Physicochemical and Nutritional Properties of Kangaroo Grass (*Themeda triandra)* and its potential as a food additive

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I certify that the work reported in this thesis is entirely my own effort, except where otherwise acknowledged. I also certify that the work is original and has not previously been submitted for assessment in any other course of study, at any other University.

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Abstract

Themeda triandra, or Kangaroo Grass (KG), is a C4 perennial grass that was ubiquitous across swathes of the Australian continent in pre-colonial times. There is strong evidence that First Nations Australians used seed from a range of native grasses, including KG, as a food source from at least 4,000 years ago.

As the Australian native food industry continues to grow, there is an opportunity to explore the use of native grass seeds as both a flour, and as a food additive, such as a thickener or a stabiliser. Currently, there are limited food stabilisers that are grown in Australia, with Manildra group supplying some wheat-based starches, but no stabilisers that are derived from a native Australian source. Similarly, there are no food thickeners currently on the market derived from native Australian plants. As food businesses in Australia expand their use of native plant products, there is an opportunity for businesses, particularly First Nations led businesses, to both broaden the range of plants used in native food products, and look beyond the traditional uses of these plants.

This study examined the physicochemical properties of KG seed, particularly in regards to how these properties would enable KG seed to act as a stabiliser in an oil-in-water emulsion, and as a thickener. By determining starch and protein contents, and testing KG seed's rheological properties compared to other, more commonly used food emulsifiers and thickeners, this thesis sought to determine whether KG seed has potential as a stabiliser or thickener.

The yield of KG seed fluctuated between 14 and 30%, depending on the method used to separate seed from the chaff. Even at its highest yield, the seed including seed coat equates to around $4800 per kilogram in raw material, and this does not include the significant time impost in processing the seed manually. This high cost of product and processing was considered when determining whether KG seed has potential as an effective stabiliser for the Australian native food market. KG seed has a starch level of approximately 10.8%, which is considerably lower than introduced species like corn and wheat. Also, while KG seed has a protein level of over 16%, which is a high level of protein compared to other grains, it only contains one protein that is known to be successful in a plant-based stabiliser. This coupled with KG seed's high material and processing cost, means it is not appropriate for use as a stabiliser or emulsifier at this time

KG seed did act similarly to corn starch in rheological tests, at both 5 and 10% KG seed to sample levels, suggesting that KG seed does have good potential as a food thickener.

The KG seed at both 5% and 10% levels achieved a similar shear thickening rate compared to corn flour at the same levels.

Whether through improving propagation, harvesting, and processing techniques the material cost of KG seed can be reduced to make it an economically viable proposition is yet to be determined, however, functionally KG seed does have the capability to act as a food thickener.

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Introduction

Themeda triandra, or Kangaroo grass (KG), is a C4 perennial grass species that occurs in all states and territories across Australia (Nie & Mitchell 2006). There is significant evidence that the seed from KG and other grass species were used by First Nations people as a food source pre-colonisation (Chivers et al. 2015).

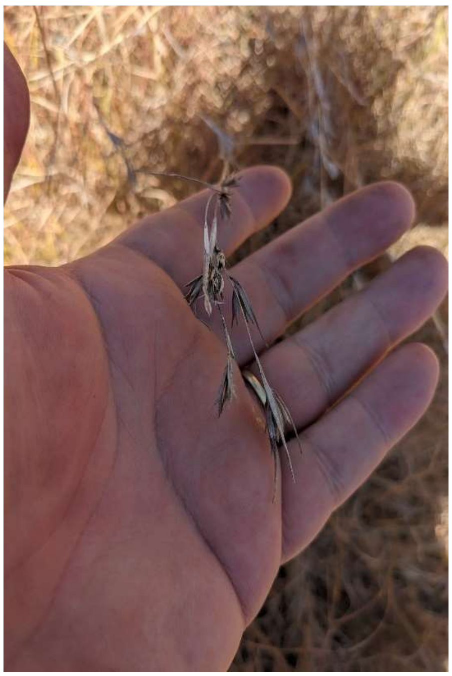

Figure 1: Kangaroo grass seed. Photo by author

As the native food industry in Australia continues to grow rapidly, there is an increased appetite for finding new native food sources, and also utilising native food sources in non-traditional ways (McCubbing 2022). The lack of a native-sourced food additive for stabilising or thickening food products means that native food businesses who would like to use such additives have no options but to either use introduced products such as xanthan gum, or not use stabilisers in their products. Therefore, there is an opportunity to assess the physicochemical properties of Australian native plant derivatives, and how that may impact their capability as a food additive.

Much of the research on KG has focused on improving propagation rates, including planting and harvesting techniques (Cole & Lunt 2005) (Briggs 2001) (Frischie et al. 2020). There is limited research on the nutritional values of KG and its seed, and no literature found on the rheological properties of KG seed when incorporated into a paste or liquid.

The small amount of literature available on the physiochemical properties of KG seed indicate that its protein levels of approximately 20% may be sufficient for it be a functional stabiliser and thickener (Cowley et al. 2023) (National Research Council 1996). This study examined physiochemical characteristics such as rheological properties, to determine if KG seed can potentially be used as a food additive.

In addition, this study examined the history of KG, and how it was used by First Nations people. This study outlines how First Nations people may benefit from the production of a commercial crop, as restitution for their stewardship over many generations. Furthermore, in determining the effectiveness of KG seed as a food additive, this study also considers some of the economic considerations in turning KG into a commercial food crop, specifically in terms of its propagation and processing requirements.

Literature Review

The History of Kangaroo Grass Pre-colonisation

KG is a species of native Australian grass, that can propagate in regions as diverse as alpine and arid regions (Nie & Mitchell 2006). It is also native to Tropical and Southern parts of the African continent, where it is commonly known as red oat grass (National Research Council 1996), and is also found across Asia and the Pacific.

It is difficult to determine how regularly KG seed was used as a food source compared to other, more easily processed native grasses by First Nations Australians, prior to colonisation. This is primarily due to the lack of robust written records from the post-colonial era, although there is some historical evidence that KG seed specifically was used as a food source (Chivers et al. 2015).

Seed grinding technology was used by First Nations Australians from at least 4,000 years ago, when environmental variability in desert regions necessitated the use of seeds in the diet (Smith 2013). There is even some evidence of seed grinding stones from a dig site in Cuddie Springs that have been dated at 30k years old (Fullagar & Field 1997).

Figure 2: Grindstone from Cuddie Springs. Australian Museum

Whether these stones were a 'once-off' event as posited by Sutton & Walshe (2021), or evidence of continued use of grass seeds in the First Nations diet for the next 26,000 years, there is little argument that grass seeds were used in the First Nations diet well prior to colonisation.

Seed grinding technology increases the palatability and digestibility of grass species (Lopes et al. 2023), and despite being time and energy consuming to process, the use of this

technology meant that First Nations people were able to live more prosperously in arid and semi -arid zones (Drake, Keitel & Pattison 2021), where other food sources were not available (Pascoe 2014). Cowley suggests that this was also the case in Southern Africa, where red oat grass was only routinely processed and eaten in times where other food sources were not available, primarily due to the significant processing requirements (Cowley et al. 2023).

Edwards and O'Connell (1995) suggest that processing enough grass seeds to provide 50% of the daily caloric requirements of a family of five would have taken up to 7 hours. Because of this, grass seeds would have been seldomly used in areas where tubers, nuts, and animal food sources were more readily available.

In the post-colonial world, grain species are the most common source of calories for humans, and advances in grain processing technology has both reduced the processing requirements , and increased the nutritional value of grains (Slavin, Jacobs & Marquart 2010). This means that seeds that were traditionally more difficult to process can now be considered seriously as alternative food sources.

Propagation and Harvesting Requirements

One major constraint on widespread propagation of KG, and in turn, its availability as a food product, is the lack of seed available for sowing. KG was ubiquitous in pre-colonial times, but its abundance decreased significantly post-colonialism, due to grazing by introduced livestock (Cole & Lunt 2005). As Snyman, Ingram and Kirkan (2013) explain, KG is a preferred fodder for livestock, due to its high crude protein level. However, KG does not tolerate heavy grazing, meaning that many populations of KG were grazed to local extinction. This also occurred in Africa, as supported by a field trial, which showed animals giving preference to KG over other grassland species (Ngugi et al. 2003). Allowing animals to graze on KG and other perennial grasses without a fallow period depletes energy reserves required for reproduction and regeneration periods, and leads to plant death (Snyman, Ingram & Kirkman 2013). Furthermore, allowing animals to graze unabated on wild grasses can damage their root systems, which again leads to significant reduction in the populations (Sanford 2019).

Field studies on using KG and other native grass species to rehabilitate ex-mining and agricultural land can help inform attempts to develop a broadacre KG crop dedicated to consumption by humans. For example, Cole and Lundt (2005), found that the hygroscopic awn attached to the seed embeds itself in the soil through cracks and crevices and Sindel et al (1993) found that leaving the seed head and awn intact lead to increased establishment rates. Windsor & Clements (2001), and also McDougall (1989), suggest that slashing and raking hay from a wild sward and then leaving for 12 months to break dormancy can provide 2 viable seeds per gram of mulch collected .

With limited populations available for seed harvesting for use in either food or rehabilitation, it is important that any wild populations are protected and harvested without damage to the plant, to ensure the seeds can be harvested multiple times.

A modified brush harvester has been used to harvest KG seed with some success (Mason 2005), with one field trial yielding 200kg of seed from 70 acres of crop, in approximately 16 hours (Briggs 2001). However, in one trial using this modified brush harvester, one third of the KG stalks were damaged significantly enough for them to not flower again (Stafford 1991). Some researchers, including Male et al (2022) suggest that using non-mechanical technology, such as a machete, is the preferred method to harvest, despite the extra time impost. It is important to note that this time impost will impact the price of the seed per kilogram, due to increased labour costs.

However, once there are more and better-established KG populations available for harvesting, mechanical harvesting could become the preferred method; as populations are less at risk from the loss of individual stalks. This will decrease the overall cost of the seed, as labour costs are reduced.

The Native Food Industry in Australia

The native food and botanical industry in Australia is projected to be worth up to $300m by 2025, and provides First Nations Australians the opportunity to partake in a lucrative industry with strong cultural connections, and also to be employed on country (NSW Treasury 2022). In terms of native grains specifically, these are currently almost solely propagated and processed for grassland restoration, rather than for food. However, NSW Treasury highlights native grains as a potential growth industry (2022), and current partnerships between universities and Indigenous-owned businesses are a positive step in utilising native grains for wider consumption. KG is a highly profitable plant for nurseries, with the current market price for KG seed up to $1600 a kilogram for nursery grade seed (Male et al. 2022).

Australian native plants are genetically selected for the Australian environment. For example, KG and other native grasses are less vulnerable to the impacts of climate change than introduced species. KG can outcompete exotic weeds (Cole & Lunt 2005), is hardy against slashing and burning (Stafford 1991), and has the ability to grow in low-nitrogen soils (Prober & Lunt 2008). In terms of climate change, an increase in CO_2 concentration may lead to increased organic matter in KG tussocks, again allowing them to outcompete exotic species, and even other native species (Hovenden 2009).

In addition to the potential economic benefit, the introduction of native foods into conventional diets can improve health outcomes for First Nations people. Vogliano et al. (2021) explain that 'harnessing traditional knowledge of nutrient-dense, agrobiodiverse foods can help improve food and nutrition security'. Their study found that health outcomes, knowledge of traditional foods, and also general nutritional literacy improved significantly after the reintroduction of native foods in the Solomon Islands, and that harnessing knowledge of traditional foods can help to improve food and nutrition security. Finally, by increasing the availability of native foods to First Nations people, they can continue their cultural connection to these foods, in addition to the health and economic benefits.

Ma Rhea (2016) argues that in post-colonial Australia, First Nations people are yet to achieve food sovereignty, and that the current food system in Australia is under threat from environmental impacts stemming from climate change. By reintroducing native food products into wider consumption and concentrating on plants which are less susceptible to the impacts of climate change, food sovereignty for First Nations people can be achieved. However, only 2% of bushfood and native botanical businesses are currently owned by First Nations Australians (NSW Treasury 2022). As Rooke describes, there have been multiple occurrences of non-Indigenous people utilising native ingredients for huge commercial gain, with little to no benefit provided to the First Nations communities who had stewardship over these plants for millennia (Rooke 2019).

Rooke (2019) describes how the gumby gumby, which has been used as a traditional medicine for generations, was patented by a non-Indigenous business. Traditional owners who derived an income from the sale of the plant were no longer able to do so, impacting Indigenous food sovereignty. Determining new uses for native food products provides an opportunity for First Nations people to benefit culturally and economically, and it is important that any knowledge gained from experiments such as those outlined in this report are shared with First Nations people in the first instance, to ensure they have an opportunity to benefit.

Native Australian fruits, spices and seeds can be combined to create many products, including dressings and sauces. Dressings and sauces are primarily oil-in-water emulsions, which consist of small oil droplets dispersed throughout an aqueous phase (Bai et al. 2017). These emulsions are not thermodynamically stable systems, so to stabilise them and prevent unwanted flocculation of the oil droplets, a food emulsifier or stabiliser can be used. These stabilisers prevent sauces and dressings from 'splitting', which can reduce the food products visual appeal, and impact mouthfeel.

There is currently no native food product readily available that can act as a stabiliser in an oil-in-water emulsion. This lack of available product means that organisations that are committed to using only either native plants, or at the least Australian grown plants in their products, do not have an option to stabilise their oil-in-water emulsion products. This thesis on KG seed and its place as both a food and a food additive was initiated due to this gap in the market.

Physicochemical Properties and Impact on Rheology

The rheology of an emulsion has a significant impact on the sensory qualities of a food product. As well as improving the emulsion stability of a product, food additives can also improve the nutritional values of a product, for example, by increasing protein or carbohydrate levels (Ma et al. 2015). Emulsion stability in an oil-in-water emulsion system can be achieved by interactions between the phases of the emulsion with starches, proteins, and other elements. Emulsion stability is a measure of how resistant an emulsion is to change in its properties, and is generally measured by an increase in droplet size of the emulsion, also known as flocculation (Traynor et al. 2013). Food additives, including emulsifiers and stabilisers, act to prevent changes in these emulsions. These emulsions form an important part of the formulation of various food types including dressings (Charcosset 2009), and if an emulsion fails, the dressing takes on a 'split' like appearance, where the oil and water phases are clearly separated.
The use of protein and carbohydrate rich seeds and pulses can act to stabilise the emulsions and reduce the chance of the emulsion splitting into two phases.

KG seed contains two major elements that are present in successful food emulsion stabilisers; starches and protein. To determine whether KG seed may be suitable for use as a food stabiliser, the levels of starch present in the seed, as well as the quantities and types of proteins present within the plant can be measured.

The starch content of KG seed is only mentioned sparingly in literature, and with varying results. Van Bilijon & le Roux found varying levels of starch in their study, between 5-10% (van Bilijon & le Roux 1986). The varying levels could be due to the starch levels in grass seeds varying during different stages of the lifecycle of the plant, as starches are used by the plant in growth stages (Zhao et al. 2018).

Starches are dispersible in water, and depending on the source of the starch and size of the starch particles, can act to stabilise oil droplets within the aqueous layer of the suspension (Keramat, Kheynoor & Golmakani 2022). Or, starches may be heated to a gelatinisation point, and incorporated into a viscous solution.

Starch granules can be especially effective in a Pickering emulsion, which is a particularly stable form of emulsion, where solid particles act against coagulation in the oil phase (Chu, Chew & Nyam 2021). Ali and Rayner found that an oil-in-water emulsion remained stable after 8 years using quinoa starches in a Pickering emulsion, with an increased concentration of starch granules correlating to a decrease in droplet size (Ali & Rayner 2020). Similarly, a 2017 study found that starches derived from corn, tapioca, sweet potato, and waxy corn starch all achieved stability in an oil-in-water emulsion system (Ge et al. 2017).

In regards to protein and its effectiveness as a stabiliser, various studies have shown that KG has a crude protein content of 9-13% (Mpiti-Shakhane et al. 2001) (Natural Resources SA Arid Lands 2018),

In a protein-based emulsifier, there are three mechanisms that can stabilise an emulsion. Proteins can adsorb to the droplets in the oil layer and form a physical barrier to prevent flocculation (steric) ; form a charged layer at the interface that acts as a repulsive force against flocculation (charge dissociation); or the solid particles can adsorb to the interface layer, again preventing flocculation (Olsmats & Rennie 2024).

Protein based emulsifiers are naturally occurring, and already accepted as food ingredients (Yan et al. 2020). Proteins such as legumin, vicilin, whey and soy can all act as protein-based stabilisers (Ji & Luo 2023). Solid particles can create a thick interface between the water and oil phases, improving stability and reducing oxidation. Furthermore, adding antioxidants to the solid particles can further decrease the risk of oxidation (Keramat, Kheynoor & Golmakani 2022).

Small molecular weight protein surfactants, which adsorb to the oil droplet surface in an oil-in-water emulsion, form a protective coating and reduce free energy in the system. This reduces the ability of the oil droplets to coalesce and form an unwanted separate layer (Yan et al. 2020).

This thesis examines both the protein content of KG seed, and the different types of protein that make up this content, to determine whether both the protein content, and the types of protein contained within KG seed, makes it suitable as a stabiliser. It also examines the starch levels of KG seed, to determine whether the starch content is sufficient to act as a stabiliser.

In addition to the stabilisation of emulsions, grains with high polysaccharide levels can be used as a thickening agent. Like stabilisers, thickeners can improve the stability of various food products, and can be made of polysaccharides such as starches (Msagati 2012). Water-soluble polysaccharides such as gelatinised starches may disperse throughout the solution, increasing the solution's viscosity, and the high molecular weight of non-soluble fibres can also increase the viscosity (Cheng & Neiss 2012). Thickeners are also used to prevent sedimentation in suspensions (Flores-Mendez & Lopez).

Just as is the case with emulsion stabilisers, there are no commercial products available from a native Australian source. By examining the rheological properties of KG seed in a solution, and comparing to the rheological properties of more common non-native thickeners such as corn flour, we can determine whether KG seed may also be an effective thickener.

Statement of Hypothesis and Project Aims

The hypothesis of this study is that KG seed will be an effective food stabiliser and thickener due to its physiochemical properties. The study aims to

1. Measure the concentration of starch in KG seed, to determine whether the starch levels in the samples are likely to lead to KG seed being an effective and economically viable stabiliser and thickener. Higher percentages of starch in the sample should correlate with stronger stabilising and thickening ability.
2. Measure the concentrations of different types of protein in KG seed, to determine whether protein levels and types present in the sample are likely to lead to KG seed being effective and economically viable stabiliser. Types of proteins tested were chosen from literature review of effective plant-based protein stabilisers (Ji & Luo 2023) (Olsmats & Rennie 2024). Higher percentages of stabilising proteins should correlate with strong stabilising ability.
3. Test the moisture content of KG seed, to determine whether it has a similar moisture content to corn starch in the thickening test, as moisture content can impact rheological properties.
4. Measure the stability of oil-in-water emulsions with varying levels of KG seed used as an emulsifier. Measure the time taken for an emulsion to separate compared to a known effective stabiliser (xanthan gum).
5. Measure the viscosity of various levels of KG seed in an aqueous solution, to compare its thickening properties compared to a known effective thickener (cornstarch).

Methods

Seed Preparation

The seed for analysis was sourced from Native Seeds Australia, based in Eurobin in Northeastern Victoria, Australia. The seed purchased was ‘nursery grade’ seed, with the seed still attached to chaff and awns.

Seed was separated from chaff and awns by hand, with the assistance of a Graintech Scientific drum sieve with a 1/8 inch aperture. Weight of separated seed was compared to chaff to measure seed vs chaff.

Seed was then milled using a ‘Hawos Billy 100’ flour mill, on setting 9 (maximum).

One 5g sample was removed after 1 pass through the mill, for use in protein analysis.

Remaining seed was milled a further two times to remove as much seed coat as possible.

Seed yield was calculated as the total amount of seed separated as a percentage of total weight including seeds, chaff, and awns.

Starch analysis

Starch content was determined by a Total Starch (alpha-amylase/amyloglucosidase) assay protocol, under the AOAC Method 996.11, using a Total Starch Kit produced by Megazyme.

Duplicates of 100mg of milled sample were placed into Corning culture tubes. Tubes were labelled ‘Sample 1’ and ‘Sample 2’.

10mL of a buffer made of 100mM pH 5 sodium acetate, and 5mM of calcium chloride was added to both samples. Samples were stirred vigorously on a vortex mixer for 5 seconds.

0.1mL of thermostable α-amylase was added to Sample 1

0.1mL more Sodium acetate (100mM ph 5.0) buffer was added to sample 2 to provide a sample blank.

Both tubes were vortexed for 3 seconds, and then transferred to a boiling water bath for 2 minutes. Samples were mixed vigorously on a vortex for 10 minutes, before returned to the boiling water bath.

After 5 minutes, the tubes were removed, and again mixed for 5 seconds on the vortex mixer, and then returned to the boiling water bath.

After another 5 minutes, the tubes were again removed and mixed for 5 seconds on the vortex mixer, before being returned to the boiling water bath.

The samples were then removed from the boiling water bath after another 5 minutes.

The tubes were then placed in a separate water bath at 50°C for 5 minutes.

0.1 mL of amyloglucosidase was then added to sample 1, and then vortexed for 3 seconds.

0.1mL of Sodium acetate (100mM ph 5.0) buffer and 5mM calcium chloride was added to sample 2, and vortexed for 3 seconds.

The tubes were then incubated at 50°C for 30 minutes.

After 30 minutes, tubes were removed and allowed to cool for 10 minutes, with the occasional inversion to ensure the samples were mixed properly.

2 mL of each solution was then transferred to separate microfuge tubes, and then centrifuged at 13,000rpm for 5 minutes. The remaining 8.2mL of incubation solution was retained.

1mL aliquot of the supernatants was then added to 12 tubes containing 4 mL of sodium acetate (100mM 5.0 ph) and calcium chloride (5mM), and then mixed.

Duplicate samples of 0.1mL of these mixtures were then transferred to 16 glass test tubes each, 32 tubes in total. 3.0mL of GOPOD reagent was then added to these tubes, and incubated at 50°C for 20 minutes.

The absorbance was then measured against the reagent blank, at 510nm.

The absorbance levels were then used in the formula $\Delta A * F * EV * \frac{D}{W} * 0.9$, where ΔA is the absorbance level of the sample against the reagent blank, F is a provided factor to convert absorbance values to μm glucose, EV is the sample extraction volume, D is the dilution level of the sample, W is the sample weight in mg, and 0.9 is the factor to convert from free glucose to anhydroglucose, which occurs in starch.

Protein Analysis

2 samples of KG seed were collected and sent to the Australian Proteome Analysis Facility (APAF), at Macquarie University, Sydney to undergo amino acid analysis. Sample 1 was whole seed, whereas the second sample was finely milled and did not contain seed coat. Samples were prepared by liquid hydrolysis for 24 hours in 6M Hydrochloric acid at 110°C.

After hydrolysis, samples were analysed on a Waters Acquity UPLC.

Amino acid profile analysis was performed as by APAF per APAF SOP AAA-001.

Differential Scanning Calorimetry

Differential scanning calorimetry was undertaken on a TA Instruments DSC 25.

2 mg of sample were weighed using a Sartorius scale and placed in the receptacle of the DSC, and hermetically sealed. The DSC was then run for a single cycle, with a heating rate of 10°C per minute up to 200°C. This experiment was done in duplicate, and undertaken to determine the gelatinisation temperature of the starch.

Thermogravimetric analysis

Thermogravimetric analysis was completed using a TA Instruments SDT650 Thermogravimetric analyser.

2mg of sample was weighed using a Satorius scale.

The sample was heated at 10°C per minute to 110°C in an anaerobic environment, and then held at 110°C for 5 minutes.

The sample was then heated at 10°C per minute to 900°C, and then held at 900°C for 15 minutes.

After 15 minutes, oxygen was introduced in the system, and then was held for a further 10 minutes.

The weight percentage v temperature curve was then used to determine moisture content.

Emulsion Experiment

To measure the stabilising effectiveness of KG in an oil/water emulsion compared to a commonly used stabiliser, samples of KG seed and xanthan gum suspensions were prepared at various ratios, as outlined in Table 1.

Table 1: Composition of samples for emulsion experiment

Sample	**Weight of additive (g)**	**Weight of water (g)**
KG seed 2.5%	0.25	9.75
KG seed 5.0%	0.50	9.50
KG seed 10%	1.00	9.00
Xanthan Gum 2.5%	0.25	9.75

All samples were then heated to 60°C, less than the starch gelatinisation temperature as determined by the DSC, to ensure the starch could still form a Pickering emulsion.

2.5g of vegetable oil was then added to each of the samples, in a plastic beaker.

The samples were then emulsified using an ANKO P_4332928 milk frother for 30 seconds each. The frother was placed at the bottom of the beaker for each sample.

After the samples were emulsified with the oil, the samples were observed until clear separation of oil droplets from the aqueous phase occurred in the sample, and the time recorded. For each sample, the experiment was repeated 3 times.

Additional samples of 5% and 10% xanthan gum to water were also prepared, but not used, as no observed separation occurred at the 2.5% ratio.

Thickener experiment

3 samples of each KG seed and corn starch were prepared at different concentrations, 5%, 10%, and 20% w/w. An Anton Paar MC502 rheometer was used to compare the viscosity of KG seed samples and viscosity of corn flour samples, with force up to 9N. A 25 mm parallel plate geometry was used to carry out a stress sweep over the range of 0.001-100Pa.

Results and discussion

Seed Processing

Seed yield varied between 13% seed to total weight of nursery grade seed separated using a drum sieve, to 31% seed to total weight separated by hand. The yields and time taken was impacted by the type of processing equipment used.

Not enough seed was procured to enable a rigorous study on the impact of processing equipment and time on the total yield, so results and graphs are not included in this report. A more rigorous study, comparing yields and time taken for hand-separated samples to samples separated using technology, such as drum sieves or mechanical separators, could help determine best-practice in processing, and research into novel techniques could decrease the costs in processing seed.

Starch analysis

Table 2: Starch analysis results

Sample	Starch level (%)
1	9.37
2	12.24

Mean of two samples is **10.81 ±1.44 %** of starch.

With the samples of KG seed measured averaging a starch content of 10.8%, the levels of starch in KG seed is considerably less than some other grass species, such as *Chloris virgata*, which can contain up to 62% starch (Zhao et al. 2018). Other grass species native to Africa and grown prolifically in Australia, such as Guinea grass, contain up to

30% starch. Other plant products commonly used as emulsifiers, such as quinoa (60-70%), and tapioca (80-90%), can contain much higher levels (Ali & Rayner 2020) (Ge et al. 2017).

Starches can form a Pickering emulsion, which is very effective in stabilising oil-in-water emulsions. The solid particles in the starches work to separate the individual droplets in the oil phases, which prevent flocculation and the forming of a separate layer of oil from the aqueous phase (Chu, Chew & Nyam 2021).

Starches, and other polysaccharides, are particularly useful against 'creaming' in an oil-in-water emulsion, and improves the sensory aspects of these emulsions to make them more attractive to consumers (Difits, Biliaderis & Kiosseoglou 2005).

It is unlikely that 10.8% starch is enough for KG seed to act as an effective stabiliser for oil-in-water emulsions, without considering interactions with other physicochemical components which may assist, such as proteins. In the Ali & Rayner (2020) and Ge et al (2017) experiments that looked closely at the effectiveness of starch based emulsifiers and their interactions with other additives, plants with much higher starch percentages were used, including quinoa. Quinoa has a starch content of between 58-68% (Sivapalan 2018), and has an average cost per kilogram of between \$2USD and \$6USD for raw material over the past decade (Shahbandeh 2023). Quinoa, and other starchy plants such as potato and tapioca that are used commonly as starch-based stabilisers, have a considerably higher starch content than KG seed, and at a much lower cost.

The high cost of KG seed is exacerbated by its processing requirements, and how difficult it is to separate the seed from the other plant material. As described, in the 'Seed Processing' results, the yield of seed from chaff and awns was a maximum of

31%. Following the separation, the seed was then milled to remove the seed coat, with another 65% of raw material lost in this process, which meant that after separation and milling, an average of 8 grams of sample remained per 100 grams of nursery grade seed. With the cost of the nursery grade seed at $1600 per kilogram, this equates to approximately $20,000 per kilogram of usable seed, which is many times higher than the cost of common starch-based stabilisers and emulsifiers.

One option to improve the likelihood of KG seed being able to act effectively as a starch-based stabiliser is to isolate the starch granules from the other elements. However, this is not an economically viable option. Even at the higher starch level recorded of 12.3%, using isolated starch would cost well in excess of $200,000 per kilogram in material costs, compared to $3-4 USD per kilogram of material costs for quinoa starch.

With a significant number of other starch based stabilisers on the market, including quinoa, that have been shown to be a reliable stabiliser, at a fraction of the cost, it is not economically viable to consider KG seed a potential source for a starch-based emulsifier.

However, quinoa is not a native Australian plant, and with other Australian grass species similarly lower in starch than quinoa, corn and the like, other components need to be considered.

Protein Analysis

1011 protein types in total were found in the sample.

Table 3: Amino acid composition and total protein content

Amino Acid	Sample 1 (whole seed) (mg/g)	Sample 2 (finely milled) (mg/g)
Histidine	2.7	4.6
Serine	5.6	9.5
Arginine	3.1	5.0
Glycine	3.8	6.3
Aspartic Acid	9.5	16.1
Glutamic Acid	20.4	36.1
Threonine	3.4	5.6
Alanine	13.0	23.3
Proline	14.7	26.3
Lysine	2.1	3.1
Tyrosine	2.5	4.1
Methionine	3.0	5.0
Valine	5.1	8.5
Isoleucine	4.5	7.8
Leucine	23.6	43.6
Phenylalanine	5.5	10.1
	122.5	**215.1**

No hydroxyproline or taurine was observed.

Total protein was 12.25% for unmilled seed, and 21.51% for milled seed with seed coat removed.

Table 4: Presence of proteins commonly found in plant-based stabilisers , based on existing literature (Ji & Luo 2023) (Olsmats & Rennie 2024)

Common plant-based protein stabiliser	**Present in sample?**
Legumin	No
Vicilin	No
Whey-style protein	No
Albumin	Yes
Soy-like protein	No

With the finely milled seed containing 21.5% protein, KG seed does have the advantage of having a higher protein content than more commonly used grass species for food such as wheat (between 8 and 14%) (Birch et al. 2023). Furthermore, if an emulsifier is used that is high in protein, this has the added benefit of increasing the protein levels in the food product, and providing additional nutritional benefits.

The concentration of the protein particles impacts the stability of an oil-in-water emulsion. If not enough particles are present, the particles cannot cover the entire droplet surface, and therefore, cannot form a stable interfacial film (Ji & Luo 2023). As well as the quantity of protein present, the type of proteins present and how they interact within the system can impact the stability of the system.

As described by Olsmats and Rennie (Olsmats & Rennie 2024) , there are three different ways that proteins act as a stabiliser in an oil-in-water emulsion. Proteins can attach themselves to the droplets in the oil phase or in the interface layer, and physically separate either the droplets from each other; the proteins may attach themselves to the oil layer, and physically separate the two layers; or the proteins can form an electric charge that again works against flocculation.
The type of protein present is also important. Ji & Lu (2023) examined four different plant-based proteins; soy, pea, quinoa and lentil, and found that the presence of legumin and vicilin proteins in the sample were associated with high stability in the oil-in-water systems. Neither legumin or vicilin were found within the KG seed samples tested; however, Ji & Lu did also suggest that albumin as found in the lentils they tested, had excellent functional properties (Ji & Luo 2023). An albumin-like protein was found in the KG seed samples tested.

Albumin is most commonly found in eggs, and can also be used as a foaming agent, therefore deriving albumin protein for stability or other food additives from a plant product would appeal to the vegan and plant-based market.

As is the case with isolating starches, isolating the proteins from KG seed and using only the proteins as a stabiliser is not viable economically. Even taking the higher protein measurement of 21.5%, the cost of isolated protein would equate to around $100,000 per kilogram.

This is compared to a more commonly used stabilisers, like xanthan gum, which was stable at 2.5% sample in the experiment, and has been shown to be stable at a level as low as 0.15 (w/v) (Traynor et al. 2013). Or, proteins derived from soy and legumes, which are considerably cheaper and have less processing cost.

The tests found that KG seed contained 8 of the 9 essential amino acids, with only tryptophan not present (*Recommended Dietary Allowances* 1989). Essential amino acids are proteins which are vital in a range of biological functions in both humans and other animals, including gene expression and metabolism (Hou, Yin & Wu 2015).

This number of essential amino acids compares favourably to some other grass species, such as wheat (Khan et al. 2014) (Erekul & Yigit 2023), pulses and some other grains. However, some other species such as quinoa, are ‘complete proteins’, containing all nine essential amino acids (Craine & Murphy 2020). The presence of essential amino acids, while not useful to its stabilising properties, means that the use of KG as a food additive can provide nutritional benefits to the food product it is used in. If KG seed is used as a more general food source in the future, such as in baked or fermented beverage products, this higher protein level is a benefit nutritionally.

Thermal behaviour of KG seed

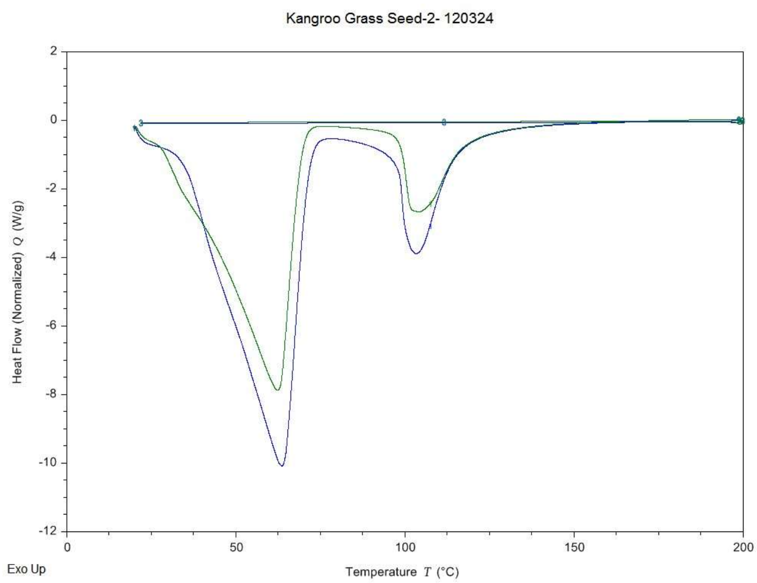

Figure 3: Graph output from DSC

A graph produced from the DSC shows that starch gelatinisation occurs at 63°C.

Starch gelatinisation temperature was measured, to determine which temperature the starches could be heated to, to ensure that its ability to form a Pickering emulsion was not compromised.

The graph also shows a change in heat flow at 100°C, which indicates the moisture has incorporated with the starch to make a paste.

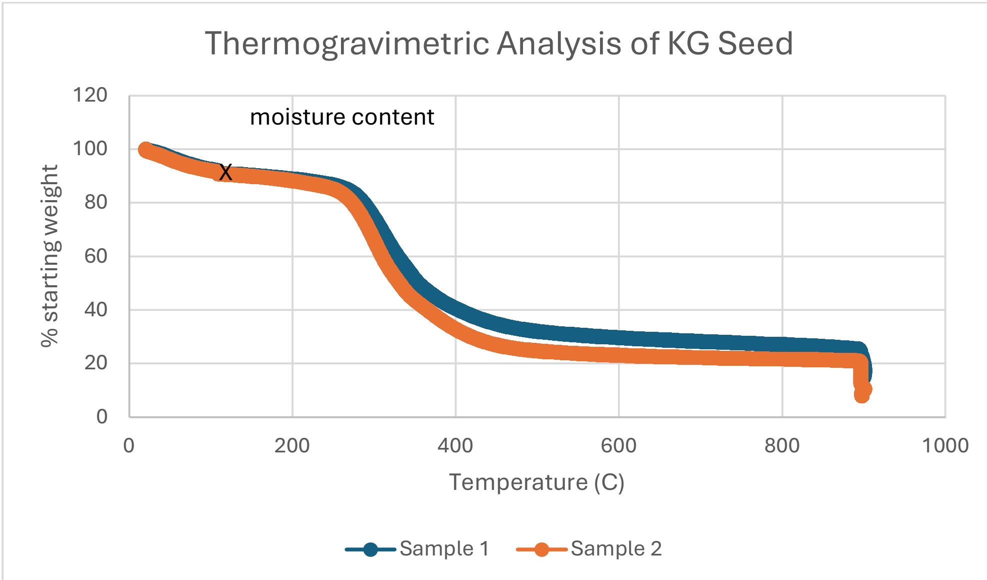

Figure 4: Graph of Thermogravimetric Analysis for two samples.

Table 5: Table of moisture content results from samples

Sample	Moisture Content (%)
1	9.3%
2	9.4%

Mean of the two samples is **9.4% ±0.05**

Lin et al (2012) suggest that the moisture content of a thickening agent can impact the degradation time of an emulsion, with samples with a higher moisture content being more susceptible to degradation within their crystalline structures (Lin et al. 2012). The thermal experiments showed that the KG seed samples had a similar moisture content to standard corn starches, so this variable should not have impacted on the results.

Stabiliser results

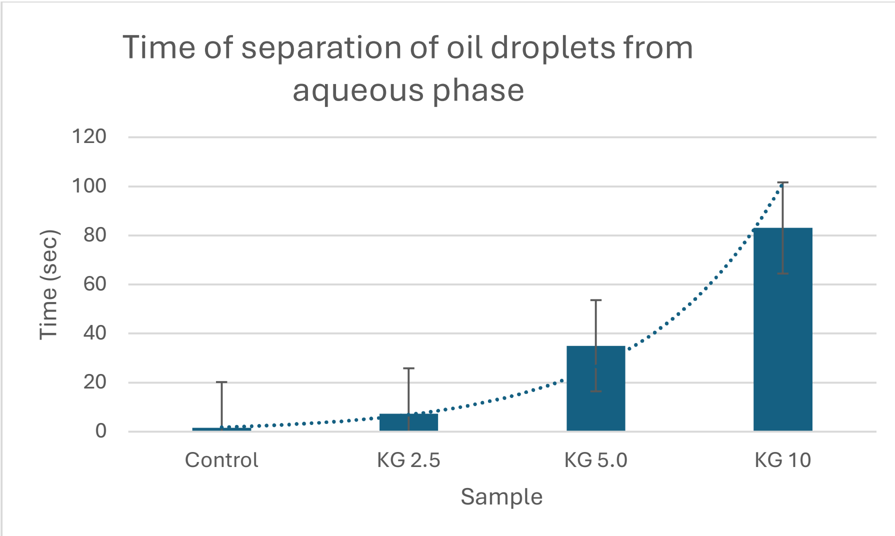

Figure 5: Table of time of separation of the samples of KG seed in an oil/water emulsion

An ANOVA test was undertaken to determine if there are statistically significant differences between the samples means, through an online calculator at https://www.standarddeviationcalculator.io/anova-calculator,

H_0 = the variance between the samples is due to random chance.

F value = 678.879. This F value shows the difference between the sample means is statistically significant, thus rejecting the null hypothesis.

For the three repeats of the Xanthan gum experiment, no separation was observed for more than 2 weeks, so emulsion was considered stable.

The higher percentage of KG seed w/w% in the samples correlated with an increased emulsion stability. However, the stability of the emulsion is not sufficient for a commercial food product, with the emulsion starting to fail after approximately 2 minutes even at the highest percentage of KG seed w/w. Commercial products require a stabiliser that will make the product shelf stable for at least weeks, if not months or longer.

Although using whole ground KG seed is not suitable, there is a possibility that isolating both the starch and protein components of the whole seed may improve suitability. However, as mentioned earlier, this is limited by the economic considerations.

Interactions with other stabilisers and further research in stability

Starches and proteins generally do not act independently within a food product; as both proteins and starches are found in the same additives regularly, and depending on the interactions between the two, can work to either assist or inhibit stablisation within an oil-in-water emulsion (Tadros 2016). Examining the interactions between the proteins and polysaccharides in the KG seed on a microscopic level may provide more information on how the two factors impact the stability of the system.

Proteins can act as an emulsifier without conjunction with another substance, but they are also greatly assisted by the addition of another emulsifier. Adding gums, such as xanthan gum or soy lecithin at small volumes greatly increases emulsion stability (Ye, Hemar & Singh 2004). For example, pea protein became a much more effective stabiliser once gum Arabic was introduced (Zha et al. 2019). With this in mind, KG and other native grasses may be more effective as a stabiliser if used in conjunction with the gums of a native plant, such as *A. mearnsii,* a native Acacia species.

However, the use of *A. mearnsii* as a food product has not been fully explored.

Acacia species, particularly *A. senegal*, produce the common stabiliser gum Arabic. Limited literature exists on the physiochemical properties of native Australian acacia species and how it relates to food stabilisation, but *A. mearnsii*, has a similar chemical structure to *A. senegal*, and according to Anjo et al, has the potential to provide a similar level of stability than the commercially available *A. senegal* (Anjo et al. 2021).

However, there is no commercial stabiliser using gum from *A. mearnsii* at this stage. *A. mearnsii* is a ubiquitous species in Australia and is commonly used in other commercial applications as a hardwood. If the gum is shown to act similarly to other species of Acacia, it may be more suitable as a stabiliser than native grain products, or, can act as a complementary stabiliser to native protein-based stabilisers.

The benefit of using a stabiliser such as KG seed in addition to gum stabiliser like gum Arabic, is that the essential amino acids contained within the KG seed are then present in the food product. This may improve the nutritional value of the food product.

Thickening results

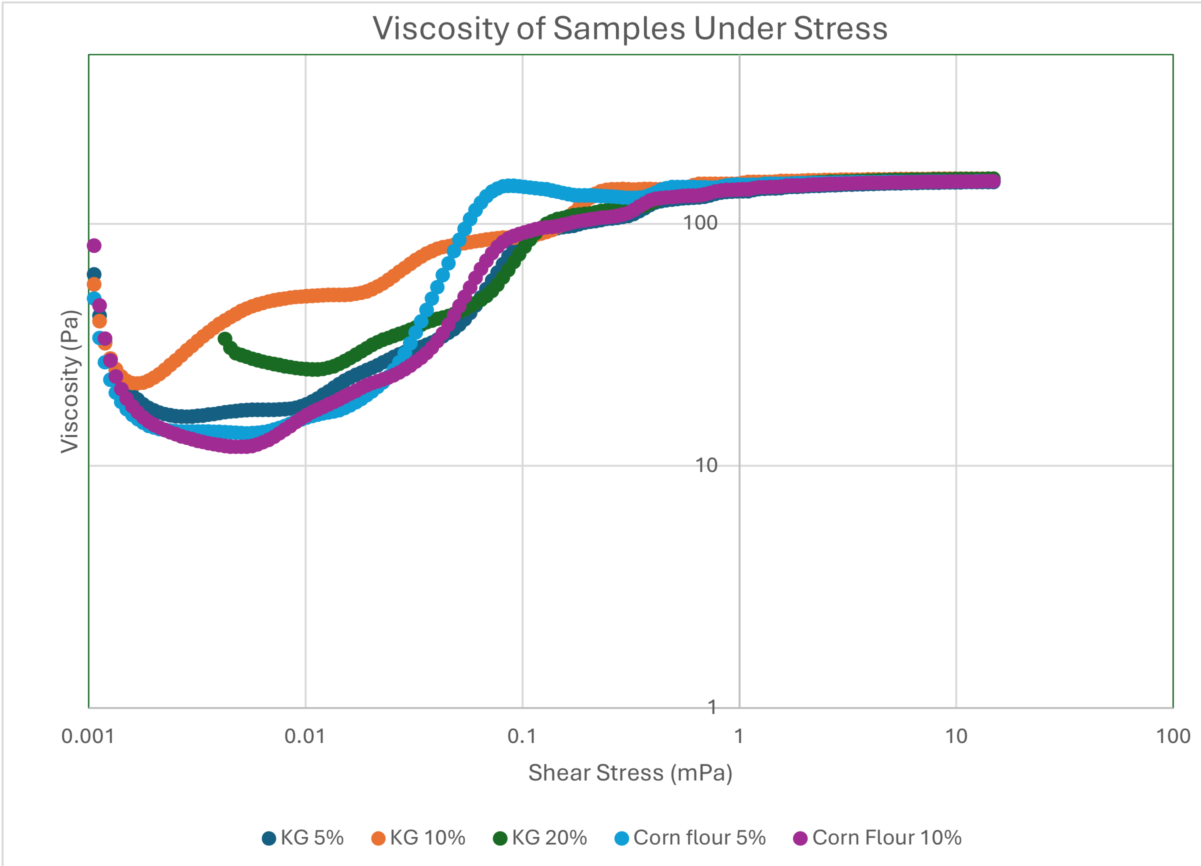

Figure 6: Comparative graph of KG seed samples at 5%, 10% and 20%, and Corn Flour samples at 5% and 10%

The rheological results indicated that the KG seed samples showed similar thickening properties to corn flour. Both KG seed and corn flour are non-Newtonian shear thickeners, meaning that viscosity increases as the sample is placed under stress. The shape of the graphs indicate, the viscosity of the samples increase until they achieve to a plateau value between 140-150 mPa.

A 20% corn flour to sample was also tested but not included on the graph, as the sample became a paste which led to an inconsistent reading.

In terms of rheological and thickening properties, KG seed shows good potential as a thickener. Rheological tests showed that, in comparison to corn flour, KG seed has similar thickening properties, although it is likely that the two substances rely on different components to provide their thickening properties.

KG seed has a high percentage of neutral detergent fibre (NDF) as a percentage of its dry weight. NDF is a measure of total fibre, both digestible and non-digestible, and up to 70% of KG's dry weight is made up of NDF (Natural Resources SA Arid Lands 2018) (Reseigh, Shuppan & Wurst 2010). NDF is non-soluble, so provides 'bulk' to an aqueous solution.

In corn flour, the most effective thickening agent is starch. More than 90% of starch produced in the United States comes from corn, due to the plants high starch levels. In an aqueous solution, starch granules swell, and eventually diffuse into the aqueous medium (Nussinovitch & Hiashima 2013). As discussed earlier, KG has limited starch, particularly in comparison to the starch levels of corn.

The experiments showed that KG seed and corn starch acted very similarly in both 5% and 10% solutions of each sample, with similar increases in viscosity measured. At 20%, KG seed again showed thickening properties, but the corn starch sample became a paste and was not able to be accurately measured, so no comparison at that level was possible.

KG seed's potential as a commercial thickener is still subject to the economic issues related to propagating, harvesting and processing the seed. However, as no further isolation of components like starch or proteins is required, this reduces both the processing time and cost, and also increases the amount of usable material as a of percentage raw material. KG seed is still many times more expensive that more common thickeners, such as corn flour, however, if harvesting, propagation and processing costs can be reduced, there is potential for KG seed to be used as a commercial thickener in the future.

In addition to improving texture of a food product to increase consumer satisfaction, thickeners are a vital tool in feeding patients with dysphagia, a swallowing condition sometimes caused by stroke. Dysphagia patients require liquids, including juices and milks, to be thickened, and NDF thickeners can be useful in achieving this (Wen, 2024). A lack of available seed for testing meant that the experiments used in this report were limited to basic thickening tests, however, some thickeners may interact differently with various food products.

Future work on the specific interactions of KG seed with different food types, such as canned soups or even juices and milk, could determine how effective KG seed is as a food additive for patients with dysphagia.

Conclusion

The use of native Australian plants in food has increased significantly over the past two decades, as challenges related to climate change and sustainability provide an opportunity for growers to introduce plants adapted to the natural environment over introduced species. Furthermore, the consumer appetite for novel and sustainable food products has also increased. This appetite has broadened to include plants such as native grains, in addition to the more common native species grown for food, such as macadamia and lemon myrtle.

KG is a species that is well-adapted to the Australian environment, and along with other native Australian seeds and grains, has been used as a food source for millennia, and can outcompete exotic species in terms of geographical spread and adaptation to climate change.

Many challenges in terms of harvesting and processing still remain, and the labour cost of harvesting and processing the seed is significant, which in turn pushes the cost of the grain far above the cost of common introduced species, like corn and wheat.

However, there has been significant development in this space over the past two decades, and if the appetite for novel and Australian-grown species continues to grow, more research and development in propagating, harvesting, and processing KG is likely, which will likely reduce the cost of the grain going forward.

Further research on best practice in harvesting and processing KG seed in particular is required, as if these imposts are reduced, the economic considerations become less of a factor in determining whether KG seed could be a viable food additive.

The use of Australian grown products is a vital consideration in the production of native food products, and Australian food businesses are increasingly focused on using either native or at least Australian grown products exclusively in their food products. With this focus comes challenges, as gaps in the market become more evident, and the opportunity for new food products is presented. Additionally, as the market grows rapidly, the issues surrounding food sovereignty for First Nations Australians becomes more significant. How an industry can support the food sovereignty of the people providing the Indigenous knowledge must be considered as the use of native Australian plants grows.

Although KG seed only contains around 10% starch, which is considerably less than other effective and economically viable starch-based emulsifiers like quinoa or tapioca starch, the level of protein and types of protein, particularly albumin, may present as an opportunity if isolation of proteins can be achieved easily.
Milled KG seed has a protein level of up to 22%, which makes it higher in protein than introduced grain species. Furthermore, KG seed contains 8 of the 9 essential amino acids, meaning it has high nutritive value.

In terms of availability of product and processing costs, other native Australian plants, such as *A. mearnsii*, have greater potential as a stabiliser than KG seed and other native grain products. More research on the effectiveness of *A. meansii,* either as a standalone stabiliser or as a compound with other protein or starch based stabilisers, could open up more economic pathways.

On the other hand, with its high level of NDF, KG seed has good potential as a food thickener. In rheological tests, KG seed acted similarly to corn flour, which is a commonly used food thickener. Although the economic limitations means that KG seed is not likely to become a commonly used thickener in the near future, if more progress is made in harvesting and processing technology, KG seed has the physiochemical properties to be a capable food thickener.

As well as improving mouthfeel and texture of food products, food thickeners are important for patients with dysphagia. More research on how KG seed interacts specifically with various food products, such as sauces, and even liquids like juices and milks, could increase the viability of KG seed as a tool to help patients with dysphagia eat and drink safely.

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