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Acacia seed proteins: Low or high quality? A comprehensive review

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Abstract

The popularity of adding value to indigenous plant protein sources has increased due to the rise in the world population, high costs of animal protein as compared to plant proteins, and an increase in the consumer awareness of the nutritional and functional roles of dietary plant protein. Seeds of acacia plants (containing over 1,350 species) have considerable amount of protein (18.25% to 35.5%) and nutritionists have shown great interest in assessing the quality and functionality of proteins from these protein-rich plants. In this review, the overall nutritional and health-promoting properties of acacia seed (AS) species are introduced. Extraction, quality, and functional properties of proteins from different AS species are discussed. Furthermore, anti-nutritional components and protease inhibitors present in AS species and the effects of processing methods applied to lower the levels of anti-nutrients are also discussed. Previous applications of AS in food formulations are highlighted. This review aims to provide updated findings that have been reported on AS proteins and to highlight areas for further studies in order to increase the utilization potential of the seeds.

KEYWORDS

Acacia seed protein, anti-nutrients, characterization, functional properties, processing

1 | INTRODUCTION

1.1 | History, classification, and botanical description of acacia seeds

The genus acacia, also known as wattle, is a large group of woody species in the subfamily *Mimosoideae* of the pea family, *Fabaceae*, and the second largest genus in the *Leguminosae* family (Sulaiman, Sadashiva, Satheesh, Goplakrishnan, & Balachandran, 2013). Acacia comprises over 1,350 species (Maslin, Thomson, McDonald, & Hamilton-Brown, 1998) that are widely distributed in warm temperate, tropical, and sub-tropical regions of the world such as the Australia-Pacific (993), Americas (185), Africa (144), and Asia (89) (Maslin, Miller, & Seigler, 2003; Figure 1a). Maslin et al. (2003) also reported current classification of *Acacia* into five main subgenera; *Acacia*, *Aculeiferum*, *Phyllodineae*, *Filicinae*, and an undescribed subgenus called "Genus x." The acacia plant is native to Australia with approximately 960 species (Maslin & McDonald, 2004; Maslin et al., 1998) found in different regions of the continent (Figure 1b). Seeds of around 40 species of Australian acacia have been used as a food source for thousands of years by

Abbreviations: AAs, amino acids; ACE, angiotensin-converting enzymes; AkCI, *Acacia karro* chymotrypsin inhibitor; AS, Acacia seed; ASP, ammonium sulfate precipitate; AvTi, *Acacia victoriae* Bentham trypsin inhibitor; CD, circular dichroism; CIA, chymotrypsin inhibitor activity; DKA, djenkolic acid; DPPH, 2,2-diphenyl-1-picrylhydrazyl; EA, emulsifying activity; ES, emulsion stability; FC, foam capacity; FRAP, ferric reducing antioxidant power; FS, foam stability; LA, lectin activity; OAC, oil absorption capacity; pI, isoelectric point; PIs, protease inhibitors; TIA, trypsin inhibitor activity; TPC, total phenolic content; WAC, water absorption capacity.

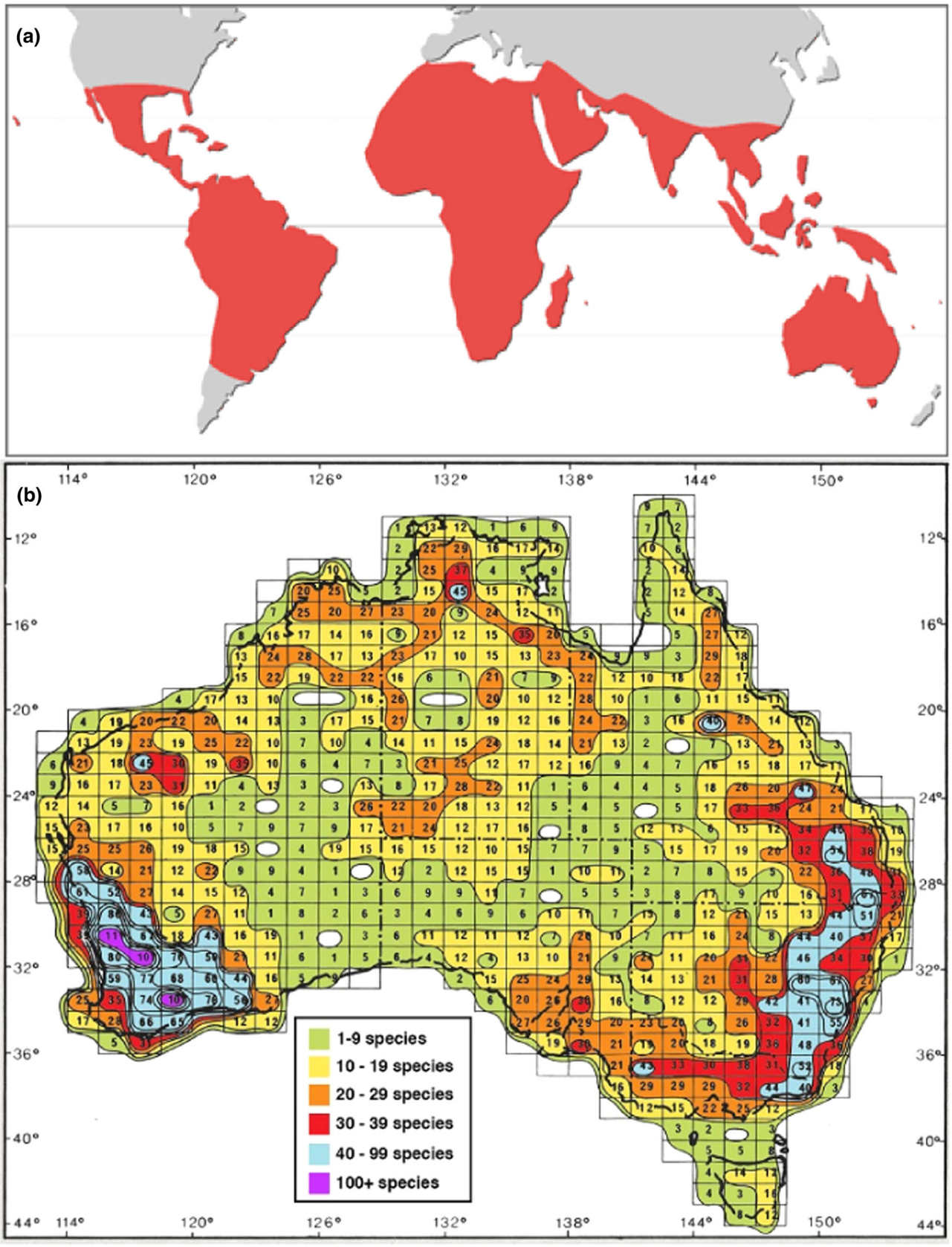

FIGURE 1 (a) Worldwide distribution of acacia (red color indicating acacia plant; Maslin et al., 2003). (b) Areas of species-richness of acacia within Australia. Numbers indicate species recorded in each 1 × 1.5 degree grid cell (http://worldwidewattle.com/infogallery/distribution/)

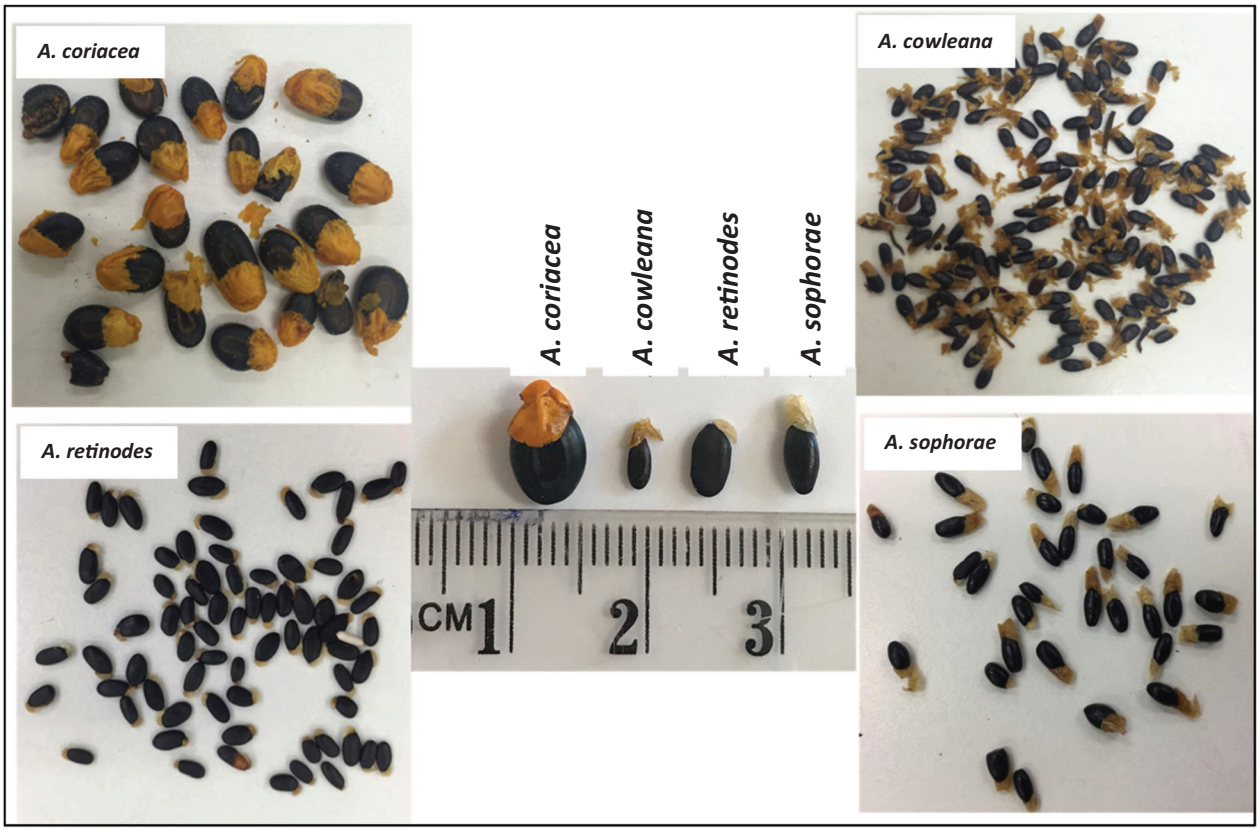

FIGURE 2 Four species of Australian acacia seeds—appearance and size comparison (Shelat et al., 2019)

Aboriginal people in Australia (Latz & Green, 1995; Lister, Holford, & Morrison, 1996). Some of the seeds such as Elegant wattle (*Acacia victoriae* Bentham) are considered by many as the food industry standard, and are available as commercial native spices (Konczak, Zabaras, Dunstan, & Aguas, 2010). As early as the late 1990's, about 10 tonnes (in 1997/1998) of *A. victoriae* Bentham seeds were harvested from the wild (Hele, 2001) and the raw and processed (roasted and milled) seeds were sold at the price of $15 and $20 to 24 per kg, respectively (Robins, 2004). For the past decade, other species have also been commonly traded and include: Coastal Wattle (*Acacia sophorae*), Sandplain Wattle (*Acacia murrayana*), Wiry Wattle (*Acacia coriacea*), Silver Wattle (*Acacia retinodes*), Golden Wattle (*Acacia pycnantha*), and Cole's Wattle (*Acacia colei*) (Cribb, Latham, & Ryder, 2005).

The seeds of some *phyllodinous* species are generally black in color, of varying length and colorful aril, usually brown (Wu & Nielsen, 2009; Figure 2). Flowering occurs mainly in late winter and spring, depending on the species and region the tree is found in (Ryder, Latham, & Hawke, 2008). As with variation of flowering, the maturation of the seeds also varies.

The chemical composition and nutritional value of acacia seed (AS) may vary according to the species, geographical location, nutrients, and soil conditions as well as year of cultivation. Generally, AS are rich in protein, fiber, and complex carbohydrates but they are low in fat and glycemic index (Agboola, Ee, Mallon, & Zhao, 2007; Hegarty, Hegarty, & Wills, 2001). As found in other legumes, protein is the most important of all these components due to its many functional characteristics such as emulsifying, foaming, and gelling properties, which makes them highly sought after for application in foods (Maslin & McDonald, 2004; Maslin et al., 1998).

1.2 | AS uses

AS, unlike other parts of the acacia plant, has significant commercial potential because of its high nutritional value and has been utilized as functional ingredients alone as well as in the development of many food and beverage products (Figure 3) such as bread, coffee, and others (Forbes-Smith & Paton, 2002; Seigler, 2002). Studies by The Commonwealth Scientific and Industrial Research Organization (CSIRO Media release 98/213, 1998) using laboratory testing and human trials have revealed that the seeds of edible acacia plants are safe for consumption. However, the seed of some species contains several anti-nutritional factors such as trypsin and chymotrypsin inhibitors (Adewusi, Falade, & Harwood, 2003; Adewusi, Falade, & Harwood, 2011; Ee & Yates, 2013), which could adversely affect the absorption of specific nutrients, and thus require appropriate processing before consumption. Presently, AS is sold commercially in a roasted form and is well-known as a value added product from

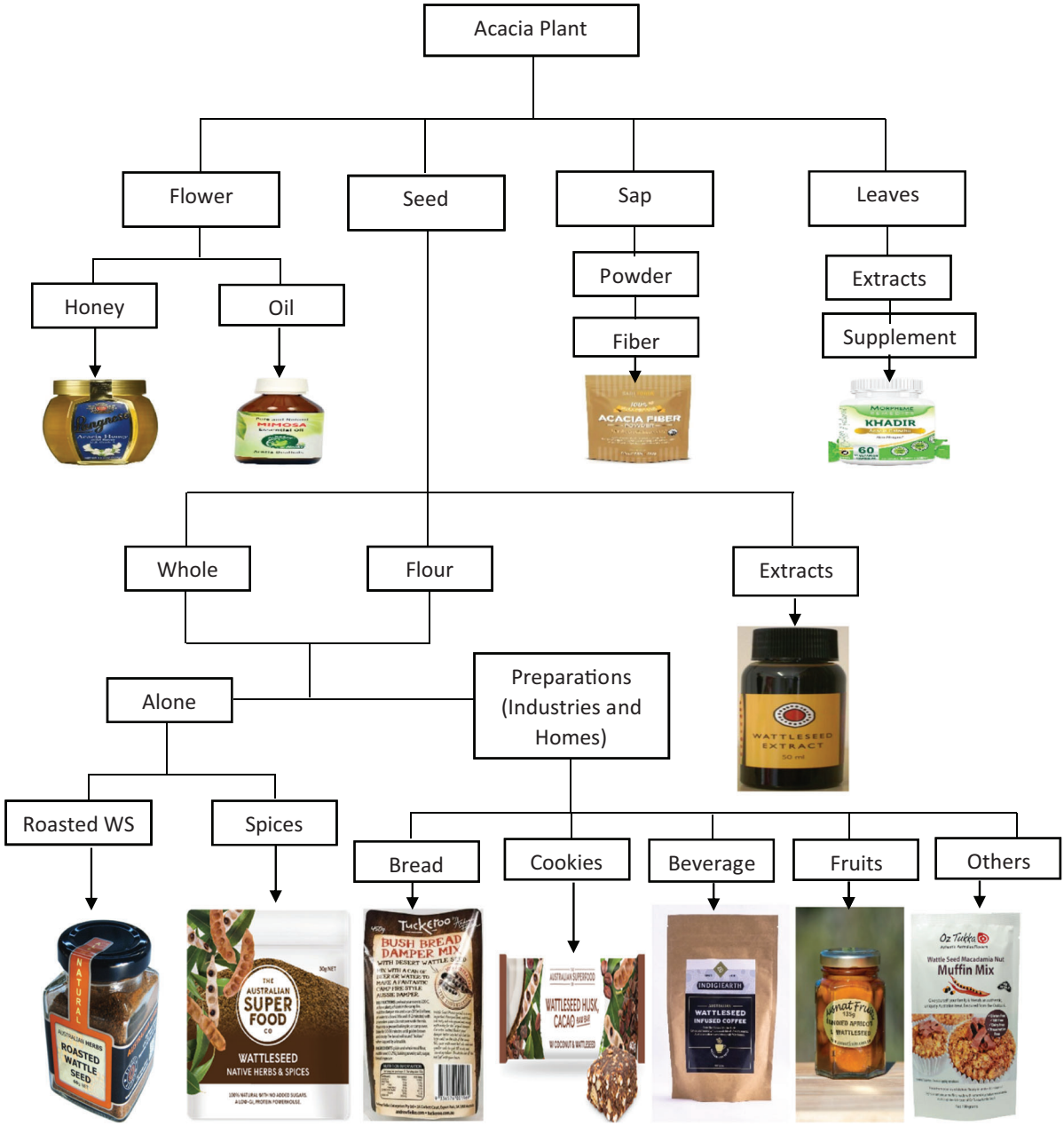

FIGURE 3 Uses of acacia plant and some products from acacia or wattle seeds

the Australian bushfood or native food industry. Generally, the roasted AS are ground and utilized as ingredients in baked foods, condiments, coffee analogue, and dairy products (Agboola & Radovanovic-Tesic, 2002; Forbes-Smith & Paton, 2002; Hegarty et al., 2001; Maslin & McDonald, 2004; Maslin et al., 1998). Moreover, inclusion of AS in diabetic and other specialty diets has been suggested due to its low glycemic index (Cribb et al., 2005).

In addition, AS extracts have been reported to exhibit several health benefits such as antioxidant properties (Abdel-Farid, Sheded, & Mohamed, 2014; Sadiq, Hanpithakpong, Tarning, & Anal, 2015), in vitro tumor inhibitory activity (Jayatilake et al., 2003; Mujoo et al., 2001), and in vitro anticancer properties (Haridas et al., 2001; Li, Davis, Haridas, Gutterman, & Colombini, 2005). Also, the protein fractions from AS possess high functional properties such as water and oil holding capacity (Agboola et al., 2007; Embaby, Swailam, & Rayan, 2018) and these could be advantageous in food formulation. Overall, AS protein and bioactive extracts could be a promising food ingredient due to its nutritional value, processing functionality, and potential health-promoting properties. In this context, this paper presents a comprehensive review of available literature on the overall nutritional and health beneficial properties of AS, protein extraction, quality and functional properties of AS proteins, anti-nutritional components, and protease inhibitors

(PIs) identified in AS as well as processing methods applied to improve its nutritional and health benefits. The present paper also identifies potential application of AS in food formulations.

2 | NUTRITIONAL COMPOSITION AND BIOACTIVE PROPERTIES OF AS

2.1 | Nutritional profile

The chemical composition of different species of AS (Table 1) reveals that it has considerable amounts of protein content ranging from 18.25% as found in *A. victoriae* Bentham (Ee, Zhao, Rehman, & Agboola, 2013) to 35.5% in *A. bilimekii* (Sotelo, Migliaro, Toledo, & Contreras, 1999). Thus, it can serve as an alternative source of protein in human diets. The seeds are also rich in crude fiber with, for example, 15.4% in *Acacia saligna* (Ee & Yates, 2013). The majority of AS contain low amounts of fat and ash, which are similar to those found in most legumes. However, a considerable amount of fat content was reported in *A. saligna* subspecies ranging from 11.5% to 13.9% (Ee & Yates, 2013). Nonfiber carbohydrates are the major macronutrient found in AS species and are typically ≥30% of the total macronutrient content (Table 1).

The seeds of acacia plants are rich in minerals, especially potassium, calcium, magnesium, and iron with the values varying among the species (Table 1). For instance, *Acacia nilotica* and *Acacia leucophloea* contribute about 10 to 11 g of potassium per day (Siddhuraju, Vijayakumari, & Janardhanan, 1996; Vijayakumari, Siddhuraju, & Janardhanan, 1994). Nutritional insecurity is often related with micronutrient deficiency (Tulchinsky & Varavikova, 2014), and these micronutrients have important functions in the normal metabolic activities of the human body (Cilla, Zanirato, Rodriguez-Estrada, & Garcia-Llatas, 2014).

Seeds of *Acacia* spp. have three times more unsaturated fatty acids than saturated fatty acids with oleic and linoleic acids being the predominant fatty acids (Table 1). For instance, approximately 66.57% linoleic acid were identified in *Acacia tortilis* (Embaby & Rayan, 2016), a value similar to those reported in other species such as *A. leucophloea* (Vijayakumari et al., 1994), *A. colei*, and *Acacia tumida* (Adewusi et al., 2003; Falade, Owoyomi, Harwood, & Adewusi, 2005). Overall, AS oils are rich in essential fatty acids that are appropriate for mixing with other vegetable oils that can be used in the confectionary industry.

2.2 | Bioactive and nutraceutical compounds

AS, apart from being consumed as foods by humans due to its favorable nutritional composition, is also rich in bioactive nonnutrient compounds known as phytochemicals that can provide health benefits (Ee, Agboola, Rehman, & Zhao, 2011; Sadiq et al., 2015; Salem, Davidorf, & Abdel-Rahman, 2011). In recent years, there has been an increased interest by the scientific community and consumers on bioactive compounds such as antioxidants present in plants due to their potential beneficial effects against various chronic diseases (Opie & Lecour, 2007). However, consumption of high dosage (>3,000 ppm) of synthetic antioxidants especially butylated hydroxyl anisole commonly added to processed foods has been reported to have carcinogenic effects in rodents (Kahl & Kappus, 1993; Williams, Iatropoulos, & Whysner, 1999). These effects may be prevented by substituting them with natural antioxidants such as those present in AS. These include phenolic acids (gallic, m- and p-digallic acid, and tannic acid) and flavonoids such as quercetin, catechin, and rutin (Sadiq et al., 2015; Salem et al., 2011).

2.2.1 | Antioxidant properties

Antioxidants naturally present in plants possess various biological properties such as therapeutic activities that can scavenge free radicals and lower oxidative stress that cause various cardiovascular diseases (Bagchi et al., 2000). Seeds of acacia plants are regarded as a good source of antioxidants for humans (Abdel-Farid et al., 2014; Sadiq et al., 2015). Different levels of bioactive compounds and antioxidant capacities have been found in AS depending on the species and extraction conditions as depicted in Tables 2 and 3.

Generally, organic solvents such as methanol, ethanol, and acetone are used to extract bioactive compounds with antioxidant activity from AS (Ee, Agboola, et al., 2011; Sadiq et al., 2015; Salem et al., 2011). An earlier study has shown much higher antioxidant levels from hydrolyzed AS flours, than those obtained in acetone extracts, thus indicating that the majority of bioactive compounds in AS exist in bound, insoluble forms that cannot be easily extracted by aqueous acetone (Ee, Agboola, Rehman, & Zhao, 2012). As shown in Table 2, there is variation in the total phenolic contents (TPC) of acacia plants depending on the parts analyzed. For instance, the pods and leaves of *A. nilotica* were found to have considerable amounts of TPS (103.68 and 136.68 mg GAE/g DW, respectively; Sadiq et al., 2015). These values are close to the TPC found in *A. leucophloea* seeds (148.7 mg GAE/g DW; Gautam, Vadivel, Stuetz, & Biesalski, 2012). Also, considerable amounts of total flavonoids have been reported in the pods and seeds of *A. nilotica*, *A. seyal*, and *A. mollissima* (Abdel-Farid et al., 2014; Jelassi et al., 2016). The presence of anthocyanins and a high 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging as well as ferric reducing antioxidant power is also found in some species (Table 2). However, in vitro antioxidant capacity from pods of *A. nilotica* (63.86%), *A. seyal* (66.67%), and *A. laeta* (42.17%) (Abdel-Farid et al., 2014) was found to be lower than some

TABLE 1 Chemical and nutritional composition of different species of acacia seeds (AS)

Components	*A. leucophloea*	*A. nilotica*	*A. colei*	*A. tumida*	*A. saligna*	*A. tortilis*	*A. victoriae Bentham*	*A. bilimekii*
Chemical composition (g/100 g DW*)								
Crude protein	26.5	23.37	22.3 to 23.4	7.9 to 23.0	28.6 to 32.6	27.21	18.2 to 18.56	35.5
Crude fat	5.13	6.66	–	–	11.5 to 13.9	9.19	5.4 to 5.6	–
Crude fiber	6.78	12.6	–	–	12.9 to 15.4	14.31	17.0	13.1
Ash	4.12	3.97	–	–	3.8 to 4.3	3.99	3.70 to 4.03	5.3
Carbohydrate	–	53.4	–	–	30.8 to 33.8	45.30	38.47 to 48.47	39.6
Mineral composition (mg/kg DM**)								
Sodium	320	276.3	70	–		480		
Potassium	10,200	11,082	9,340	9,140		4,480		
Calcium	3,140	2,375	2,740	1,684		705		
Magnesium	2,610	2,618	2,900	2,610		565		
Manganese	40	37.3	80	3,140		38.9		
Iron	220	245	310	544		67.9		
Copper	20	25.1	6	7		7.4		
Zinc	60	27.0	30	22.5		37.6		
Fatty acid composition***								
Palmitic	17.0	18.1	11.4	9.4		12.31		
Stearic	5.80	7.80	3.7	6.9		11.88		
Oleic	22.7	28.8	18.0	22.1		7.74		
Linoleic	51.1	38.2	55.9	50.1		66.57		
Arachidonic	–	1.30	1.4	4.1		1.50		
References	Vijayakumari et al. (1994)	Siddhuraju et al. (1996)	Adewusi et al. (2003); Falade et al. (2005)	Adewusi et al. (2003); Falade et al. (2005)	Ee and Yates (2013)	Embaby and Rayan (2016)	Ee et al. (2013); Agboola et al. (2007)	Sotelo et al. (1999)

*Dry weight.
**Dry matter.
***Percentage of total fatty acids.

commonly consumed legumes such as germinated broad bean (92.46%) (Saleh, Hassan, Mansour, Fahmy, & El-Bedawey, 2019), chickpea (72.80%), lentil (81.65%), and faba beans (95.16%) (Salem, El-Bostany, Al-Askalany, & Thabet, 2014).

2.2.2 | Bioactive compounds

Table 3 presents the bioactive compounds identified in different parts of the acacia plant. Sadiq et al. (2015) reported on the identification of different bioactive compounds in ethanolic extracts of different parts (pods, leaves, and barks) of *A. nilotica*, which revealed phenolic compounds from two major families: gallic acid and tannins; and some other flavonoids (mostly catechin, quercetin, and rutin). This also agrees with the findings reported on the methanolic extracts of *A. nilotica* pods (Salem et al., 2011). However, succinic acid and gallic acid were found in abundance in the seeds of *A. victoriae* Bentham (Ee, Agboola, et al., 2011). Gallic acid is a phenolic acid with a strong antioxidant capacity and thus has been considered as a potential source of functional food ingredients (Sethiya, Trivedi, & Mishra, 2014). Also, quercetin, another major bioactive compound present in AS, is a flavonoid with a structure containing a double bond in the C ring and a 4-oxo group, which increases its antioxidant capacity with in vitro and in vivo proven anticancer effects (Hashemzaei et al., 2017; Rauf et al., 2018). Extraction temperature is another main factor that can influence the kind and amount of bioactive compounds extracted from the plant matrix because some bioactive compounds are sensitive to heat. However, this was not evaluated in previous studies

TABLE 2 Antioxidant capacity and anthocyanins in different species of acacia seed extracts

Species	Parts	TPC	TFC	DPPH	Anthocyanins	RP	MC	FRAP	References
A. nilotica	Pods	9.39 to 103.68	23.01 to 29.03	63.86	8.29	2.01			Abdel-Farid et al. (2014); Sadiq et al. (2015)
	Barks	62.03	45.5						Sadiq et al. (2015)
	Barks	9.20 to 16.5	2.14 to 4.93↑	18 to 45					Sultana, Anwar, and Przybylski (2007)
	Leaves	136.49	37.53						Sadiq et al. (2015)
A. seyal	Pods	10.11	28.25	66.67	11.63	0.50			Abdel-Farid et al. (2014)
A. laeta	Pods	6.21	7.36	42.17	6.98	0.07			Abdel-Farid et al. (2014)
A. cyclops	Seeds	12.84	7.25						Jelassi et al. (2016)
A. mollissima	Seeds	11.78	15.25						Jelassi et al. (2016)
A. cyanophylla	Seeds	2.63	3.60						Jelassi et al. (2016)
A. spp	Seeds	0.8						17.8*	Konczak et al. (2010)
A. victoriae	Seeds	1.19	0.40*						Ee, Agboola et al. (2011)
A. leucophloea	Seeds	148.7		52.08				32.3**	Gautam et al. (2012)
A. auriculiformis	Seeds	10.6					4.7 to 65.9	827.5***	Sathya & Siddhuraju (2013); Loganayaki, Siddhuraju, and Manian (2011)
A. ferruginea	Seeds						72.5		Loganayaki et al. (2011)
A. sp. (species not specified)	Seeds	2.66		81.17					Sommano, Caffin, and Kerven (2013)
A. cyanophylla	Seeds	1.87 to 1.97	0.29 to 0.49↕						Youzbachi et al. (2012)

Note. TPC (total phenolic contents): mg GAE/g extract.
TFC (total flavonoid contents): mg quercetin equivalent/g extract; mg catechin/g of flour↑; g RE/kg DW↕
DPPH (2,2-diphenyl-1-picrylhydrazyl): % DPPH scavenging at 100 μg/mol
Anthocyanin content: μmol/g extract
RP: Reducing power at 100 μg
MC (Metal chelating): mg EDTA eq/g extract
FRAP (Ferric reducing antioxidant power):
*μmolFe^{2+}/g DW
**mmol Fe(II)/g extract
***mM FeSO4/mg

during extraction of bioactive compounds from AS, and thus requires further investigation.

3 | PROTEIN EXTRACTION

Prior to extraction, AS are typically ground to flour, defatted using hexane as solvent and air dried at room temperature in order to maximize protein extraction (Embaby et al., 2018). The size of flour granules used during extraction can influence the extraction yield (Russin, Arcand, & Boye, 2007) but the effect of this for AS has not been reported in previous studies.

3.1 | Alkaline, aqueous, and ethanol extraction of protein

Generally, protein extraction involves mixing of the seed flour and solvent at a given ratio followed by stirring for a given period of time to solubilize the proteins. The mixture will then be centrifuged and proteins in the supernatant will be precipitated by adjusting to acidic pH using a dilute acid. Last, the precipitated proteins will be centrifuged, washed to remove salts, neutralized, and freeze-dried. Several factors could influence the extraction yield of protein and these include: type and concentration of extraction solvent, period of mixing, centrifugation setting, and pH used for protein precipitation (Tan, Mailer, Blanchard, & Agboola, 2011). Alkaline (NaOH) solution is still the most typical solvent used in the extraction of protein from AS followed by precipitation with a dilute acid (HCl; Embaby et al., 2018). This method is based on the high solubility of most plant proteins in alkaline media as compared to acidic media. The protein content and extraction yield of AS protein isolates have differed, probably due to differences in species and extraction conditions. Embaby et al. (2018) produced protein

TABLE 3 Bioactive compounds identified in different species of acacia seed

Species	Parts	Extraction conditions	Compounds reported	References
A. sp. (species not specified)	Seeds	80% aqueous methanol/1.0% HCl (v/v)	Trace amounts of chlorogenic acid, quercetin hexoside, rutin hexoside, and kaeferol were identified	Konczak et al. (2010)
A. victoriae	Seeds	Aqueous acetone (70%) or aqueous methanol (80%), constant stirring for 2 hr at room temperature	Succinic and gallic acid were the predominant phenolic acids	Ee, Agboola et al. (2011)
A. nilotica	Pods	Methanol and ethanol	Gallic acid, catechin, methyl gallate, catechin 5-O-gallate, gallocatechin 5-*O*-gallate, 1-*O*-galloyl-*β*-D-glucose, 1,6-di-*O*-galloyl-*β*-D-glucose, and *m*- and *p*-digallic acid	Salem et al. (2011)
	Pods	Ethanol (80%, v/v), shaking incubator for 48 hr at 200 rpm	Gallic acid, catechin, tannin acid, rutin, iso-quercetin, and quercetin	Sadiq et al. (2015)
	Leaves	Ethanol (80%, v/v), shaking incubator for 48 hr at 200 rpm	Gallic acid, catechin, rutin, iso-quercetin, quercetin, apigenin, and kaemferol	Sadiq et al. (2015)
	Barks	Ethanol (80%, v/v), shaking incubator for 48 hr at 200 rpm	Gallic acid, catechin, tannic acid, rutin, iso-quercetin, hydroquinine, eriodictyol, quercetin, apigenin, and kaempferol	Sadiq et al. (2015)
A. sp. (species not specified)	Seeds	Mixture of methanol, acetone and water (7:7:6, v/v/v)	Naringenin	Sommano et al. (2013)

isolates from seeds of *A. tortilis* using a 1:20 (w/v) flour to solvent ratio, adjusted to pH 11 with 1 N NaOH, stirred for 2 hr, followed by centrifugation and precipitation of protein from the supernatant at pH 4.5 with 1 N HCl. Although a high protein content of isolates (91.8%) was obtained, the yield for this extraction was not reported and would have been helpful in evaluating the economic importance of this process.

In another study that compared different extraction solvents (water, 0.1 M NaOH or 70% ethanol), *A. victoriae* Bentham flour was separately dispersed in each of the solvents (1:10 w/v), stirred for 1 hr followed by centrifugation at 3,000 × *g* and protein precipitated from the supernatant (pH 3.85 with 1 M HCl; Agboola et al., 2007). The water-, alkali-, and ethanol-soluble extracts yielded 34.49, 11.33, and 1.79 g of solids per 100 g of sample, respectively, indicating more water-soluble components in the whole WS. Moreover, the water, alkali, and ethanol extracts obtained contained 26.0%, 52.7%, and 13.0% protein per gram of sample, respectively. The results confirmed that alkali extraction conditions are necessary to enhance protein extraction.

3.2 | Extraction of specific protein fraction

Agboola and Aluko (2009) prepared fractionated AS protein isolates by using 50 mM tris-HCl buffer (1:10 flour to buffer, pH 8.1, room temperature), stirred for 1 hr, followed by centrifugation, fractionation of the supernatant with ammonium sulfate (25%, 50%, and 75%), dialysis of the precipitate (molecular weight cutoff 10,000 Da) of the supernatant against tris-HCL buffer (pH 8.1 at 4 °C, 48 hr), and freeze-drying. The fractions were purified using fast protein liquid chromatography. The fractions obtained were found to have substantial amounts of essential amino acids (AAs) especially lysine and leucine. However, the tertiary structures of the purified protein fractions were more vulnerable to changes in the ionic strength, pH, and temperature used (Agboola & Aluko, 2009), suggesting that further studies should be performed on the utilization of *A. victoriae* seed protein as a functional food ingredient under these conditions. Also, the authors did not report the yield and protein contents of each fraction obtained using this method.

4 | PROTEIN PROFILE AND CHARACTERISTICS

4.1 | AA composition and sequence

4.1.1 | Protein and major protein fractions

AAs are the building blocks of proteins and the composition of AAs, especially essential AAs, determines protein quality. Generally, glutamic and aspartic acid are the major AAs found in most plant proteins, and similar findings were reported in

TABLE 4 Amino acid composition of different species of acacia seed protein and protein fractions

Amino acids (g/100 g protein)	*A. leucophloea*	*A. nilotica*	*A. colei*	*A. tumida*	*A. victoriae Bentham*			*A. tortilis*	*A. bilimekii*
					Whole seeds	Protein fractions	AvTI		
Essential amino acids									
Histidine	2.20	4.10	7.6	7.1	3.07	3.17	1.86	3.41	2.58
Isoleucine	3.59	2.88	8.8	8.4	3.21	3.35	3.82	2.25	2.66
Leucine	6.96	8.16	17.2	16.1	6.87	9.25	7.62	10.4	6.69
Lysine	6.46	6.42	14.3	15.2	7.01	9.13	7.01	3.32	3.87
Methionine	0.47	0.90	3.2	2.6	1.60	0.99	0.75	0.00	0.95
Phenylalanine	4.43	4.10	9.9	8.9	3.59	4.50	3.27	3.12	2.79
Threonine	4.32	3.02	8.3	8.0	4.04	4.68	3.34	2.76	3.37
Valine	4.84	5.62	11.6	13.4	7.08	4.73	4.55	3.49	3.68
Tryptophan	ND	1.87			2.12	0.75	1.17		0.29
Nonessential amino acids									
Arginine	5.43	5.40	15.1	15.2	5.83	5.73	5.98	4.22	12.99
Alanine	6.51	7.11	9.3	9.0	4.05	5.39	3.58	4.22	
Aspartic acid	10.2	14.7	19.0	21.9	12.1	11.1	10.3	3.01	9.46
Glutamic acid	14.6	18.8	30.5	30.5	15.7	14.4	13.3	3.29	12.90
Glycine	8.11	8.30	11.8	12.1	8.10	5.14	4.09	2.04	
Proline	5.60	ND	8.9	9.9	4.48	6.28	4.84	1.14	2.87
Serine	5.81	3.86	12.5	12.3	7.06	6.34	3.36	3.03	5.07
Tyrosine	1.71	2.14	8.4	7.9	2.58	4.36	3.44	2.78	
References	Vijayakumari et al. (1994)	Siddhuraju et al. (1996)	Adewusi et al. (2003)	Adewusi et al. (2003)	Agboola and Aluko (2009)	Agboola and Aluko (2009)	Ee, Zhao et al. (2011)	Embaby and Rayan (2016)	Sotelo et al. (1999)

Abbreviation: AvTi, *Acacia victoriae* Bentham trypsin inhibitor.

AS but their levels vary depending on the species (Table 4). Larger amounts (approximately 30%) of these AAs were identified in *A. colei* and *A. tumida* (Adewusi et al., 2003). Justification of the potential nutritional value of AS proteins as a source of essential AAs can be achieved by comparison with the Food and Agricultural Organization's (FAO) reference pattern (WHO/FAO/UNU, 2007). The seeds of many species of acacia plants were found to be rich in essential AAs and their values are higher than the FAO reference with the exception of tyrosine (Table 4). However, studies have shown that *A. tortilis* (Embaby & Rayan, 2016) and *A. bilimekii* (Sotelo et al., 1999) exhibited lower levels of isoleucine, lysine, and threonine as compared to the FAO references. The differences between the AAs in different AS species could be due to plant species or regional differences or protein extraction method. Moreover, the sulfur-containing AA, methionine is the most conspicuous limiting AA in AS protein.

In a study on AA profile of protein fractions of *A. nilotica* seeds, the albumin and globulin seem to be a good source of threonine (157 and 114 g/100 g protein, respectively), lysine (145 and 109 g/100 g protein, respectively), and isoleucine (113 and 149 g/100 g protein, respectively) with high essential AA scores as compared to total seed proteins (Siddhuraju et al., 1996). This agrees with the reported protein fractions of *A. victoriae* Bentham (Agboola & Aluko, 2009). Although legume seeds such as are rich in protein and essential AA, their protein digestibility and utilization are poor due to the presence of anti-nutritional components (Liener, 1994). Roasting affects all amines and AAs bound within protein, by degradation through the Maillard reaction from which a complex series of poorly characterized products are formed or pyrolysis at higher temperatures (Hodge, 1953).

Processing of *A. colei* seeds with water to reduce the anti-nutrients (phytates and tannins) levels was found to improve the AA compositions (as %) of the seed as compared to whole *A. colei* seed and this was attributed to loss of some protein fractions during processing (Adewusi et al., 2003). However, Adewusi et al. (2003) found that the AA profile of the processed *A. colei* seeds was less balanced than that of *A. tumida*. In contrast, roasting (180 °C, 0 to 10 min) of *A. colei*, *A. saligna*, and *A. torulosa* seeds has been reported to cause a significant reduction in the amount of essential AAs

present in the species (Boughton, Reddy, Boland, Roessner, & Yates, 2015).

4.1.2 | Protease inhibitors

PIs present in plant, especially leguminous seeds, protect the plant against micro-organisms and insects as well as serve as storage proteins (Norton, 1991; Srinivasan, Giri, Harsulkar, Gatehouse, & Gupta, 2005). However, PIs specifically lower the ability of the human body to absorb and utilize food proteins by inhibiting the activity of protein-digesting enzymes in the gut. Several studies have isolated and characterized different PIs from different species of AS because these are known to influence their AA composition and sequence (Ee, Zhao, Rehman, & Agboola, 2008, 2011; Patthy, Molnár, Porrogi, Naudé, & Gráf, 2015).

The AA profile of the purified trypsin inhibitor's (Acacia victoriae Bentham trypsin inhibitor [AvTi]) fraction of *A. victoriae* Bentham seeds revealed that glutamate, aspartate, leucine, and lysine were the major AAs (Ee, Zhao, et al., 2011). Similar results were found in *A. victoriae* Bentham seeds (Agboola & Aluko, 2009), although some of the aliphatic and polar uncharged AA, such as serine, glycine, valine, and methionine, were lower in the AvTi fraction. These variations were expected because the AA analysis of the former was conducted on whole seeds unlike that on the AvTi fraction.

Another study on the AA sequence of the major chymotrypsin inhibitor present in the seeds of *A. karro* revealed that *A. karro* chymotrypsin inhibitor (AkCI) exhibit more than 60% sequence similarity with other PIs from the *Mimosideae*'s group (Patthy et al., 2015). Similarly, Ee, Zhao, Rehman, and Agboola (2009) found that the AvTi fraction's AA sequence is homologous with *Glycine max* (soybean) Kunitz-type trypsin inhibitor (Odani, Odani, Ono, & Ikenaka, 1979) and the sequences were matched at two positions (2 – 10 and 65 – 75). The identical AAs found in all the sequences were proline, leucine, isoleucine, glycine, phenylalanine, and aspartate.

4.2 | Molecular weight of AS proteins

4.2.1 | Protein and major protein fractions

Analysis of SDS-PAGE profile (Figure 4a) of crude protein isolate obtained from *A. victoriae* Bentham showed about 12 bands (molecular mass: 10 to 145 kDa) and two bands (62 and 125 kDa) under reducing and nonreducing conditions, respectively (Agboola & Aluko, 2009). This was further reduced to a single band of molecular mass of 62 kDa suggesting that the 125 kDa band was most likely a dimer with each subunit (62 kDa) joined presumably by one or more disulfide bond(s). Agboola et al. (2007) reported similar findings on the purified protein fraction of *A. victoriae* Bentham seeds.

Similarly, more protein bands (Figure 4b) were found in extracts of *A. saligna* subspecies under reducing conditions as compared to nonreducing conditions (Ee & Yates, 2013). The authors also found that water soluble AS proteins were mainly polypeptides with molecular weights lower than 66 kDa, which agrees with those reported in *A. victoriae* Bentham (Agboola et al., 2007; Ee et al., 2013; Ee, Rehman, Agboola, & Zhao, 2009). However, processing such as soaking and roasting of the AS degraded the proteins into fragments smaller than 6.5 kDa (Ee & Yates, 2013). Previous studies have also reported the breakdown of proteins from AS, mucuna, red peanuts, and red kidney beans by thermal treatment (Ee et al., 2013; Ejigui, Savoie, Marin, & Desrosiers, 2005; Mugendi et al., 2010).

In another study, molecular weights of water, alkali, and ethanol extracts of proteins from *A. victoriae* Bentham were compared and it was observed that major bands in the range of 27 to 61 kDa were found in both water and alkali extracts (Agboola et al., 2007). However, a very faint band was observed in the ethanol extracts, probably because of their low protein concentration and purity. The authors therefore suggested that the proteins in the alkali-soluble extracts were most likely conjugated multiple units of the water extract's protein, which were decreased to similar sizes during the SDS-PAGE analysis, that might have reduced the disulfide bonds.

4.2.2 | Protease inhibitors

Analysis of SDS-PAGE pattern of AkCI showed a single band under nonreducing conditions and was found to exhibit both chymotrypsin and trypsin inhibitory activities as determined through synthetic peptide substrates (Patthy et al., 2015). These authors also reported two polypeptide chains with molecular weight of 15.3 and 5 kDa yielded by reduction and alkylation of this protein, respectively.

Also, glycoprotein analysis of purified *A. victoriae* Bentham protein extracts by gel electrophoresis showed at least four glycoprotein bands (molecular weight: 45 and 60 kDa and less than 20 kDa) in the crude extract, salt-precipitated, and ion-exchange protein fractions, whereas purified AvTi revealed only one band and a lower degree (2.06%) of glycosylation (Ee, Zhao, et al., 2011). The authors therefore suggested that AvTi is a glycosylated protein as indicated by the appearance of a single magenta band after the addition of Schiff's aldehyde reagent.

4.3 | Isoelectric point

Isoelectric point (pI) is the pH at which protein is least soluble and this intrinsic property of protein is important in evaluating the utilization of proteins especially in food processing. Also, knowing the pI of a seed's protein prior to protein extraction may increase the recovery of the protein.

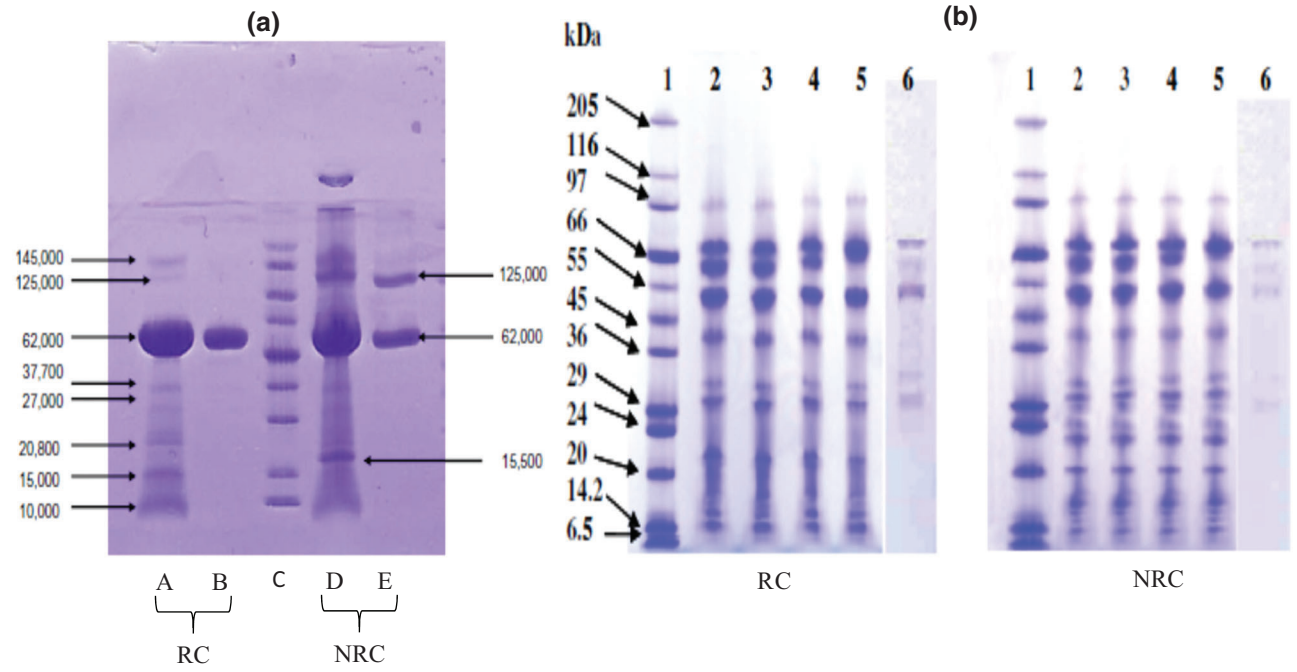

FIGURE 4 SDS-PAGE electrophoretograms of protein products from (a) *A. victoriae* Bentham seed, reproduced with permission from Agboola and Aluko (2009), and (b) *A. saligna* subspecies seed, reproduced with permission from Ee and Yates (2013) A and D: crude protein isolates; B and E: purified major fractions; C: protein standard; RC: reducing conditions; NRC: nonreducing conditions; lane 1: sigma wide range molecular mass marker from 6.5 to 205 kDa as labeled; lane 2: *A. saligna* subsp. *saligna*; lanes 3: *A. saligna* subsp. *pruinescens*; lane 4: *A. saligna* subsp. *stolonifera*; lane 5: *A. saligna* subsp. *lindleyi*; lane 6: typical soaked and boiled wattle seed extract

The pI is usually determined by checking the solubility of the protein over a wide range of pH, mainly from acidic to alkaline regions. Previous studies on AS only focused on the pI of the seed's protein in relation to protein extraction and not in terms of functionality of foods or molecular structure of the protein. For instance, pI of 3.85 and 4.5 was used during extraction of protein isolates from *A. tortilis* seed (Embaby et al., 2018) and protein extracts from *A. victoriae* Bentham seed (Agboola et al., 2007), and these are similar to the pI of 4.5 found in walnut (Mao & Hua, 2012) and lentil (Joshi, Adhikari, Aldred, Panozzo, & Kasapis, 2011). Generally, the pI of most pulses is between 4 and 6, and plant proteins are precipitated at this pH during protein extraction due to low solubility of proteins at pI (Boye, Zare, & Pletch, 2010). More studies are required on the impact of food processing conditions such as ionic concentration and pH on the characteristics and functional properties of WS proteins.

4.4 | Circular dichroism spectra

Circular dichroism (CD) is a valuable tool for rapid examination of the structure and folding properties of proteins in solution. This technique provides information on stability of the protein's structure. Analysis of ultraviolet-CD spectra of protein extracted from *A. victoriae* Bentham seeds revealed that the protein consists mainly of equal values (39%) of β-sheets and random structures, followed by β-turns (19%) and relatively low amount of α-helix (3.6%) (Agboola & Aluko, 2009). Generally, protein fractions such as globulins from different plant protein sources including soybean, pea, buckwheat, lentil, sunflower, and red bean possess β-sheets as the predominant secondary structure while they typically have low levels of α-helix (Carbonaro, Maselli, & Nucara, 2012; Choi, & Ma, 2005; Ellepola, Choi, & Ma, 2005; Marcone, Beniac, Harauz, & Yada, 1994; Meng, & Ma, 2001). For instance, β-sheets (20% to 30%) and random coil (40% to 60%) are the major secondary structure of sunflower proteins (helianthinin), with less than 10% α-helix recorded (González-Pérez et al., 2004; Rahma & Rao, 1981; Sripad & Rao, 1987; Suryaprakash & Prakash, 2000). The abundance of β-sheets in the secondary structure of pulse proteins has been reported to lower the digestibility of the protein because it limits the access of proteolytic enzymes (Yu, 2005). Agboola and Aluko (2009) also found the structures of protein extracts from *A. victoriae* Bentham seed to be stable to environmental changes such as pH (3 to 9), ionic strength, and temperature. A great effect of calcium was observed on the CD spectrum with the positive peak around 210 nm changing to negative peak that is typical of increasing α-helical structures (Schmid, 1990). However, the effect of calcium did not actually imply that there were significant alterations in the fraction of secondary structures. It is most likely that the determination algorithm of available structure was not appropriate for protein fractions of AS because a

much higher protein concentration (10 mg/mL) was utilized in order to obtain a reasonable signal, probably due to a considerable degree of glycosylation (Agboola & Aluko, 2009). A comparison of the structural stability of plant proteins after processing revealed an extensive denaturation and subsequent increase of nonstructured protein of helianthinin after heat treatment (105 to 110 °C) at pH 7.0 (González-Pérez et al., 2004), whereas the native structure of sunflower albumin was very stable against pH modification and heat treatment (González-Pérez, Vereijken, Van Koningsveld, Gruppen, & Voragen, 2005) and minimal structural changes were observed in soy glycinin against pH and ionic strength modifications (Lakemond, de Jongh, Hessing, Gruppen, & Voragen, 2000). Generally, the pH of food products ranges from 3.0 to 7.0 and ionic strength varies from 0.02 to 0.2 M (Lakemond et al., 2000); thus, the stability of AS proteins over these range of pH and ionic strength makes it a very attractive group of proteins because minor structure modifications are expected to occur during food processing and could therefore have functional food applications.

4.5 | Surface hydrophobicity

Surface hydrophobicity (S_0) is an important property of protein in assessing a protein's functional properties such as emulsifying properties and interfacial tension, which play a vital role in the manufacturing of foods (Haskard & Li-Chan, 1998). The value of S_0 can be influenced by environmental conditions used in processing such as pH and ionic strength. Agboola and Aluko (2009) demonstrated that S_0 value in *A. victoriae* Bentham seeds decreases as the pH rises, whereas it increases with calcium ion concentration. Similar findings have been reported in cowpea globular proteins (Aluko & Yada, 1995; Mwasaru, Muhammad, Bakar, & Man, 1999), which was attributed to the protein charge being masked at high pH and a rise in ionic strength with calcium ions (Kinsella, Damodaran, & German, 1985). Therefore, the low S_0 obtained at low pH should enable the protein to form a very stable emulsion in acidic foods (Agboola & Aluko, 2009). More research is required to examine the hydrophobicity of protein isolates from AS or the changes induced in aqueous environments, or by different solvents, and proteolytic enzymes.

4.6 | Solubility

Proteins from most legumes have maximum solubility at alkaline pH. Gradual decreases in solubility occur near the pI, which is usually between pH 3.5 and 5 for most legumes (Adiamo, Gbadamosi, & Abiose, 2016; Yu, Ahmedna, & Goktepe, 2007). Ee, Rehman, et al. (2009) studied the protein solubility of unprocessed and processed *A. victoriae* Bentham extracts over a wide range of pH and reported that it increased with pH between 4.0 and 9.0, for all the extracts. An acidic pH of 4 was reported as the isoelectric point due to the low solubility measured at this pH and this pH was also close to the pI values found in *A. tortilis* seed (Embaby et al., 2018). Similar findings were reported in protein extracts of other leguminous seeds such as cowpea, faba bean, chickpea, lentil, and dry bean where a change in the pH from acidic to the alkaline region resulted in an increase in protein solubility (Carbonaro, Cappelloni, Nicoli, Lucarini, & Carnovale, 1997; Prinyawiwatkul, Beuchat, McWatters, & Phillips, 1997).

The solubility profile of untreated and treated (soaking, soaked/heated, and roasted) *A. victoriae* Bentham seed flours showed that all the treated samples had higher protein solubility (above 90%) compared to the raw seeds (Agboola, Ee, & Huhn, 2012). This was attributed to the significant changes in the extracted protein due to the heat treatments, thus resulting in more soluble proteins being extracted. In addition, an increase in solubility after soaking may be due to the removal of some anti-nutritional factors, such as polyphenols, the presence of which is known to affect the physicochemical properties of plant proteins, particularly their solubility (Agboola et al., 2012).

4.7 | Morphological analysis

Scanning electron micrographs (Figure 5) of raw *A. tortilis* seed and protein products from the seed revealed that the protein concentrate had small flake-like particles with a porous type-morphology, whereas an intact flake-like structure was found in the protein isolates (Embaby et al., 2018). Similar microstructures have been reported for AS flour (Embaby & Rayan, 2016) and protein products from groundnut (Jain, Prakash, & Radha, 2015) and walnut (Mao & Hua, 2012). The authors reported that the porous and flake-like structure in plant protein products contributes to increased protein solubility and digestibility (Jain et al., 2015; Mao & Hua, 2012).

5 | FUNCTIONAL PROPERTIES

5.1 | Water and oil absorption capacity

Water absorption capacity (WAC) is an essential processing parameter that can influence viscosity and is important in baking applications, bulking, and consistency of products (Niba, Bokanga, Jackson, Schlimme, & Li, 2002). Oil absorption capacity (OAC) is also an important processing factor because oil acts as flavor enhancer and improves the palatable texture of foods. Protein is the major chemical component that can affect OAC because protein is composed of both hydrophilic and hydrophobic parts, and thus could have implications on the functional properties of flours (Jitngarmkusol, Hongsuwankul, & Tananuwong, 2008). The WAC and OAC

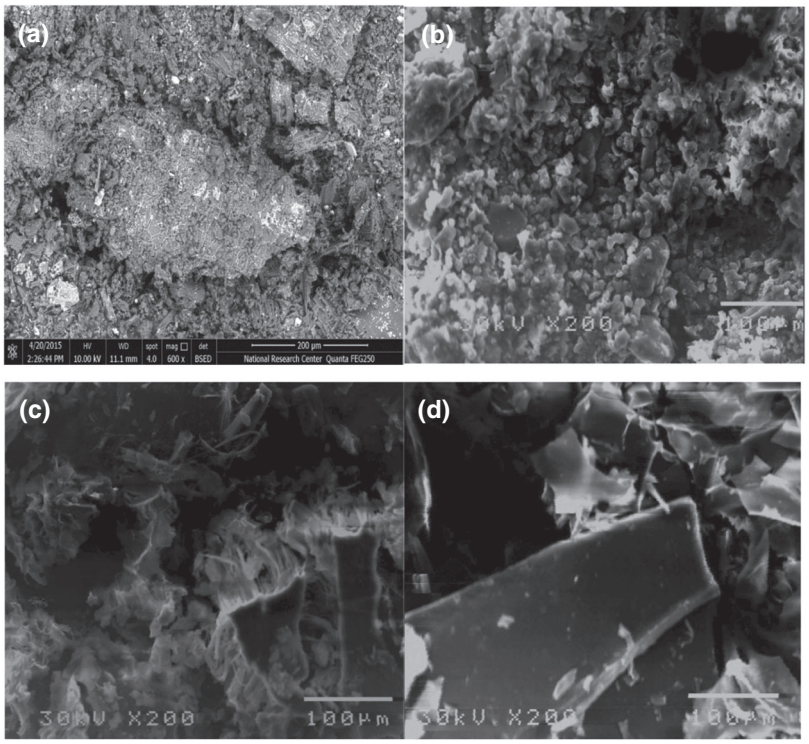

FIGURE 5 Scanning electron microscope pictures for *Acacia tortilis* (a) seed flour, reproduced with permission from Embaby and Rayan (2016), (b) defatted flour, (c) protein concentrate, and (d) protein isolates, reproduced with permission from Embaby et al. (2018)

of *A. tortilis* grown in Egypt were found to be 3.17 g/g and 1.28 mL/g, respectively (Embaby & Rayan, 2016) and the values are comparable to WAC and OAC of soybean (1.3 and 1.1 g/g, respectively) and pea (1.7 and 1.2 g/g, respectively) protein isolates (Fernández-Quintela, Macarulla, Del Barrio, & Martínez, 1997). The high WAC found in AS may be attributed to the substantial amount of protein (27.21%) in the seed surrounding the starch granules, thereby increasing the capacity of the flour to absorb water (Embaby & Rayan, 2016). In another study on an *A. tortilis* variety, preparation of protein isolates from the seeds further enhanced the WAC and OAC as compared to the protein concentrate (Embaby et al., 2018). The authors attributed this to exposure of additional binding sites because the protein isolate has a high ability to swell, dissociate, and unfold as compared to protein concentrate and defatted flour. However, insufficient disruption of the structure of the protein concentrate and the presence of other nonprotein components in the concentrate can impair this process (Jain et al., 2015). The high WAC and OAC reported in *A. tortilis* protein isolates (Embaby et al., 2018) as compared to commercial soy protein isolate (Jain et al., 2015) implies that proteins from WS could be a suitable food ingredient in the formulation of foods such as breads and cakes where hydration and shortening properties are required (Shevkani, Singh, Kaur, & Rana, 2015).

5.2 | Emulsifying properties

Emulsifying properties of protein flours are often evaluated using two main indices; emulsifying activity (EA) and emulsion stability (ES). Generally, EA is used to evaluate the quantity of oil that can be emulsified per gram of protein, whereas ES determines the emulsion's ability to resist structural changes over a specific period of time. Proteins form a film around oil droplets that are dispersed in an aqueous medium, thus, preventing changes in structure caused by flocculation, coalescence, sedimentation, or creaming. Therefore, the ability of proteins to act as emulsifiers is dependent on the ratio of hydrophilicity to hydrophobicity and structural constraints that influence the ease with which they can unfold to skin around or form a film in dispersed oil droplets (Boye et al., 2010). In addition, the strength of the interfacial protein film is also important during emulsion formation.

Analysis of the EA of *A. tortilis* seed proteins showed that defatted flour had the highest EA (59.60%), followed by protein concentrates (54.01%) and isolates (49.56%) (Embaby et al., 2018). However, these values are lower than the range of EA (61.14% to 93.20%) found in legume flour such as lima bean, small red bean, mung bean, lentil, and chickpea (Du, Jiang, Yu, & Jane, 2014) as well as lupin (74.0%) (Lqari, Vioque, Pedroche, & Millán, 2002), thus, indicating relatively poor emulsion activity of *A. tortilis* seed protein. The variation in the EA of the legumes is related to the protein contents (soluble and insoluble) and other components, such as starch, fat, and sterol contents, of the legume flours (Du et al., 2014). Similar trends in the decrease in EA of *A. tortilis* seed as protein levels increased have been reported in protein concentrates and isolates from legumes such as groundnut (Jain et al., 2015) and cashew nut (Ogunwolu, Henshaw, Mock, Santros, & Awonorin, 2009). At low

protein concentration, adsorption of protein is diffusion controlled because it will spread over the surface before it can be absorbed. However, movement of proteins does not take place at high protein concentrations due to an activation energy barrier (Sze-Tao & Sathe, 2000), thus the EA is reduced with an increase in protein concentration. Furthermore, as the protein concentration is reduced, the shearing involved in emulsifying properties causes a greater degree of unfolding of polypeptides supported by the hydrophobic interaction of the peptide chains with the lipid droplets, causing a larger surface area of protein to be made available thereby improving the efficiency of emulsification (Tsai, Cassens, & Briskey, 1972). Emulsifying properties may also be affected by protein conformation, droplet size, interfacial tension, net charge, and viscosity (Carvalho, Garcia, & Amaya-Farfán, 2006), as high viscosity may prevent sufficient interactions of proteins with the oil droplet surface. Proteins may increase the viscosity of the continuous phase that reduces the rate of movement of oil droplets in the system (Sikorski, 2001).

The impact of different extraction methods (water, alkali, or ethanol) on emulsion of whole *A. victoriae* Bentham seeds revealed that water extracts, even at very low protein concentrations (0.17% to 1.12%), formed stable emulsions, with up to 50% canola oil addition; these emulsions were affected by pH (4 to 9), ionic strength (0.25% to 1% NaCl), and retorting (115 °C for 30 min) (Agboola et al., 2007).

The results of emulsifying properties of raw and processed *A. victoriae* Bentham seed showed that a larger particle size was observed in emulsions containing 50% oil as compared with 20% oil at pH 4 and 7 (Ee, Rehman, et al., 2009). However, emulsions formed with 20% oil using processed seed extracts were generally less stable (30% to 50% separation at pH 4) than those containing 50% oil content (maximum separation of 25% at pH 4) (Ee, Rehman, et al., 2009). This could be attributed to considerable high viscosity of the dispersion medium that leads to emulsion with higher kinetic stability (Benichou, Aserin, & Garti, 2002). Although high oil contents have large droplet size, the oil level could have resulted in a hindering separation phenomenon whereby the droplets are physically prevented from joining and separating (Darling & Birkett, 1987).

Also, emulsions from the raw seed extracts were found to be very stable at both 20% and 50% oil levels, especially at pH 7 (Ee, Rehman, et al., 2009). Similarly, observations were reported in water extract and ammonium sulfate precipitate (ASP) from raw *A. victoriae* Bentham but at concentrations between 1% and 5% (w/v) (Agboola et al., 2012). However, destabilization of the emulsion formed from processed AS flours at low extract concentrations occurs within 7 days at 25 °C. Conversely, ASP-stabilized emulsions were very stable at all concentrations and conditions in the study (Agboola et al., 2012). To avoid the conflicting results outlined above, the most practical way to determine emulsification efficiency is by measuring the changes in average and distribution of particle size as reported by Ee, Rehman, et al. (2009).

5.3 | Foaming properties

Foam capacity (FC) and foam stability (FS) are the two most frequently used indices to measure foaming properties. Formation of foam occurs when proteins unfold to an interfacial skin that entraps air bubbles in suspension and prevents their collapse. Foam formation plays a vital role in food applications such as whipped toppings, beverages, and cakes. Generally, FC is measured by high-speed homogenization of proteins dispersed at specified concentrations to induce formation of foam and is expressed as the percent increase in volume after homogenization, whereas FS measures the change in the volume of the foam over a defined period of time, mostly, 0 to 30 min (Boye et al., 2010).

Low FC was observed in raw *A. victoriae* Bentham seeds and no changes in FC were reported after processing (soaking/heat treatment; Ee, Rehman, et al., 2009). This agrees with Agboola et al. (2012) who reported poor FC in water and ASP extracts from *A. victoriae* Bentham seeds. Similarly, low FC was found in whole and defatted *A. tortilis* flour (Embaby & Rayan, 2016; Embaby et al., 2018). The ability of flour to form foam depends on the amount of the flexible protein molecules in the flour, which may reduce the water's surface tension (Sathe, Deshpande, & Salunkhe, 1982). However, there was an inverse relationship between the FC and FS with the *A. tortilis* species having FS of between 67.2% and 78.15% in whole flour and protein isolates (Embaby et al., 2018). The enhanced FS observed could be due to the formation of small air bubbles surrounded by a thicker, more flexible protein film that might not be easy to collapse. Furthermore, weakening of the hydrophobic interaction and increase in protein solubility and flexibility due to high charge on the protein molecule may results in increase in FC and FS (Lawal, Adebowale, Ogunsanwo, Sosanwo, & Bankole, 2005). Consequently, it may increase the possibility of the protein to spread faster on the air–water interface, encapsulate air particles, and enhance foam formation (Lawal et al., 2005). Nevertheless, the FC (39.58%) and FS (78.15%) values reported in *A. tortilis* seed protein isolates (Embaby et al., 2018) are lower than the FC and FS observed in commonly consumed legumes such as protein isolates from soybean (350% and 95.24%, respectively) and pea (333.3% and 100%, respectively) at pH 8 (Barac, Pesic, Stanojevic, Kostic, & Bivolarevic, 2015) as well as different lines of kidney bean (83% to 121% and 90% to 95%, respectively) and field pea (87% to 132% and 94% to 96%, respectively) at neutral pH (Shevkani et al., 2015). The poor foaming properties of protein products from seeds of *Acacia* species as compared to other plants may limit their use as foaming agents in food formulations.

6 | ANTI-NUTRITIONAL FACTORS

Like most legumes, AS also contain several anti-nutritional compounds that can reduce the absorption of nutrients if they are not removed through appropriate processing. High levels of anti-nutrients such as phytic acid and trypsin inhibitor were found in *A. nilotica* seed (Siddhuraju et al., 1996) and were comparable with that of a white variety of *Cicer arietinum* (Khan, Zaman, & Elahi, 1988). Anti-nutritional analysis of *A. victoriae* Bentham seed extracts using different extraction methods (dehulled cotyledon, freeze-dried water, and freeze-dried alkali extracts) showed different levels of trypsin inhibitor activity (TIA) and chymotrypsin inhibitor activity (CIA), with all treatments exhibiting higher TIA than CIA per gram of extract (Ee et al., 2008). Unlike other extraction methods, freeze-dried alkali extracts only showed CIA, which was attributed to destruction of TIA under alkaline conditions. Ee and Yates (2013) found that TIA is the predominant PI in the seeds of different subspecies of *A. saligna*, which agrees with previous findings on *A. victoriae* Bentham seeds (Ee et al., 2008).

Analysis of lectin activity (LA) or haemagglutinating activity of raw *A. nilotica* seed against erythrocytes from human blood group showed that the seed had LA that ranged from 10 (O blood group) to 162 (B blood group) hemagglutinating unit (HU)/mg protein (Siddhuraju et al., 1996). These values are comparable to the LA of lentils (10.91 to 11.07 HU/mg dry matter [DM]) and bean (87.69 to 88.59 HU/mg DM) but lower than that of soybean (692.82 HU/mg DM) observed against rabbit erythrocytes (Shi, Arntfield, & Nickerson, 2018). Although seed differences could be responsible for the variation, the method of analysis and blood group of the animal used in the studies may also cause variations in LA of the pulses.

Also, AS contain nonprotein AAs such as djenkolic acid (DKA), which may have potential anti-nutritional or toxic effects (Bell, 2003). It has been reported that continued exposure to DKA could result in formation of kidney stones (Areekul, Muangman, Bohkerd, & Saenghirun, 1978). This was attributed to the ability of DKA to precipitate in the acidic environment of the kidney (Barceloux, 2008). A study on the levels of DKA in different species of AS (Table 5) showed that substantial amounts of DKA were identified in each of the AS species, with *A. colei* (0.49 g DKA/100 g sample) and *A. saligna* (1.85 g DKA/100 g sample) having the lowest and highest values, respectively (Boughton et al., 2015). The levels of DKA in the AS species are similar to those found in djenkol bean (1 to 2 g DKA/100 g sample) (Areekul, Kirdudom, & Chaovanapricha, 1976; Lucas, Guerrero, Sigales, & Sotelo, 1988) and these values are higher than the amounts (0.15 and 0.30 g DKA, respectively) reported to cause illness after consumption (Areekul et al., 1976; Lucas et al., 1988). Therefore, the high anti-nutritional factors present in some varieties of AS could make it unsafe for human consumption and this could limit the incorporation of some of these species in the diets of humans as well as in food formulations if not properly processed. Also, more studies need to be conducted to determine the presence of allergens in AS similar to those found in other legumes.

6.1 | Processing methods to remove anti-nutritional factors and enhance antioxidant capacity

Several processing techniques have been applied to AS to remove or reduce their anti-nutrient compounds (Table 6). The application of thermal treatment such as dry heating and autoclaving has been reported to significantly reduce the levels of phytic acids, trypsin inhibitor, total free phenols, and tannins in *A. nilotica* seeds (Siddhuraju et al., 1996). Also, greater reduction in LA in respect of both A (by 56% and 78%) and B (55% and 78%) blood groups was observed in *A. nilotica* seeds after subjecting the seeds to a dry heat treatment and autoclaving, respectively (Siddhuraju et al., 1996). The authors attributed this to the breakdown of hemagglutinins (proteins) into their subunits or conformational changes in their native structure which might be important for the activity of haemagglutinins (Vijayakumari et al., 1994).

Soaking is one of the traditional processing methods that has been applied to *A. victoriae* Bentham seeds and was found to increase the TIA content but decreases the level of CIA (Table 3; Ee et al., 2008). However, application of heat (100 °C) for 30 s to the soaked samples was found to be effective in inactivating both TIA and CIA. The authors focused mainly on TIA and CIA inactivation because of the known high susceptibility of other enzymes such as lipoxygenase and amylase to heat treatment (Whitaker, 1996). Furthermore, significant reduction in the level of DKA in different species of WS after processing (dehusking, roasting, germination and soaking) has been reported with roasting (optimum conditions: 180 °C, 6 min) being the most effective causing more than 90% reduction in DKA content in *A. colei*, *A. saligna*, and *A. toruolsa* (Boughton et al., 2015). Although soaking of *A. saligna* subspecies overnight followed with 2-min boiling has been reported to significantly reduce their PI activity by greater than 75%, high-density (clear) bands were observed in the trypsin activity gel (Ee & Yates, 2013). This observation demonstrated the possible occurrence of other coexisting inhibitory factors, such as natural phenolic compounds (Gonçalves, Soares, Mateus, & De Freitas, 2007), which might have been greatly removed during soaking (Ee & Yates, 2013). Also, a significant reduction in TIA and CIA content to negligible values was observed in samples roasted for 2 min or longer (Ee & Yates, 2013). This agrees with that

TABLE 5 Anti-nutritional components and phytohaemagglutinating activity (PA) of different species of raw acacia seed

Species	Antinutrients				PA		References
	TIA (TIU/mg protein)	CTIA (CIU/mg protein)	DKA (g/100 g)	SC (%)	Erythrocytes from HBG	HA	
A. nilotica	38.3	51.2	18.5		A (104) B (162) O (10)		Boughton et al. (2015); Siddhuraju et al. (1996)
A. victoriae Bentham	170 to 618.69	20 to 60					Ee et al. (2008, 2013)
A. saligna subspecies	2,500 to 3,300	120 to 150	1.90	2.6 to 3.0			Ee and Yates (2013); Boughton et al. (2015)
A. colei			0.49				Boughton et al. (2015)
A. tumida			0.94				Boughton et al. (2015)
A. torulosa			1.74				Boughton et al. (2015)
A. bilimekii	13.3			1		2	Sotelo et al. (1999)

Abbreviations: TIA, trypsin inhibitor activity; CIA, chymotrypsin inhibitor activity; DKA, djenkolic acid; SC, saponin content; PA, phytohemagglutinating activity (HU/mg protein); HA, hemagglutinating activity; HBG, human blood group.

TABLE 6 Effect of pretreatment methods on antioxidant capacity and anti-nutrient levels in different species of acacia seed

Species	Treatment	Conditions	Observation	References
A. auriculiformis	Dry heating	Oven (160 °C; 15 min)	Decrease in antioxidant capacity of processed samples with greater effect noticed in dry-heated samples	Loganayaki et al. (2011)
	Pressure cooking	Seeds: water (1:3, w/v); Pressure cooker (20 min)	Significant decrease in total phenolic and tannin contents during pressure cooking	
A. nilotica	a. Soaking in alkaline/tamarind solution + cooking b. Sprouting + oil frying c. Open-pan roasting		All treatments reduced total free phenolics and tannins except "b"; L-Dopa was reduced by all treatments except "c"; Phytic acid was reduced by all treatments	Vadivel & Biesalski (2012)
A. victoriae Bentham	Roasting	200 °C for 5, 10, 20, and 30 min	Elimination of trypsin inhibitor activity after 20 min	Ee et al. (2013)
	Roasting	200 °C for 5, 10, 20, and 30 min	Increased antioxidant capacity of the extract; destroyed the ACE-inhibitory activity; reduced level of cytotoxicity	Ee et al. (2012)
	(a) Soaking (b) Moist treatment (c) Soaking + Heat treatment	Seeds: water (1:10, w/v); overnight Water bath (100 °C, 0 to 120 s)	Treatment (b) inactivate trypsin inhibitor than chymotrypsin inhibitor; Treatment "c" enhanced the inactivation of both inhibitors	Ee et al. (2008)
A. saligna, A. colei, A. torulosa	Roasting	180 °C; 0 to 10 min	Totally eliminated djenkolic acid after 10 min of roasting	Boughton et al. (2015)

reported on *A. victoriae* Bentham, where 20 min of roasting was found to completely eliminate TIA (Ee et al., 2013).

Processing of some legumes including *A. nilotica* by soaking in either tamarin or alkaline solution and cooking have been reported to markedly reduce the total free phenolics in the seeds of these plants by 26% to 40% and 44% to 67% after tamarind and alkaline solution treatments, respectively (Vadivel & Biesalski, 2012). The authors also

observed a similar effect when the seeds were subjected to open pan roasting but with moderate loss of L-Dopa (by 10% to 17%) and phytic acid (by 12% to 20%). This was in agreement with previous findings on antioxidant capacity of *A. auriculiformis* seeds subjected to soaking, dry-heating, and autoclaving (Sathya & Siddhuraju, 2013). On the other hand, increase in total free phenols of *A. nilotica* seed by 18% was observed after subjecting it to sprouting and oil-frying but with diminishing effect on phytic acid (by 53%) and L-Dopa (by 34%) (Vadivel & Biesalski, 2012). Similarly, an increase in antioxidant levels has been reported in roasted *A. victoriae* Bentham seeds but roasting for 5 min was found to destroy their angiotensin-converting enzyme (ACE)-inhibitory activity and lowered their cytotoxicity level (Ee et al., 2013). The increase in antioxidants was attributed to either thermal dissociation of soluble conjugated and insoluble bound phenolics (Dewanto, Wu, & Liu, 2002) or formation of phenolic degradation products with antioxidant capacity (Yanagimoto, Ochi, Lee, & Shibamoto, 2004).

Although AS are well-known for their high nutritional quality that makes them suitable for human consumption, processing of the seeds is also equally important to minimize the amount of anti-nutritional compounds. Thus, any new processing technique, such as innovative nonthermal treatments, for AS should be investigated in order to create a clear pathway for their inclusion into human foods without adversely affecting their nutritional and sensory properties.

7 | CYTOTOXICITY

Bioactive proteins in plants may possess common defense functions over a range of targets such as antibacterial, antifungal, and PI activities (Wang & Ng, 2006; Wang et al., 2006). For instance, PIs isolated from some species of AS have been reported to possess health beneficial properties such as antifungal properties in *A. plumose* (Lopes et al., 2009) and antitumor and anti-proliferative activities in *A. nilotica* (Meena et al., 2006). However, a study revealed that the cytotoxicity of *A. victoriae* Bentham seed did not possess the above activities and the authors suggested that the PIs from the species had a more specific functionality in terms of ACE inhibition and cytotoxicity (Ee et al., 2012). Ee and co-workers also reported that roasting of the seeds for 30 min significantly decreased their cytotoxicity levels, with >100% reduction in cytotoxicity levels observed in seeds roasted for 30 min as compared to the raw and other roasted seeds. The relatively low versatility of AS in terms of their biological activities may be attributed to the low diversity of phenolic compounds (Ee et al., 2012). The phenolic fraction in AS is dominated by only two phenolic acids, namely, succinic and gallic acid, the amount of which increased up to 10-fold after roasting (Ee, Agboola, et al., 2011).

8 | YIELDS, MARKETS, AND ECONOMICS OF PRODUCTION OF AS

In the last two decades, the annual demand for acacia/wattle seed was estimated at between 12 and 20 tons, with about $12 to $25 per kg of clean seed as farm-gate price (Simpson & Chudleigh, 2001). Also, the market for AS is mainly for inclusion in bush food products, marketed on a basis of novelty (Simpson & Chudleigh, 2001), and this has not changed. Currently, there is no accurate information with regard to the annual global production of acacia/wattle seed and the market demand for the seed in Australia is met by around six processors of wattle seed, who source their own seed by directly harvesting wild trees or cultivating their own plantation of wattle trees (Anonymous, 2017). A yield from 10 to 15 kg of seed could be obtained from a mature wattle tree and about 100 kg of AS harvested from 20 to 30 trees are typically marketed by native food companies in Australia, indicating the small number of trees involved within an established enterprise (Anonymous, 2017). However, this also highlights the growth potential of the sector, particularly amongst indigenous people, not only in Australia but also worldwide where *Acacia* spp. occur (Figure 1). Seed production also varies both within and between *Acacia* spp. due to various factors such as pests, diseases, flowering and seeding patterns, and harvesting techniques (Simpson & Chudleigh, 2001). Previously, it was estimated that to reach 1,000 tons of annual demand for AS from the bush food industry, about 800 ha of acacia need to be planted, assuming an average yield of 1.25 t per hectare (Simpson & Chudleigh, 2001). Currently, there is high demand for the seed with more than 2,000 wattle trees and annual production of 500 kg produced per farm (Anonymous, 2019); however, these values are incomparable with the annual global seed production of commonly consumed legumes such as soybeans (352,643,548 metric tons), peas, dry (16,205,448 metric tons), and lentils (4,700,998 metric tons) (FAOSTAT, 2019). However, large-scale markets of AS including vegetable proteins and starches would be possible, although, for the seed to compete in high volume markets, the cost of AS will need to be substantial less than $1 per kg (Simpson & Chudleigh, 2001). Previous economic analysis has suggested that this production cost may be attained at annual yields of 2 kg/tree combined with a tree density of 625 trees/hectare, if a "fingers-type" shaker, a low cost harvesting technique, can be developed (Simpson & Chudleigh, 2001). Moreover, AS may be competitive provided some unique properties of the seeds are discovered from the different species (Simpson

& Chudleigh, 2001). Also, development of more mainstream markets for AS and identifying numerous potential products from the seed are important in order for extensive plantings of acacia trees to be feasibly undertaken (Simpson & Chudleigh, 2001).

9 | FOOD FORMULATIONS

Several studies have reported the application of wattle seed in the formulation of food products especially baked goods, beverage, and meat products. Krishna Kumar, Bejkar, Du, and Serventi (2019) evaluated the effect of incorporating 1% each of flax seed and four species (*A. dealbata*, *A. decurrens*, *A. terminalis*, and *A. verniciflua*) of wattle seed powders on the textural properties of gluten-free bread. The authors found that the addition of all the seed powders decreased the bread's crumb hardness by 30% to 65% and enhanced specific loaf volume by 50%. This textural improvement was attributed to high water absorption capacity and emulsifying properties of the seeds. However, they noticed dark particles on bread formulated with wattle seed and this could be due to the dark color of the seed. Further studies should be conducted investigating the changes in sensory attributes of the bread after AS addition as well as developing methods to remove the seed color, without compromising the nutritional and processing characteristics of the seeds.

Improvement in quality attributes and oxidative stability of pork frankfurters using 0.10% extracts from *Acacia catechu* (AC) seeds and other plant seeds during refrigerated storage (20 days) was investigated (Wagh, Chatli, Ruusunen, Puolanne, & Ertbjerg, 2015). Sea buckthorn (SB) and AC extracts were found to be most effective in reducing the rate of lipid oxidation as compared to control, grape seed, fenugreek seed, and green tea-formulated frankfurters. The presence of high bioactive compounds in the extracts could be responsible for stabilizing the oxidation of the samples. Also, increase in redness (a^*) color ordinates of the frankfurters formulated with SB and AC extracts was observed during storage unlike in other extracts where it decreased. This could be due to higher oxidative stability of the SB and AC extracts as compared to other samples. Overall, antioxidant extracts from AC could be utilized in maintaining the quality attributes of frankfurter during storage (Wagh et al., 2015).

Sensory analysis of an AS-chocolate beverage at different inclusion levels (AS/chocolate: 50/50% and 15/85%) indicated that the consumer preference was in the following order: 100% chocolate >15% AS and 85% chocolate >50% AS and 50% chocolate (Forbes-Smith & Paton, 2002). Although The aroma and nutty flavor of 15% AS and 85% chocolate was mostly liked by the panelists, they objected to the bitterness, which affected the strength of aftertaste and overall liking of AS-flavored beverages. Therefore, 3% to 5% AS addition was recommended for taste and economic reasons (Vic Cherikoff Food Services Pty Ltd; www.cherikoff.net).

10 | CONCLUSIONS

Although protein is the major component of ASs that could serve as substitute for animal protein, the high anti-nutritional components present in the seeds could render it unsafe for human consumption. High thermal processing methods such as roasting commonly applied to lower the anti-nutrient levels in AS have caused a significant reduction in essential AAs and disruption of the native structure of AS protein. Also, the use of traditional processing methods such as soaking and germination was not efficient to lower the anti-nutrient levels. Therefore, additional studies on the application of innovative nonthermal methods to reduce the anti-nutrient levels in AS without causing any detrimental effects on the seed's protein quality should be investigated. The structure and functional properties of the proteins obtained from the seeds after treatment should be assessed. Furthermore, studies have shown that some legumes contain allergic compounds that can pose a high health risk to some categories of people. However, no studies have been conducted to determine the presence of allergens in AS, and thus research on this aspect is essential.

The high protein content in AS could be advantageous in improving the health beneficial properties by modifying the protein structure through enzymatic treatments. Thus, further studies could focus on subjecting AS proteins to enzymatic hydrolysis to produce peptides that may possess high bioactive activities. The bioactive peptides obtained should be further purified to enhance the activity of the peptides and compared with commercially available peptides from legumes such as soybean peptides. Bioactive peptides from plants exhibit antioxidant, antimicrobial, anticancer, and anti-hypertensive activities. Therefore, such studies will provide valuable information on possible production of AS protein that are both safe for human consumption and exhibit functional and health-beneficial properties for potential use in food formulations as well as broadening the market potential of AS in the food industry.

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Oladipupo Adiamo prepared and wrote the manuscript. Michael Netzel, Louwrens Hoffman, and Yasmina Sultanbawa corrected, revised, and improved the manuscript.

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