity and health disorders at elevated concentrations. Most toxic and antinutritional effects in feed can be mitigated using various processing methods, such as soaking, autoclaving, and thermal treatments. However, a significant drawback of heat treatments is the loss of nutritional constituents, including proteins, fatty acids, and other bioactive compounds. Recent research indicates that fermentation and enzymatic methods, individually or in combination, are highly effective in eliminating antinutritional factors while preserving the nutritional value of the feed.

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AUTHOR CONTRIBUTIONS

Conceptualization, methodology, and investigation: V.J, S.N, and D.D; table preparation: V.J and S.N; writing-original draft preparation: V.J, S.N, and D.D; writing-review and editing: S.N and D.D. All authors have read and agreed to the published version of the manuscript.

DATA AVAILABILITY STATEMENT

Data contained within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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TEHNIKE OBRADE UKLANJANJA ANTINUTRIJENATA IZ ULJANIH POGAČA KAO NUSPROIZVODA KOJI SE KORISTE U ISHRANI ŽIVOTINJA

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Sažetak: Savremena proizvodnja hrane za životinje bazira se na iskorišćenju nusproizvoda iz prehrambene industrije i ekonomičnijem pristupu na temeljima cirkularne ekonomije i održive proizvodnje. Nusproizvodi iz industrije ulja, poput uljanih pogača, pokazali su se kao vredne sirovine zbog svojih nutritivnih karakteristika. Međutim, njihova upotreba u industriji hrane za životinje je ograničena, usled prisustva antinutrijenata kao što su: glukozinolati, cijanogeni glikozidi, fitinske kiseline, saponina, sinapina, tanina i inhibitora enzima. Ovi antinutrijenti negativno utiču na apsorpciju nutrijenata, svarljivost i senzorske karakteristike hrane za životinje. Hemijska svojstva ovih jedinjenja se značajno razlikuju, a njihova koncentracija varira u zavisnosti od vrste biljke. Ove razlike otežavaju pronalaženje univerzalne i efikasne metode za njihovo smanjenje i uklanjanje. Stoga je cilj ovog rada da pruži pregled na različite antinutrijente prisutne u pogačama, njihove koncentracije, kao i na negativne efekte koje mogu da imaju na zdravlje životinja i usvojivost hrane za životinje. Takođe, dat je uvid i u tradicionalne metode za uklanjanje ovih jedinjenja, uključujući termičke tretmane, autoklaviranje, natapanje i hemijske tretmane, kao i na savremene tehnike poput fermentacije i enzimskih metoda.

Ključne reči: *antinutrijenti, uljana pogača, ishrana ljudi i životinja, proteini*

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