

## Review

## Food processing to reduce antinutrients in plant-based foods

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### Abstract

Antinutrients such as phytic acids, tannins, saponin, and enzyme inhibitors are phytochemicals that can decrease the bioavailability of micro- and macronutrients, thus causing them to be unavailable for absorptions in the digestive system. Antinutrients are a major concern especially in countries where plant-based commodities such as wheat, legumes, and cereals are staple foods, for the antinutrients can cause not only mineral deficiencies, but also lead to more serious health issues. Although various thermal and non-thermal processing methods such as cooking, boiling, and fermentation processes have been practiced to decrease the level of antinutrients, these processes may also undesirably influence the final products. More advanced practices, such as ozonation and cold plasma processing (CPP), have been applied to decrease the antinutrients without majorly affecting the physicochemical and nutritional aspects of the commodities post-processing. This review will cover the types of antinutrients that are commonly found in plants, and the available processing methods that can be used, either singly or in combination, to significantly decrease the antinutrients, thus rendering the foods safe for consumption.

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### Introduction

Nutrients are beneficial compounds found in foods that can improve our health (Popova and Mihaylova, 2019), whereas antinutrients, or more commonly known as antinutritional factors, are well-known for their ability to impair or interfere with certain biochemical reactions in our body (Shigaki, 2015), and decrease the bioavailability of nutrients in foods (Samtiya *et al.*, 2020; Kaspchak *et al.*, 2018). Sinha and Khare (2017) mentioned that antinutrients are normally formed from the use of pesticides and fertilisers, but they could also exist naturally in plants (Sinha and Khare, 2017), in the form of evolved chemicals or secondary metabolites (Mohan *et al.*, 2016) that are used by plants as their defence system against pests and predators (Popova and Mihaylova, 2019). For instance, cyanogenic glycosides, one of the most abundant antinutrients found in cassava, play a vital role in the plant's defence to prevent diseases and deter herbivores (Shigaki, 2015). However, Sousa *et al.* (2015) proved that the susceptibility of soybean crops to pathogens is

negatively correlated to the concentration of antinutrient compounds. Therefore, it can be interpreted that in general, antinutrients exist in plants to perform specific functions for the plants, and are not meant for human consumption.

According to Samtiya *et al.* (2020), the presence of antinutrients in foods can be reduced through various technology and processing methods. The types, structures, and chemical properties of the antinutrients will determine the most effective treatment that can be applied to decrease their levels (Vikram *et al.*, 2020). Commonly, heat treatments such as autoclaving and microwave-heating are the most effective ways to decrease the level of antinutrients, especially in leafy green vegetables, where the presence of heat can rupture the cell wall of plants, thus leaching out the soluble antinutrients (Natesh *et al.*, 2017; Samtiya *et al.*, 2020). However, some antinutrients such as phytate or phytic acid are heat-stable, thus other physical methods should be used for their reduction (Samtiya *et al.*, 2020). For example, phytic acid can be found abundantly in bran fraction or outer layer of seeds (Ertop and Bektaş,

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2018), and it has been suggested that phytic acid in seeds can be removed through the milling process (Sinha and Khare, 2017).

Another approach that can be applied to reduce the level of antinutrients is fermentation. Fermentation is a processing method that converts carbohydrates into simpler molecules by fermenting microorganisms. It is a common practice in countries such as South America and Africa, due to their dependency on agricultural products such as cereals and legumes as their staple foods (Nkhata *et al.*, 2018). Fermentation is not only capable of decreasing antinutrient levels and increasing food safety, but also capable of enhancing the food's nutritional quality (Samtiya *et al.*, 2020). Mohapatra *et al.* (2019) reported that fermentation helped amplified the content of essential amino acids such as tryptophan and methionine. Due to the ability of antinutrients to reduce the bioavailability of micronutrients, fermentation provides effective mechanisms to interrupt the interactions between micronutrients and antinutrients through several interactions. The exact mechanisms of the degradation of antinutrients by fermenting microorganisms will be discussed further in the subsequent subtopics.

The common thing about the use of advanced technologies in reducing the level of antinutrients is that they are not extensively studied. There is still a lack of scientific research on the safety and efficacy of innovative technologies such as pressurised, electromagnetic, acoustic, and genetic modification technologies. However, several pieces of literature have demonstrated the potential of these technologies to decrease antinutrient content in foods (Yan *et al.*, 2012; Hamid *et al.*, 2017; Pan *et al.*, 2019; Popova and Mihaylova, 2019). In this review, the type of antinutrients that are present in foods, their effects on human health, and the processing methods to reduce them will be discussed. This review will also cover various traditional and innovative processing methods that can be applied to reduce antinutrients, the advantages of these processing technologies, and the effects of these processing technologies on other bioactive compounds and phytochemicals that exist in plant-based foods.

#### *Effects of common antinutrients on human health*

The antinutrients listed in Table 1 have one thing in common; they decrease the bioavailability of certain minerals or nutrients for absorption. Overconsumption of these antinutrients may lead to

the occurrence of more severe diseases in humans, sometimes indirectly. For instance, high intake of food that is rich in oxalate, such as leafy green vegetables, promotes the formation of oxalate-calcium complex (Table 1), which will lead to diseases such as nephrolithiasis (Alealign and Petros, 2018) and hyperoxaluria (Mitchell *et al.*, 2019), which are a symptom of kidney stone disease. Since oxalate will bind to calcium, this will also hinder the absorption of calcium in the body, thus leading to diseases such as hypocalcaemia, a condition where the level of calcium in the blood is low (Young *et al.*, 2008).

As described in Table 1, oxalic acid and phytic acid possess the same mechanism, whereby they can bind with minerals such as calcium, zinc, magnesium, and potassium, thus forming an insoluble protein-mineral complex (Gupta *et al.*, 2013; Borquaye *et al.*, 2017). When complexes formed are insoluble, it will render the micronutrient unavailable for solubilisation in the small intestine, thus blocking its absorption. Tannin, saponin, and lectin can also interfere with the absorption of nutrients, but through a different mechanism. Instead of forming a complex with micronutrients, they bind to the lining of the human gut in the gastrointestinal tract and promote inflammation, thus preventing the absorption of nutrients and minerals (Natash *et al.*, 2017; Fleck *et al.*, 2019; Samtiya *et al.*, 2020). Therefore, these antinutrients should be reduced (if not completely removed) as they can cause numerous severe health problems associated with deficiencies of minerals and nutrients (Young *et al.*, 2008; Murphy *et al.*, 2017; Samtiya *et al.*, 2020).

The presence of antinutrients is commonly found in commodities such as cereals, grains, seeds, and legumes. In one study, the protein contents varied from 9.89 to 21.5%, with cereals having the lowest protein content, and legumes having the highest (Rajnicova and Galova, 2019). Some of the antinutrients such as phytic acid, tannins, glucosinolates, and enzyme inhibitors are known to decrease protein digestibility (Borse *et al.*, 2016; Coscueta *et al.*, 2017; Yahia, 2017; Wang *et al.*, 2019). For example, phytic acid can form complexes with proteins, either by competing for mineral factors needed to activate enzymes, or by forming insoluble complexes governed by the formation of electrostatic linkages between phytic acid and basic lysine, histidine, and arginine residues (Mohan *et al.*, 2016; Joye, 2019). Trypsin inhibitor also exhibits the same

**Table 1.** Common types of antinutrients, and their impacts on human health.

<b>Antinutrient</b>	<b>Source</b>	<b>Mechanism</b>	<b>Reported effect</b>
Phytic acid	Nuts, seeds, grains	Chelates metals/minerals such as zinc, copper, and ferum (Gupta <i>et al.</i> , 2013)	Mineral deficiency, decreases protein digestibility, decreases starch utilisation, dwarfism, hypogonadism (Ye <i>et al.</i> , 2016; Murphy <i>et al.</i> , 2017; Wang <i>et al.</i> , 2019)
Tannins	Pomegranates, seeds, berries, cocoa beans	Bind to protein in food, bind to gut digestive enzyme (Natesh <i>et al.</i> , 2017)	Decreases protein digestibility, decreases digestive processes (Yahia, 2017)
Oxalic acid and oxalates	Nuts, seeds, leafy greens	Bind with minerals such calcium, magnesium, and potassium, bind to nutrients in the genitourinary tract (Borquaye <i>et al.</i> , 2017)	Mineral deficiency, nutrient deficiencies, gut lining damage, leads to hypocalcaemia (Tadele, 2015; Samtiya <i>et al.</i> , 2020)
Glucosinolates	Cabbage, cauliflower, broccoli, kale	Compete and inhibit accumulation of iodide in the thyroid gland (Possenti <i>et al.</i> , 2017)	Decreases protein digestibility, decreases iodine absorption, decreases synthesis of thyroxin, impairs metabolism (Borse <i>et al.</i> , 2016)
Saponin	Legumes, tea leaves, <i>Allium</i> species, seeds	Inhibit activities of digestive enzymes, disrupt membrane cholesterol of erythrocyte (Lee <i>et al.</i> , 2015; Ercan and El, 2016; Fleck <i>et al.</i> , 2019)	Decreases nutrient absorption, indigestion disorders, haemolysis (Addisu and Assefa, 2016; Kregiel <i>et al.</i> , 2017; Fleck <i>et al.</i> , 2019)
Enzyme inhibitors (trypsin and amylase inhibitor)	Cereals, legumes	Inhibit the enzyme by blocking the active site, form protein-protein complex or protein-carbohydrate complex	Incomplete digestion of protein and carbohydrate, delayed growth, digestive-related issues (Coscueta <i>et al.</i> , 2017)
Lectins	Cereals, legumes, nuts	Attach to red blood cells, bind to carbohydrates, attach to the lining of guts/small intestine (Panacer and Whorwell, 2019; Samtiya <i>et al.</i> , 2020)	Agglutinates red blood cells, interferes with carbohydrate digestion, mineral and nutrient deficiency (Samtiya <i>et al.</i> , 2020)

mechanism, whereby it can bind to the  $\epsilon$ -amino groups of lysine, thus reducing the digestibility of the protein (Shi *et al.*, 2017; Velickovic and Stanic-Vucinic, 2018).

Despite the various reports on the damaging effects of antinutrients (Table 1), some conflicting reports were also found which indicated that some of these antinutrients or their hydrolysis products may or may not be advantageous to human health. For example, a glucosinolate compound, progoitrin, when hydrolysed into goitrin, is known for its antithyroid properties, and associated with goitre formation following long-term exposure (Bischoff, 2016). However, several other hydrolysis products of glucosinolates, especially isothiocyanates, have become an interesting subject in the medical field due to their anticarcinogenic activity under appropriate concentrations (Possenti *et al.*, 2017). Similarly, at low levels, lectins, phytic acid, and phenolic compounds such as saponin and enzyme inhibitors, have shown antidiabetic properties by reducing the blood glucose and cholesterol levels (Popova and Mihaylova, 2019). This might be caused by the ability of phytic acid to disrupt intestinal lipase activity, which will help to decrease the level of cholesterol in the body, thus reducing the chances of getting cardiovascular diseases (Abdulwaliyu *et al.*, 2019).

#### *Traditional methods to reduce antinutritional factors in plant-based foods*

Many studies have reported that antinutrients in food can be reduced through traditional processing methods such as milling, soaking, cooking, autoclaving, irradiation, germination, enzymatic treatment, and fermentation (Natesh *et al.*, 2017; Sadhu *et al.*, 2017; Thakur *et al.*, 2019; Vikram *et al.*, 2020; Alsalman and Ramaswamy, 2020). Samtiya *et al.* (2020) mentioned that most antinutrients are water-soluble; therefore, sometimes a process as simple as soaking can reduce the antinutrient levels by leaching them out into the soaking medium. Various processing techniques can be used to decrease the antinutrient level, either alone or in combination (Sihag *et al.*, 2015; Ertop and Bektaş, 2018; Popova and Mihaylova, 2019).

Vanga *et al.* (2017) classified physical processing methods (such as thermal; wet and dry heat, and non-thermal; soaking, germinating, and dehulling) and fermentation as traditional processing methods, whereas processes such as ozonation and

cold-plasma processing as innovative technologies. Traditional technologies are generally inexpensive and simple to make, apply, maintain, and repair. Among all traditional methods, many literatures proposed that fermentation is the superior method to reduce the antinutritional factors in foods, and improve food ingredients' organoleptic, nutritional, and shelf-life attributes (Tamang *et al.*, 2016; Samtiya *et al.*, 2020).

#### *Application of fermentation to reduce antinutrients in plant-based foods*

Fermentation of food is an anaerobic process that involves the chemical conversion of carbohydrates into lower molecular weight compounds through the help of microorganisms such as bacteria, yeasts, moulds, or combination of these (Vuppala and Murthy, 2015). In Table 2, many studies have proven that fermentation is an effective method to reduce antinutrient levels in foods (Sharma *et al.*, 2015; Olagunju *et al.*, 2018; Kitum *et al.*, 2020). In foods, lactic acid bacteria (LAB) are the most common microorganisms used or present during fermentation. LAB have been implicated in the production of phytase, which is the enzyme to degrade phytic acid (Nuobariene *et al.*, 2015; Rollán *et al.*, 2019). Additionally, it helps in maintaining the pH at around 4.0 to 5.5 (Demir *et al.*, 2018; Lamid *et al.*, 2018), which is the optimum pH required for maximal activity of phytase (Samtiya *et al.*, 2020). Ogodo *et al.* (2019) found that LAB fermentation of maize flour decreased the levels of antinutritional factors more significantly than spontaneous fermentation. However, in another study, a different result was obtained whereby the tannin and phytate contents in *Phaseolus vulgaris* L. showed a larger reduction when spontaneous fermentation was applied, as compared to fermentation with *Lactobacillus plantarum* BFE 5092 (Kitum *et al.*, 2020). Therefore, the reduction of antinutrient concentration is likely to be dependent on the type of food, rather than the species of the microflora.

As earlier mentioned, microbial fermentation plays a major role in reducing the antinutrient concentration in foods, as demonstrated in kinema (Sharma *et al.*, 2015), *Xuan Mugua* (Shang *et al.*, 2019), *Hura crepitans* (Gbadamosi and Osungbade, 2017), *Tamarindus indica* L. seeds (Olagunju *et al.*, 2018), and rambutan seeds (Mehdzadeh *et al.*, 2015; Chai *et al.*, 2019). Most fermentation may lead to an

**Table 2.** Reduction of antinutrients via fermentation in plant-based foods.

Antinutrient	Food	Microflora	Period	Findings	Reference
Tannin	Bambara nut ( <i>Voandzeia subterranean</i> L.)	<i>Rhizopus</i> spp. ( <i>R. oligosporus</i> , <i>R. oryzae</i> , <i>R. nigricans</i> )	0, 12, 24, 36, 48, 60, and 72 h	Decreased 93.55%	Ola and Opaleye (2019)
	Cassava product ( <i>gari</i> )	N/A	0, 24, 48, 72, 96, and 120 h	Decreased 30.51%	Olaoye <i>et al.</i> (2015)
	Kariya ( <i>Hildergardi barteri</i> ) seeds	N/A	24 - 96 h	Decreased 24.54%	Fawale <i>et al.</i> (2017)
	Kinema – a <i>Bacillus</i> -fermented alkaline food	<i>Bacillus subtilis</i> DK-W1 (MTCC 1747)	48 h	Decreased 100%	Sharma <i>et al.</i> (2015)
	<i>Xuan Mugua</i> fruits	LAB (not specified)	3, 8, and 34 h	Decreased 78%	Shang <i>et al.</i> (2019)
	<i>Hura crepitans</i> seeds	N/A	24, 48, 72, and 96 h	Decreased 18%	Gbadamosi and Osungbade (2017)
	<i>Tamarindus indica</i> L.	<i>B. subtilis</i> , <i>B. licheniformis</i> , <i>B. pumilus</i>	24, 48, and 72 h	Decreased 75%	Olagunju <i>et al.</i> (2018)
	Cocoa butter alternatives from fermented rambutan seeds	N/A	8 d (mixing interval every 48 h)	Decreased 47%	Chai <i>et al.</i> (2019)
	<i>Phaseolus vulgaris</i> L.	<i>L. plantarum</i> BFE 5092	24, 72, and 120 h	Tannin content was 109.50 and 54.04 mg/100 g in inoculated fermentation (IF) and spontaneous fermentation (SF), respectively.	Kitum <i>et al.</i> (2020)
	Maize flours	<i>L. plantarum</i> , <i>L. rhamnosus</i> , <i>L. nantesii</i> , <i>L. fermentum</i> , <i>L. reuteri</i> , <i>Pediococcus acidilactici</i> , <i>L. brevis</i>	0, 12, 24, 36, and 48 h	Decreased with increasing fermentation period with LAB-consortium than spontaneous fermentation	Ogodo <i>et al.</i> (2019)
Phytic acid/ phytate	Bambara nut ( <i>Voandzeia subterranean</i> L.)	<i>Rhizopus</i> spp. ( <i>R. oligosporus</i> , <i>R. oryzae</i> , <i>R. nigricans</i> )	0, 12, 24, 36, 48, 60, and 72 h	Decreased 69.60%	Ola and Opaleye (2019)
	Cassava product ( <i>gari</i> )	N/A	0, 24, 48, 72, 96 and 120 h	Decreased 78.63%	Olaoye <i>et al.</i> (2015)
	Kinema – a <i>Bacillus</i> -fermented alkaline food	<i>Bacillus subtilis</i> DK-W1 (MTCC 1747)	48 h	Decreased 71%	Sharma <i>et al.</i> (2015)
	<i>Tamarindus indica</i> L.	<i>B. subtilis</i> , <i>B. licheniformis</i> , <i>B. pumilus</i>	24, 48, and 72 h	Decreased 50%	Olagunju <i>et al.</i> (2018)
	<i>Phaseolus vulgaris</i> L.	<i>L. plantarum</i> BFE 5092	24, 72, and 120 h	Phytates were at 242.52 and 163.43 mg/100 g in IF and SF, respectively.	Kitum <i>et al.</i> (2020)

	Maize flours	<i>L. plantarum</i> , <i>L. rhamnosus</i> , <i>L. nantesis</i> , <i>L. fermentum</i> , <i>L. reuteri</i> , <i>Pediococcus acidilactici</i> , <i>L. brevis</i>	0, 12, 24, 36, and 48 h	Decreased with increasing fermentation period with LAB-consortium than spontaneous fermentation	Ogodo <i>et al.</i> (2019)
Oxalate	Bambara nut ( <i>Voandzeia subterranean</i> L.)	<i>Rhizopus</i> spp. ( <i>R. oligosporus</i> , <i>R. oryzae</i> , <i>R. nigricans</i> )	0, 12, 24, 36, 48, 60, and 72 h	Decreased 74%	Ola and Opaleye (2019)
	Cassava product ( <i>gari</i> )	N/A	0, 24, 48, 72, 96, and 120 h	Decreased 70.67%	Olaoye <i>et al.</i> (2015)
	Kariya ( <i>Hildergardi barteri</i> ) seeds	N/A	24 - 96 h	Decreased 19.68%	Fawale <i>et al.</i> (2017)
	<i>Hura crepitans</i> seeds	N/A	24, 48, 72, and 96 h	Decreased 21%	Gbadamosi and Osungbade (2017)
Trypsin inhibitor	Bambara nut ( <i>Voandzeia subterranean</i> L.)	<i>Rhizopus</i> spp. ( <i>R. oligosporus</i> , <i>R. oryzae</i> , <i>R. nigricans</i> )	0, 12, 24, 36, 48, 60, and 72 h	Decreased 84.59%	Ola and Opaleye (2019)
	Cassava product ( <i>gari</i> )	N/A	0, 24, 48, 72, 96, and 120 h	Decreased 78.35%	Olaoye <i>et al.</i> (2015)
	Kinema – a <i>Bacillus</i> -fermented alkaline food	<i>Bacillus subtilis</i> DK-W1 (MTCC 1747)	48 h	Decreased 61%	Sharma <i>et al.</i> (2015)
	<i>Tamarindus indica</i> L.	<i>B. subtilis</i> , <i>B. licheniformis</i> , <i>B. pumilus</i>	24, 48, and 72 h	Decreased 86%	Olagunju <i>et al.</i> (2018)
	Maize flours	<i>L. plantarum</i> , <i>L. rhamnosus</i> , <i>L. nantesis</i> , <i>L. fermentum</i> , <i>L. reuteri</i> , <i>Pediococcus acidilactici</i> , <i>L. brevis</i>	0, 12, 24, 36, and 48 h	Decreased with increasing fermentation period with LAB-consortium than spontaneous fermentation	Ogodo <i>et al.</i> (2019)
Saponin	Kariya ( <i>Hildergardi barteri</i> ) seeds	N/A	24 - 96 h	Decreased 27.78%	Fawale <i>et al.</i> (2017)
	<i>Hura crepitans</i> seeds	N/A	24, 48, 72, and 96 h	Decreased 44%	Gbadamosi and Osungbade (2017)
	Cocoa butter alternatives from fermented rambutan seeds	N/A	8 d (mixing interval every 48 h)	Decreased 67%	Chai <i>et al.</i> (2019)

increase in nutritional or functional values of the foods, such as an increase in antioxidant level (Sanjukta *et al.*, 2015; Gbadamosi and Osungbade, 2017; Verni *et al.*, 2019), protein level (Olagunju *et al.*, 2018), development of flavour, and reduction of complex molecules (Olasupo *et al.*, 2016; Chai *et al.*, 2019). Certain fermentation may even form newly nutrients or bioactive compounds unavailable in the original substrate, such as  $\gamma$ -aminobutyric acid (GABA) in soy sauce (Wan-Mohtar *et al.*, 2020) and vitamin K in *natto* (Tsukamoto *et al.*, 2000).

#### *Conventional physical processing to reduce antinutrients in plant-based foods*

Table 3 describes the different types of physical processing that are commonly used to reduce the antinutrient level in foods with the inclusion of heat. In Table 3, different heat treatments, such as blanching, autoclaving, extrusion cooking, roasting, steaming, and boiling, are mostly effective in reducing or degrading the antinutritional factors in fooda. Heat may disrupt the plant's cell wall to allow leaching out of antinutrient compounds (Natesh *et al.*, 2017; Bellande *et al.*, 2017), or simply by denaturing their structures (Alam *et al.*, 2016; Adeleke *et al.*, 2017; Csapó and Albert, 2019). In extrusion cooking, the denaturation effect on trypsin inhibitor is also due to the shear force applied during the extrusion process, thus leading to the deformation of the compound structure. The extent of the effectiveness of the process is highly dependent on the temperature used, the type of plants, and its genetic constituent or characteristics (Dagostin, 2016).

Although heat was generally shown to reduce antinutrients, the use of dry heat such as roasting may selectively increase antinutrients while decreasing others (Bueno-Borges *et al.*, 2018; Sahni *et al.*, 2021). Liu *et al.* (2017) proposed that the increase in antinutrient levels despite being exposed to heat was due to a better extraction of the antinutrients after processing. The reduction of tannin and phytic acid contents in beans was reported to be greater in steamed beans (31.1 and 46.1%, respectively) as compared to roasted beans (4.25 and 18.42%, respectively) (Nakitto *et al.*, 2015). The medium used in the steaming process allows the antinutrients to leach out, thus reducing their contents (Miano and Augusto, 2018). However, as compared to tannin and phytic acid contents in non-treated boiled beans, boiled beans showed lower reduction of these

antinutrients (7.24 and 10.81%, respectively). Additional pre-treatment before heating, such as the de-hulling process, can significantly improve the reduction of antinutrients (Nakitto *et al.*, 2015), likely due to the greater access of heat, and its subsequent leaching capabilities.

#### *Irradiation to reduce antinutrients in plant-based foods*

Irradiation of food is the exposure of food to ionising radiation such as alpha, beta, gamma, energetic electrons, or x-ray for a certain period (Lima *et al.*, 2019). The effectiveness of irradiation ultimately depends on the dose used on the target material, and time of exposure. For example, inhibition of vegetable sprouting, delaying of fruit ripening, and insect disinfections usually require low doses of irradiation (as low as below 1 kJ/kg), while irradiation doses ranging from 1 to 10 kJ/kg are required for reduction of microbial loads, and high irradiation dose (ranges from 10 to 50 kJ/kg) is used for commercial sterilisation of low acid foods and elimination of viruses (Ehlermann, 2016). Several literatures have also demonstrated that irradiation dose as low as 2.0 kJ/kg was able to reduce the level of antinutrients in most grains and beans; and is regarded as one of the best and safe post-harvest methods in extending the shelf-life of the commodities, and minimising the antinutrients (Abdalla *et al.*, 2015; Mahmoud *et al.*, 2016; Popova and Mihaylova, 2019).

Table 4 summarises the impact of food irradiation on the antinutrient contents. Khosravi *et al.* (2016) proposed that the reduction of antinutrients is attributable to the ability of gamma irradiation to hydrolyse the chemical bonds of the antinutrients, while retaining the nutritional composition of the foods (Devi *et al.*, 2018). However, Devi *et al.* (2018) also reported that gamma irradiation may alter crude protein, crude fibre, and crude fat contents in *Citrus jambhiri*, where the values showed a decreasing trend when higher doses (0.75 - 1.0 kJ/kg) were applied. As irradiation carries high-energy molecules, such a process can interact with biomolecules and decrease their content. In rare circumstances, it may increase certain nutrients such as ascorbic acids (de Figueiredo *et al.*, 2014) or carbohydrates (Bamidele and Akanbi, 2013). Nevertheless, numerous researches have shown that the macronutrient content of foods is found to be fairly stable against irradiation; however,

**Table 3.** Physical processing techniques to reduce antinutrients in plant-based foods.

Item	Antinutrient	Method	Findings
<i>Dioscorea dumetorum</i> (bitter yam) (Egbuonu and Nzewi, 2016)	Tannin	Blanching at 100°C for 3, 6, 9, 12, 15, and 18 min	-62.6% (tannin) and -67.43% (alkaloids) at 18 <sup>th</sup> min. Reduction of many nutrients and proximate composition.
<i>Amaranthus viridis</i> seed flour (Olawoye and Gbadamosi, 2017)	Tannin, oxalate, saponin	Autoclaving at 121°C for 15 min; Blanching at 75°C for 5 min	-56.52% (tannin), -45.5% (oxalate), and -40.57% (saponin) during autoclave. -44.2% (tannin), -42.93% (saponin), and -32.46% (saponin) during blanching
<i>Crotalaria laburnoides</i> Klotzsch leaves (Mwakalukwa et al., 2016)	Tannin, phytate, oxalate	Blanching at 80°C for 15 min	-53.02% (phytate) and -24.44% (oxalate). Insignificant tannin reduction
Alfalfa (Sahni et al., 2020)	Tannin, phytic acid, trypsin inhibitor, lectin	Wet heat processing at 110°C for 10 min	-12.04% (tannin), 14.89% (phytic acid), and -85.97% (trypsin inhibitor), -100% (lectin).
<i>Sesbania aculeate</i> (dhaincha) (Sahni et al., 2021)	Tannin, phytic acid, trypsin inhibitor, saponin, lectin	Wet heating at 100°C for 10 min; Dry heating (roasting) at 120°C for 20 min; Extrusion cooking at 150°C, 150 rpm, and 25% moisture content	+7.6% (phytic acid), -9.80% (tannin), -80.78% (trypsin inhibitor), -11.51% (saponin) during wet heating. +2.53% (phytic acid), -75.81% (trypsin inhibitor), -7.89% (saponin) during dry heating. +4.92% (phytic acid), -100% (trypsin inhibitor), -23.68% (saponin) during extrusion cooking. Tannin was not significantly affected by dry and extrusion heating, while lectin was eliminated in all treatments
Beans (Nakitto et al., 2015)	Tannin, phytic acid	Boiling, Dehulled and steamed (DS), Dehulled and roasted (DR), Whole beans steamed (WS), Whole beans roasted (WR)	Tannin and phytic acid were undetected in both DS and DR. -7.24% (tannin) and -10.81% (phytic acid) by boiling. -4.25% (tannin) and -18.42% (phytic acid) in WR. -31.1% (tannin) and -46.1% (phytic acid) in WS.
Sacha inchi seeds (Bueno-Borges et al., 2018)	Tannin, phytic acid, trypsin inhibitor, saponin	15 min roasting at 80°C (T80), 120°C (T120), and 160°C (T160).	Tannin was undetected in all treatments. +35.30% (saponin), -90.86% (phytic acid), -94% (trypsin inhibitor) at T160
Cassava – Tigernut composite flour extruded snack (Adebowale et al., 2017)	Tannin, phytate, oxalate	Extrusion cooking of flours of different compositions at 80°C, moisture content 27%, and a screw speed of 60 rpm.	- 32.42% (tannin), - 21.65% (phytate), - 24.10% (oxalate), and - 34.21% (hydrogen cyanide)
<i>Oryza sativa</i> L. flours (Albarracín et al., 2019)	Phytic acid	EBR - Extrusion cooking of brown rice at 160°C, screw speed was 150 rpm; ESR - Extrusion cooking at 160°C of soaked rice (in lactic acid solution at 45°C for 24 h); EGR - Extrusion cooking at 175°C of germinated rice (at 35°C for 24 h)	-65% (phytic acid) in ESR. -14% (phytic acid) in EBR. Highest antioxidant activity recorded in EGR.



Cereal bran (Kaur <i>et al.</i> , 2015)	Phytic acid, trypsin inhibitor, polyphenols, oxalates	Extrusion cooking at different temperatures and moisture content. The screw speed was 400 rpm.	-54.51% (phytic acid), -36.84% (oxalate), -72.39% (trypsin inhibitor), and -73.38% (polyphenol) at 140°C and 20% moisture content
Whole rice grain (Albarracín <i>et al.</i> , 2015)	Phytic acid	Soaked in lactic acid solution at 45°C for 24 h; Extrusion cooking at 160°C with 16.5% moisture content, screw speed was 150 rpm.	-52.41% (phytic acid) in soaked rice. -77% (phytic acid) is soaked and extruded rice
Foxtail millet grains (Sharma <i>et al.</i> , 2018)	Tannin, phytic acid	High pressure soaking at 200, 400, and 600 MPa. Each pressure is operated at 20, 40, 60, and 80°C for 30, 60, 90, and 120 min.	-67.87% (phytic acid) at 400 MPa (40°C). The highest reduction of tannin is observed at 600 MPa (80°C for 120 min).
Chickpeas (Alsalman and Ramaswamy, 2020)	Tannin, phytic acid	Pre-soaked and without pre-soaking under high-pressure treatment at 200 and 500 MPa up to 6 cycles	-76.6% (tannin) and -85.71% (phytic acid) with soaking. -77.52% (tannin) and -81.51% (phytic acid) without soaking
<i>Canavalia ensiformis</i> (Ojo <i>et al.</i> , 2018)	Tannin, phytic acid, trypsin inhibitor, saponin	Boiling at elevated pressure (80 KPa); Steamed at elevated temperature (80 KPa)	-62.97% (phytic acid), -75.99% (saponin), and -3.49% (tannin) when boiled at elevated pressure. -56.41% (phytic acid), -74.29% (saponin), and 61.27% (tannin) when soaked at elevated pressure. -63.26% (phytic acid), -78.83% (saponin), and -64.69% (tannin) when boiled at normal pressure. -57.02% (phytic acid), -72.40% (saponin), and -61.41% (tannin) when soaked at normal pressure. Complete inactivation of trypsin inhibitor at all treatments

**Table 4.** Advantages of irradiation in reducing antinutrients in plant-based foods.

Antinutrient	Food	Treatment	Dose	Findings	Reference
Tannin	Mung bean ( <i>Phaseolus vulgaris</i> )	Infrared radiation	1000 W at 40 kg/h	Decreased 92.51%	Padmashree et al. (2016)
		Soaking and cooking followed by gamma irradiation	1, 5, and 10 kJ/kg	Tannin was reduced when soaking and cooking were performed before irradiation	Lima et al. (2019)
	<i>Mucuna deeringiana</i>	Gamma irradiation	2, 5, 10, 15, and 25 kJ/kg	Tannin increased as irradiation dose increased	Tresina et al. (2018)
	Whole flours of sorghum cultivars Dabar, Wad Ahmed, and Karamaka	Gamma irradiation followed by fermentation and cooking	5, 10, and 15 kJ/kg	Tannin was reduced when combination of treatments was applied	Abdalla et al. (2015)
	Kachai lemon ( <i>Citrus jambhiri</i> Lush)	Gamma irradiation	0.25, 0.5, 0.75, and 1.0 kJ/kg	Tannin decreased as irradiation dose increased	Devi et al. (2018)
<i>Pennisetum glaucum</i> L.	Gamma irradiation	0.25, 0.50, 0.75, 1.0, and 2.0 kJ/kg	Tannin decreased	Mahmoud et al. (2016)	
Phytic acid/ phytate	Mung bean ( <i>Phaseolus vulgaris</i> )	Infrared radiation	1000 W at 40 kg/h	Decreased 57.14%	Padmashree et al. (2016)
		Soaking and cooking followed by gamma irradiation	1, 5, and 10 kJ/kg	Phytic acid was reduced when soaking and cooking were performed before irradiation	Lima et al. (2019)
	<i>Mucuna deeringiana</i>	Gamma irradiation	2, 5, 10, 15, and 25 kJ/kg	Phytic acid decreased as irradiation dose increased	Tresina et al. (2018)
	Whole flours of sorghum cultivars Dabar, Wad Ahmed, and Karamaka	Gamma irradiation followed by fermentation and cooking	5, 10, and 15 kJ/kg	Phytate was reduced when combination of treatments was applied	Abdalla et al. (2015)
	<i>Pennisetum glaucum</i> L.	Gamma irradiation	0.25, 0.50, 0.75, 1.0, and 2.0 kJ/kg	Decreased phytic acid	Mahmoud et al. (2016)
Rice bran for broiler's chicken diet	Gamma irradiation	100 kJ/kg	Decreased 98%	Khosravi et al. (2016)	
Trypsin inhibitor	Mung bean ( <i>Phaseolus vulgaris</i> )	Infrared radiation	1000 W at 40 kg/h	Decreased 100%	Padmashree et al. (2016)
	<i>Mucuna deeringiana</i>	Gamma irradiation	2, 5, 10, 15, and 25 kJ/kg	Trypsin inhibitor decreased as irradiation dose increased	Tresina et al. (2018)
	Rice bran for broiler's chicken diet	Gamma irradiation	100 kJ/kg	Decreased 98%	Khosravi et al. (2016)
Phyto-haemagglutinins	<i>Mucuna deeringiana</i>	Gamma irradiation	2, 5, 10, 15, and 25 kJ/kg	Phyto-haemagglutinins decreased as irradiation dose increased	Tresina et al. (2018)

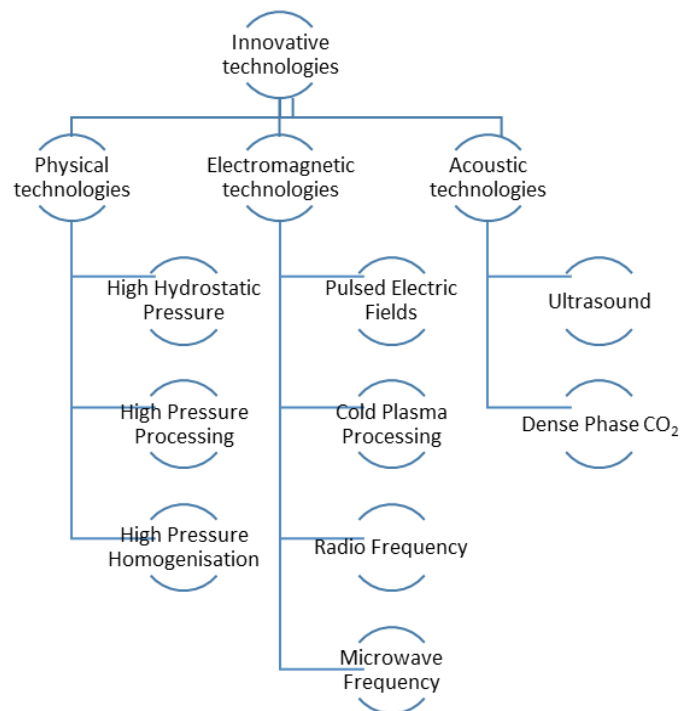
it depends on the dose applied, the composition of the food, and the environment of irradiation such as the surrounding temperature, and the presence or absence of oxygen (Jan *et al.*, 2020).

Like nutrients, irradiation can also affect antinutrients. Tresina *et al.* (2018) demonstrated that gamma irradiation on *Mucuna deeringiana* succeeded in decreasing the value of antinutrients studied (phytic acid, trypsin inhibitor, and hydrogen cyanide) as the irradiation dosage increased. However, the level of tannin was found to increase proportionally with the irradiation dose. The increasing trend was probably due to its high extractability, while the decrease in the other components was caused by the rupture of the protein structure of the antinutrients (Tresina *et al.*, 2018) or its chemical bonds within the protein (Mahmoud *et al.*, 2016). It was also found that gamma irradiation was able to gradually increase protein fractions (albumin and globulin) with increasing radiation dose. This rupture may improve the *in vitro* protein digestibility, as observed with *Pennisetum glaucum* (up to 40%; Mahmoud *et al.*, 2016), *M. deeringiana* (up to 25%; Tresina *et al.*, 2018), and canola seed oil (up to 24%; Anwar *et al.*, 2015). However, irradiation was shown to be less effective under lack of hydration, as the ability of the antinutrients to leach out into the medium is significantly hindered (Miano and Augusto, 2018).

Interestingly, high gamma radiation may result in the increase of beneficial compounds. At dosages of 0 - 10 kGy, the greatest increase in phenolic and flavonoid contents in faba seeds were detected when the samples were irradiated at 9 kGy (Ali *et al.*, 2019). This was also observed by Marathe *et al.* (2016), when *Phaseolus vulgaris* was treated with gamma irradiation at 0.25 - 10 kGy. Both the phenolic content and antioxidant activity of the samples were enhanced (5 - 10%) when irradiated at 10 kGy. Mun'im *et al.* (2017) also reported that gamma-irradiated *Peperomia pellucida* leaves showed increased total phenolic compound (TPC), DPPH free radical scavenging activity, and angiotensin converting enzyme (ACE) activity when irradiated at 10 kGy. Jamshidi *et al.* (2014) explained that the increase in the total phenolic contents when the samples are exposed to gamma irradiation is due to the breaking of polyphenolic components by irradiation. Gamma irradiation is also known to increase the phenylalanine ammonia lyase activity responsible for synthesising polyphenol acid.

### Innovative technologies to reduce antinutrients in plant-based foods

In recent years, the food industry is becoming more competitive and dynamic, focusing on developing high-quality and freshly produced foods. Consequently, the food sector has demonstrated an increased interest in the development of innovative technologies which have the potential to improve or replace traditional processing processes. These innovative technologies have been shown to result in the production of higher-quality, better-targeted food items at a lower environmental cost, hence boosting the added value of the products that meet many, if not all, of the modern consumer's demands (Barba *et al.*, 2018; Muthukumarappan and Knoerzer, 2020). Barba *et al.* (2018) classified innovative technologies into several groups, among them are (1) physical technologies, (2) electromagnetic technologies, and (3) acoustic technologies (Figure 1). These technologies can be used to reduce antinutrients in food products while retaining other beneficial nutritional contents.



**Figure 1.** The innovative technologies and their grouping. (adapted from Barba *et al.*, 2018).

#### Physical technologies

Balakrishna *et al.* (2020) explained that high pressure processing (HPP) is an advanced technology that subjects foods to high hydrostatic pressure (in the order of hundreds to thousands of atmospheric pressure). As described in Table 4, when *Canavalia*

*ensiformis* was treated with boiling and soaking processes under atmospheric and elevated pressure, it was found that boiling showed a greater reduction in the antinutrient level as compared to soaking, and this result is attributable to the heat that is present during the boiling process (Ojo *et al.*, 2018). It was also found that the reduction of phytic acid, saponin, and tannin contents was mostly higher in treatments (boiling and soaking) at elevated pressure than at atmospheric pressure. Ojo *et al.* (2018) also mentioned that the total inactivation of trypsin inhibitor in thermal processing is due to the denaturation of the chemical structure of the compound. In a more recent study by Alsalman and Ramaswamy (2020) on the reduction of phytic acid, although significant in both pre-soaked and non-pre-soaked beans, the greater reduction was observed when the chickpeas were pre-soaked before HPP at 500 MPa for two cycles. Deng *et al.* (2015) attributed the reduction to the activation of hydrolytic activity of phytase enzyme which has transpired during the soaking process. Tannin, on the other hand, had the greatest reduction when pre-soaking was not performed before HPP, with a reduction of 77.52% at 200 MPa for six cycles. However, the tannin content in foxtail millet was the lowest when HPP was applied at 600 MPa for 120 min at 80°C (Sharma *et al.*, 2018). These disparities can be attributed to the different number of cycles performed, and the different plant species.

#### *Electromagnetic technologies*

Dielectric heating (DH) treatments, such as radiofrequency and microwave radiation, rely on the radiation in the electromagnetic field spectrum that heats significantly quicker than conventional thermal approaches. Radiation for radiofrequency is higher than microwave radiation; therefore, the effects of radiofrequency heating are more impactful than microwave heating (Zhong *et al.*, 2015; Barba *et al.*, 2019). DH treatment was often more effective in reducing the level of trypsin inhibitor, phytic acid, tannins, and saponin (Zhong *et al.*, 2015) AS compared to HPP. Pérez-Grijalva *et al.* (2018) reported that blackberry juice subjected to microwave processing (453 W) for 60 s contained the highest level of polyphenols and monomeric anthocyanin. The increase was due to the ability of microwave radiation to induce cell walls to expand and tear, allowing for more antioxidant chemicals to be extracted. Interestingly, it was found that the highest

phenolic content and radical scavenging activity was reported when *Pisum sativum* L. (green peas) were subjected to lower microwave drying power at 100 W, as high microwave power levels (300 and 450 W) increased the temperature, thus leading to irreversible chemical changes in the phenolic compounds (Chahbani *et al.*, 2018).

The new cold plasma processing (CPP) is now one of the fastest emerging technologies. It offers many advantages, and is of much interest in the food processing industry (Sadhu *et al.*, 2017; Pan *et al.*, 2019). It may replace thermal processing in reducing antinutrients (Ekezie *et al.*, 2017) while saving energy and retaining better nutritional content (Alves Filho *et al.*, 2020). In a study conducted by Sadhu *et al.* (2017), CPP was observed to reduce the content and trypsin inhibitor activity and phytic acid content in *Vigna radiata* (mung beans) as compared to untreated germinated beans for 24 h. The outcome of the study is in agreement with the findings by Li *et al.* (2017) who showed that the trypsin inhibitor activity in soybean was reduced by 86.1% when subjected to CPP. Although no clear correlation was found, the ability of CPP to induce germination and breaking the antinutrients via ion bombardments on the seeds surface might be the contributing factor. It can also be caused by an increase in protease due to the faster absorption of water, thus increasing the seed coat permeability which results in higher enzyme activity (Ekezie *et al.*, 2017; Sadhu *et al.*, 2017; Pan *et al.*, 2019). Besides, several literatures have also reported on the effects of CPP on bioactive compounds of foods, such as the increase in the level of pigments, phenolic compounds, vitamin C, carotenoids, antioxidant activity, and B vitamins in fruit juices/beverages (Rodríguez *et al.*, 2017; Paixão *et al.*, 2019; Silveira *et al.*, 2019). However, it is important to determine the most suitable parameters to perform CPP. This is because overexposure to the plasma (higher flow rate and prolonged processing time) may result in the degradation of some of the bioactive compounds like vitamin C and polyphenols (Rodríguez *et al.*, 2017; Silveira *et al.*, 2019).

Pulsed-electric field (PEF) is a technology that can be defined as applying high-voltage pulses to food matrices using two electrodes. The intensity of the electric field, the pulse form, width, frequency, the total treatment time, the electrode arrangement, and the temperature are among the processing parameters that should be focused on to ensure the effectiveness of the technology. Barba *et al.* (2019)

also mentioned that the majority of PEF research concentrate on food decontamination, but its effect on biological cells has also been extensively studied, primarily in the areas of food processing, such as bioactive chemical extraction or pre-treatment. Recently, there is a growing interest in the application of PEF in reducing the level of antinutrients. Liu *et al.* (2018) found that PEF considerably lowered the oxalate levels in both the outer and inner areas of oca tubers. This reduction could have been due to the change in the conformation of the plasma membrane caused by the application of electric pulses (Cholet *et al.*, 2014; Liu *et al.*, 2018; Barba *et al.*, 2019). In addition, due to PEF ability to inactivate browning enzymes, the browning index of PEF-treated apricot juice was found to be lower than that of unprocessed samples. Additionally, PEF-treated samples always showed a higher yield of extraction of bioactive compounds within a shorter time, such as the phenolic compounds, flavonoids, and antioxidant activity (Rahaman *et al.*, 2020).

#### *Acoustic technologies*

Acoustic technology is well known for transforming mechanical or electrical energy into sound energy with the help of transducers (Barba *et al.*, 2019). Ultrasound is a term used to describe sound waves that exceed the audible frequency range (*i.e.*, greater than 20 kHz) (Bhargava *et al.*, 2021). When the ultrasound travels through food media, the acoustic wave generates a series of compressions and rarefaction in the medium particles (Barba *et al.*, 2019; Bhargava *et al.*, 2021). When the ultrasound power is high enough, cavitation bubbles form, which interact with the local media and neighbouring cavitation bubbles before collapsing and releasing massive amounts of energy, causing chemical, mechanical, and physical changes of the medium (Feng *et al.*, 2011; Barba *et al.*, 2019). Several literatures have demonstrated the effectiveness of ultrasound technology, such as inactivating enzymes in post-harvest treatment (O'Donnell *et al.*, 2010), elimination/reduction of microorganisms (Cameron *et al.*, 2009; Nicolau-Lapeña *et al.*, 2019), and reduction of drying time (Kowalski *et al.*, 2016).

The use of ultrasound in reducing antinutrients in plant-based foods has also been studied (Huang *et al.*, 2008; Yadav *et al.*, 2021). The application of ultrasound technology on the hydration of finger millet showed a reduction of 66.98 and 62.83% of phytate and tannin, respectively, as compared to the

raw sample. Similarly, ultrasound treatment also managed to significantly reduce trypsin inhibitor activity (specifically Kunitz-trypsin inhibitor) up to 55% (Huang *et al.*, 2008). In addition, the reduction of trypsin inhibitor level was proportional to the ultrasonic amplitudes and sonication durations utilised, and it was found that Kunitz-trypsin inhibitor lost its inhibitory effect faster than Bowman-Birk trypsin inhibitor. This is because the resistance of Bowman-Birk trypsin inhibitor towards ultrasound is higher, most likely due to the larger amount of sulfhydryl composite and disulphide linkages in its structure (Huang *et al.*, 2008). As explained by Huang *et al.* (2008) and Yadav *et al.* (2021), the effect of bubbles in the microcavities produced by the ultrasound waves increases significantly, thus resulting in the generation of free radicals that induced the conformational changes in the structure of antinutrients and inactivation of enzymes, such as phytase. Higher total phenolic content was also recorded when finger millet was treated with ultrasound as compared to the raw and conventionally hydrated samples (Yadav *et al.*, 2021). Although ultrasound is known to improve the quality of its final product by improving its texture, firmness, organoleptic properties, and aids in extending its shelf-life at high frequency, the free radicals generated by the acoustic waves may cause negative physical and chemical effects on the foods. Among the adverse effects are lipid oxidation which often induces off-odour and off-flavour, protein denaturation, and reduction of total phenolic contents due to ascorbic acid degradation (Yadav *et al.*, 2021). Therefore, it is essential to investigate the optimum intensity and synergy of ultrasound before its application on foods.

#### *Others*

Genetic modification (GM) is one of the most advanced yet most controversial methods in reducing the level of antinutrients in foods (Zhang *et al.*, 2016; Raman, 2017). In mitigating the occurrence of antinutrients in foods, Gupta *et al.* (2013) explained that phytic acid content could be reduced through GM by cloning the genes of phytase enzyme, which can then be used in the production of transgenic plants with over-expression of phytase enzyme genes (Gupta *et al.*, 2013). For example, the levels of phytic acid and trypsin inhibitor in GM rice crops (Agb0102 and Agb0103) were insignificant, and biologically similar as compared to non-GM rice crops (Oh *et al.*,

2016). This finding could probably be due to the fact that Agb0102 and Agb0103 were not modified and explicitly bred to reduce the level of antinutrients, but for resveratrol synthesis (Agb0102) and drought tolerance (Agb0103).

Ozone (O<sub>3</sub>) is produced from oxygen during irradiation reactions or lightning, and is an allotropic form of oxygen (Mohammadi *et al.*, 2017). Oxygen gas, in its stable form (O<sub>2</sub>), splits into highly reactive singlet oxygen, which has a high tendency to react with other oxygen molecules, thus forming ozone. In 2001, the Food and Drug Association (FDA) gave ozone treatment in food processing a GRAS (Generally Regarded as Safe) approval. Since then, ozone has been extensively used in the food industry, and is an excellent antimicrobial agent in food processing (Varol *et al.*, 2017; Pandiselvam *et al.*, 2019). Aside from its rapid action and strong oxidative behaviour, ozone is also widely used in the food processing and preservation industry due to its competency in leaving no hazardous compounds in food products by quickly decomposes into molecular oxygen (Pandiselvam *et al.*, 2017). Regarding the removal or reduction of antinutrients in food, ozone has been proven to significantly reduce the amount of tannin in grain sorghum flours by changing and disrupting the conformation of tannin molecules, thus affecting the capability of tannin molecules to bind to proteins (Yan *et al.*, 2012). The use of ozonation was also shown in an unrelated food-based application such as wastewater treatment in the tannery industry (Sivagami *et al.*, 2018; Sigona *et al.*, 2020).

## Conclusion

In general, this review discussed the common types of antinutrients found in plants, their health implications, and the processing methods that can be applied to lower the presence of antinutrients in plant-based foods. Both fermentation and thermal treatments can be effective. Nevertheless, non-thermal processing such as irradiation, HPP, and CPP can also be beneficial in lowering the level of antinutrients in plant-based foods in a way that will not significantly alter the final products. Several studies have also concluded that combining two or more processing methods is more effective than the use of single processing method in lowering the level of antinutrients in plant-based foods. Some of the advanced methods, such as GM and ozonation, are

relatively new, but possess tremendous potential in lowering the antinutrients in plant-based foods.

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