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FINAL REPORT

"Greenleaves" – Wet extraction of leaf protein from fresh sprouted broccoli stems and other green leafy vegetable waste

ENDFOODWASTE COOPERATIVE RESEARCH CENTRE
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Industry Partner Foreword

The Leaf Protein Co. was founded to increase human and planetary health by commercialising earth's most abundant protein, found in edible leafy materials. A large component to increasing the sustainability of our food systems is to reduce the waste produced in our food chain. This project sought to determine our ability to use the "waste" leafy material identified at 2 different parts of our food system, at farm gate and at retail. The collaboration with Perfection Fresh and Woolworths to supply this material at farm gate (Perfection Fresh) and retail (Woolworths) has been invaluable to this research. We're grateful that some of the leading businesses in the Australian food industry have joined us in the search for innovative solutions for more sustainable food ingredients.

Our early work developing our leaf protein extraction protocol indicated the ability to extract valuable plant protein ingredients. This project was the next step to understand the end-to-end process to acquire input material, extraction at scale and the application of leaf protein into acceptable food products.

The research helped us understand the logistical challenges of collecting waste material, and the stability of supply of the type of material, such as whether sufficient leaf material could be collected at farmgate. It also highlighted the hurdle of how to process a mixture of leafy material from retail to produce a standard output ingredient.

The most promising result from the project was the ability to include both the protein and fibre fractions from the extraction process into desirable finished food products. We were particularly excited with the overwhelmingly positive response of attendees at evokeAg 2022 when tasting the "fibre" crackers that were developed from this project.

The technical impact of this project has been to reinforce the importance of an optimal extraction process with extraction efficiency above 50% to ensure commercial viability. We've taken the learnings of the extraction inefficiencies identified in the project, to improve on and optimise at our pilot plant.

The commercial impact is our ability to market all outputs from the extraction process, particularly as protein not captured in the isolate protein output is often "lost" in the fibre fraction. We've seen from this project that the fibre fraction has strong potential as a food ingredient, aligning with current market trends for increased wellness.

We're looking forward to taking the technical and commercial learnings to our pilot plant in our continued efforts to commercialise earth's most abundant source of protein.

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Fern Ho

CEO and Co-founder

The Leaf Protein Co

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Woolworths is proud to have been part of this innovative project, which aims to reduce food waste across the supply chain by transforming green leafy waste into valuable food ingredients. At Woolworths, we are dedicated to sustainability and continually seek new opportunities to improve our operations and minimise waste. This collaboration with Perfection Fresh, The Leaf Protein Co, the South Australian Research and Development Institute (SARDI), and End Food Waste CRC is a significant step towards creating a more sustainable food system in Australia.

This initiative aligns with our broader sustainability strategy, which focuses on minimising food waste, reducing our environmental footprint, and supporting the circular economy. We are excited about the future possibilities this project opens up and look forward to exploring how these innovations can be integrated into Woolworths' broader sustainability efforts. By turning food waste into a valuable resource, we are helping to build a more sustainable and efficient supply chain for our customers, our partners, and the planet.

Woolworths remains committed to driving positive environmental outcomes and supporting research and development projects that align with our values of sustainability and innovation.

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Mischa Hutchinson

Food Waste Program Manager

Woolworths360

At Perfection Fresh, sustainability has always been at the heart of our operations, and we are thrilled to have been part of this pioneering project aimed at reducing food waste and extracting value from green leafy waste. As one of Australia's largest fresh produce growers and the leading producer of broccolini, we believe that in a world striving for sustainability, it is crucial to explore new ways to extract maximum value from every part of the plant.

This collaborative project with Woolworths, The Leaf Protein Co, the South Australian Research and Development Institute (SARDI), and Fight Food Waste CRC is a key example of how partnerships across the supply chain can turn challenges into opportunities. By assessing the viability of recovering protein, fibre, and other components from green leaf waste, we are working toward a solution that not only addresses food waste but also creates valuable food-grade ingredients from materials that would otherwise be discarded. This aligns perfectly with our commitment to innovation and delivering fresh, sustainable, and nutritious food products to consumers.

By participating in this project, Perfection Fresh is reinforcing its dedication to reducing food waste and contributing to a more sustainable agricultural future. We look forward to exploring further opportunities to integrate these innovative processes into our operations, ensuring that we maximise the value of our produce while supporting global sustainability goals.

We are proud to have partnered on this project and believe that the insights gained will pave the way for future advancements in reducing food waste and creating a more circular economy in the fresh produce industry.

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Michael Liddell

Head of Packaging & Sustainability

Perfection Fresh Australia Pty Ltd

Executive Summary

This Project achieved several important and positive goals for the potential to upcycle certain green leaf and stem food waste from cabbage, cauliflower, sprouted broccoli and lettuce to create two product outcomes that could utilise almost 100% of the biomass collected using well-established processing methodologies and equipment at a laboratory scale. The product outputs were a 0.5-2% yield of wet biomass protein isolate powder containing 60-70% protein, and a fibre-rich fraction which ranged from 15% yield of wet biomass as a dried powder, up to 95% yield as a wet food ingredient. The Project successfully demonstrated that the fibre-rich fractions could relatively easily be included as ingredients into a wide range of food products at levels of 5-50% (in dry and wet formats) without detrimental impacting on the appearance, volume, texture and shape of the food product, and with sensory outcomes that were widely assessed as positive. The Project also demonstrated the potential for effective through value-chain collaboration from a food grower, a food processor, a food retailer and a plant protein innovation start-up company to help increase the amount of green leaf material that could enter the food system as value-added ingredients and reduce the amount of biomass being composted or used as animal feed. The logistical and practical challenges that would be faced if this laboratory scale approach were to be upscaled to a commercial scale became obvious as this Project progressed. Collecting fresh green leaf material from a large number of supermarkets spread across a metropolitan area could supply enough green leaf biomass to sustain a facility to process that material into the upcycled products, but it brings several hurdles. First, the approach would only be possible if each of the supermarkets taking part in the program had sufficient space “behind the scenes” to install a new cool room to store that green leaf biomass until it was picked up. Second, the expense of running sizeable, refrigerated vehicles to do a daily pick up and preserve the fresh quality of that biomass would be substantial. Third, a processing facility to upcycle the biomass would have to be located fairly close to the metropolitan centre which would make the land (if available) expensive. Fourthly, the requirements for water and electricity to process the biomass and the cost of the energy needed to dry the protein isolates and/or fibre-rich fractions would be substantial. Finally, whilst the product outputs are of good nutritional composition, have interesting and useful techno-functional properties (e.g. foaming and water holding capacity) there is limited perceived ‘superfood’ marketing opportunity in cabbage, broccoli or lettuce that might attract consumer interest that would support a premium price for the fibre-rich fractions. The global mega-trend of improving health through better balancing intestinal microbiota through prebiotic foods (especially fermented foods, and products with resistant starch and soluble fibre) and probiotics should start to gain more momentum in Australia soon, and these fibre-rich food ingredients could be well positioned to take advantage of that increased level of consumer and food manufacturer interest.

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1. Introduction

Edible green leafy waste is removed for different reasons (such as quality, appearance, compliance) throughout the supply chain post-harvest. CSIRO data conservatively suggest the volume is at least 128kT of leafy green waste produced nationally every year (Ambiel et al., 2019). Perfection Fresh (Liddell, pers. comm.) indicates that sprouted broccoli production alone creates 580-800T/week of waste material. This material is composted, returned to the soil or fed to animals. Woolworths stores each collect about 100kg/week (Hutchinson, pers. comm.) of a large variety of leafy green vegetable waste – outside leaves from lettuce, cabbage and cauliflowers that are removed by staff before display, wilted Asian greens, celery and herbs, prepacked bags of spinach, rocket and lettuce leaves that reach their best before dates. These are used for animal feed or as compost instead of going to landfill.

Previous work has shown that up 50% of the dry weight of the leaf mass of certain vegetables is protein (Davys et al., 2011). If this material is quickly processed by wet extraction it results in high yield and quality of the protein powder which can be sold to food manufacturers as an alternative to animal sources of protein. Other components of the leaf material, such as fibre, can also be captured and sold. In this way, green leaves that would otherwise go to waste (landfill, compost) or animal feed can be transformed into valuable food ingredients. The aim of this Project was to demonstrate at laboratory scale that this upcycling of green leaf biomass can include opportunities for protein and fibre-rich food ingredients.

During the course of this project The Leaf Protein Co built and equipped a new pilot scale protein extraction facility on an alfalfa farm at Biloela in Queensland in order to co-locate with a source of fresh leaf biomass. This gave rise to the opportunity to evaluate potential uses of alfalfa fibre-rich biomass in food applications and was a logical follow-on from the Woolworths green leaf collection work.

2. Methodology

No ethics approval was needed for the sensory assessment part of the project since none of the food products developed in the project were categorised as ‘novel foods’ and they were all manufactured from food grade ingredients.

2.1 Sourcing and preparation of the green leaf material

Woolworths: Approvals were given by the Woolworths360 Food Waste Program Manager and the National Operations – Supermarkets, Sustainability Lead to engage with the Marryatville store (only 5.6km from the Waite campus) to arrange for weekly pickups of fresh green leaf material from the fresh produce section. Green leaves are removed from fresh produce in the preparation area behind the scenes before it goes on display in the store to make the produce more visible to customers, and to reduce the amount of green leaf material that would be removed by customers in store which could become a slip hazard and look untidy. A 100L green wheelie bin was purchased and put in a cooled area for produce storage together with signs that informed staff in the fresh produce section what it was for and how the bin was to be used (Figure 1). Each Monday during the trial, green leaves that were removed from produce were put into the wheelie bin until it was full. When the researcher came to pick up the material at about 4.30pm the bin was wheeled to the receivals bay roller door.

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A B C

Figure 1. The Greenleaves Project at the Woolworths Marraytville store.

A) the wall sign with instructions of what is to be done, B) the wheelie bin used to collect the green leaf materials, and C) the full wheelie bin on collection day.

In the receivals bay the green leaf material was removed from the wheelie bin by hand and transferred to a 120L esky in the back of a vehicle and then driven 15min to Waite where the esky was put into a 4ºC walk-in cool room overnight. The following day the green leaf material was weighed, washed in potable cold tap water, trimmed (leaf was removed from larger cauliflower or cabbage stalks) by hand or with a knife, and either sorted by type: cauliflower, cabbage (green), cabbage (red), lettuce, bok choy; or combined, and weighed again to get a measure of leaf yield (Figure 2).

A B C

Figure 2. Pre-processing stages of the Woolworths green leaf material.

A) The sizeable cauliflower stalks and stems that could not be used in this trial as they were too large to chop, B) washed cauliflower leaves removed from the stems, and C) washed lettuce leaves prior to shredding and juicing.

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Perfection Fresh: Freshly chopped (5-7cm long) chunks of sprouted broccoli stems were delivered by Perfection Fresh staff to the Waite campus, packed with crushed ice into plastic bags inside cardboard boxes (each 2kg), where they were immediately put in the walk-in cool room (Figure 3). The following day the chopped stems were rinsed in cold potable tap water and then processed for extraction.

Figure 3. The fresh sprouted broccoli stem material as delivered by Perfection Fresh.

2.2 Preparing the material for extraction

Each type of Woolworth green leaf was coarsely chopped using a 4L Robot Coupe Blixer, the coarsely chopped leaves were then passed through a juicer (Breville juice fountain) with the resulting green juice mixed with extraction buffer and retained for further protein extraction (Figure 4). The pulpy solids ejected from the juicer were retained as the 'fibre' fraction.

A B

Figure 4. Processing of the Woolworths green leaf materials.

A) the shredded cauliflower leaf material after being chopped in the Robot Coupe Blixer, and B) leaf juice in extraction buffer from red and green cabbage leaves.

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The rinsed sprouted broccoli stems were loaded into a Robot Coupe CL50 Veg Prep Ultra machine with a 2mm slicing disc. The sliced stems were reloaded into the machine and sliced with the same blade another one or two times; on some occasions the 3rd (final) slicing blade was a 1mm disc. This gave the stem material a relatively uniform particle size (Figure 5A). The finely chopped sprouted broccoli stem was used fresh, frozen into 1L foil takeaway containers for later use ((Figure 5B) after thawing or put onto a stainless-steel tray to be dried in a food dehydrator (see below).

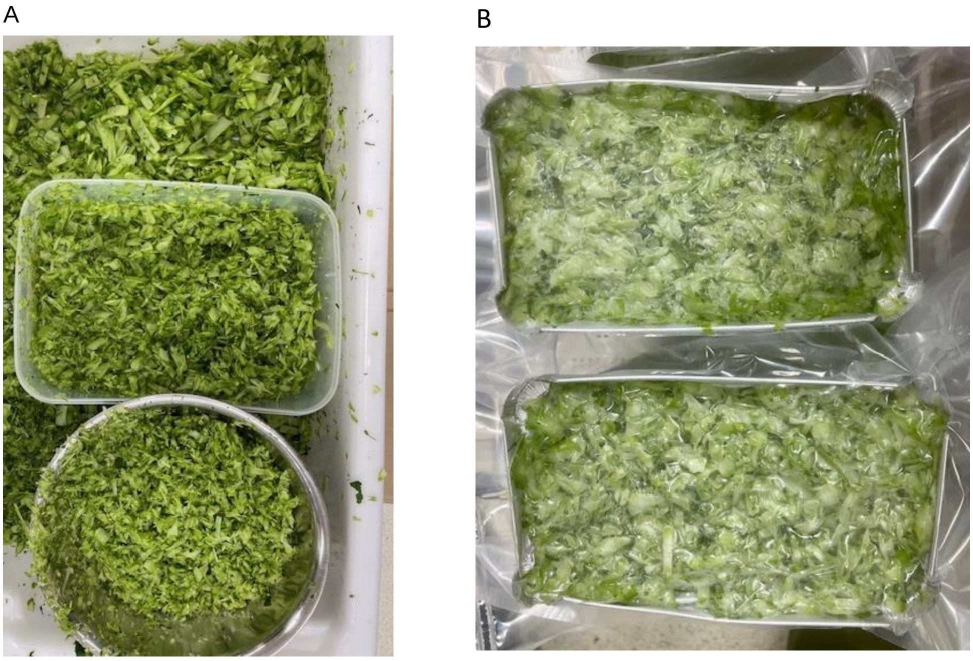

Figure 5. Wet sprouted broccoli stem materials after processing.

A) showing the effects of (top) single, (middle) double and (bottom) triple passes through a Robot Coupe CL50 Veg Prep Ultra machine fitted with a 2mm disc slicer, and B) triple-sliced sprouted broccoli stem vacuum packed into foil trays ready for blast freezing and frozen storage.

2.3 Protein isolation from the leaf material

The precise details of the extraction methodology are commercial in confidence information of The Leaf Protein Co. but the basic process was an initial extraction step in an alkali solution, after which the solids were removed and the remaining liquid was then acidified to precipitate the protein. The composition of the extraction buffer, ratio of biomass/juice to extraction buffer, temperatures, pHs and mixing durations were based on published protocols developed to extract RuBisCo protein from leaf material (recently reviewed by Narwaz et al., 2024) but the non-confidential steps are described below.

The greenleaf juice and extraction buffer solution was made alkaline (caustic soda NaOH food grade, Orku QLD) and mixed for a period of time (Ohaus overhead mixer) to solubilise the proteins. The extracted material was then filtered through a layer of muslin cloth supported on a large mesh sieve. When the liquid was no longer free-running through the mesh the cloth was 'balled up' around the fibre and squeezed to release the available liquid. The fibre ball was put on a stainless-steel tray and broken up before going into the dehydrator (Figure 6). The liquid was put into 750mL containers and centrifuged (Thermo Scientific Megafuge ST Plus) at 4,000rpm for 10mins to spin down the remaining starch and fine fibre particles. The supernatant (containing the solubilized protein) was decanted into a clean 10L plastic container, and the relatively small amount of starch/fine fibre pellet material was discarded.

A B C

Figure 6. The fibre-rich material remaining after the alkali extraction step.

A) the wet fibre in the muslin cloth filter, B) the semi-dry fibre material after it was rolled into a ball in the cloth and squeezed by hand, and C) the semi-dry fibre fraction spread out on a tray ready to go into the dehydrator.

After the solubilized protein extract had been acidified and gently mixed with concentrated hydrochloric acid (Scharlau, Chem-Supply SA) the container was put onto a mid-level shelf in the walk-in cool room overnight for the precipitating protein to settle (Figure 7). The following day a plastic siphon tube was carefully inserted into the container and the bulk of the clear supernatant was removed to a container placed on the floor. The precipitated material was resuspended in the remaining supernatant and allocated to 750mL centrifuge containers that were then spun at 4,000rpm for 10 mins. The clear supernatant was decanted off and the dense creamy precipitated material was spooned out and consolidated. The consolidated precipitate was blast frozen and freeze dried (VirTis AdVantage Pro; SP Scientific) for 5 days (Figure 7C). Several successful attempts were made to spray dry (Buchi, mini spray drier B-290) about 0.7L of alfalfa protein (heat precipitated) solution (Figure 8).

A

B

Figure 7. A red and green cabbage juice blend after protein precipitation was induced by heat and/or acidification (IEP).

A) shows the samples shortly after the protein precipitation step, B) shows the same samples in the fridge at 18 hours later. The protein is mostly green but there is also smaller amounts of white RuBisCo protein. The clear red supernatant was removed by siphoning and the precipitated material was then centrifuged to further concentrate the protein.

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A

B

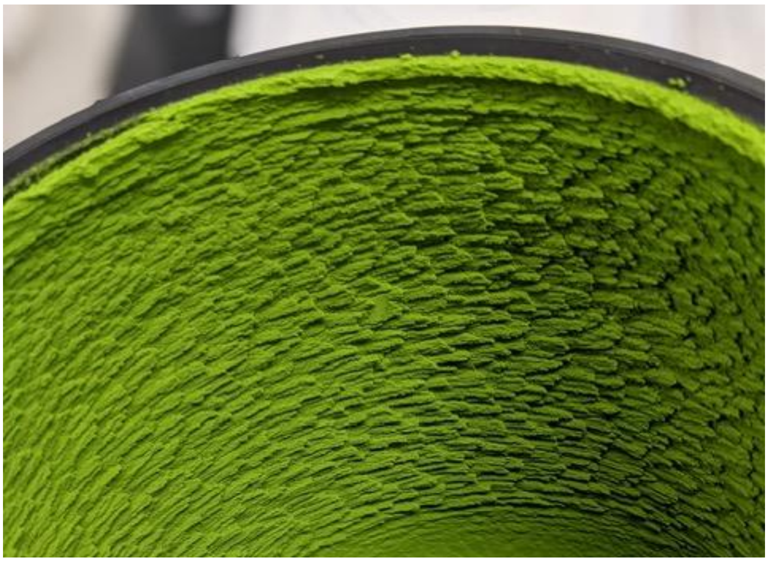

Figure 8. Freeze and spray drying of the protein isolates.

A) blast frozen concentrated precipitates from (left) lettuce and (right) cabbage; B) alfalfa protein after spray drying.

2.4 Drying of fibre

The 'fibre' fractions from the Woolworths green leaf material, and the finely chopped sprouted broccoli stem material was put onto stainless steel trays and loaded into a food dehydrator (Benchfoods 16CUDG). During drying the uppermost surface of the material tended to form a brown 'crust' and a slight toasted flavour if the temperature was too high, so a temperature of 40ºC was used to minimise this but it meant that the material needed >72hr drying time and regular declumping and redistribution on the trays each

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day to dry fully. Fully dry material was put into a blender (Ninja 1000W) for about 30sec to reduce the particle size to a powder. Finer powders were produced after passing the material through a stone mill (Mockmill 200)(Figure 9). Particles of the resulting material were separated into coarse and fine categories by passing through a 360µm mesh sieve (Resch).

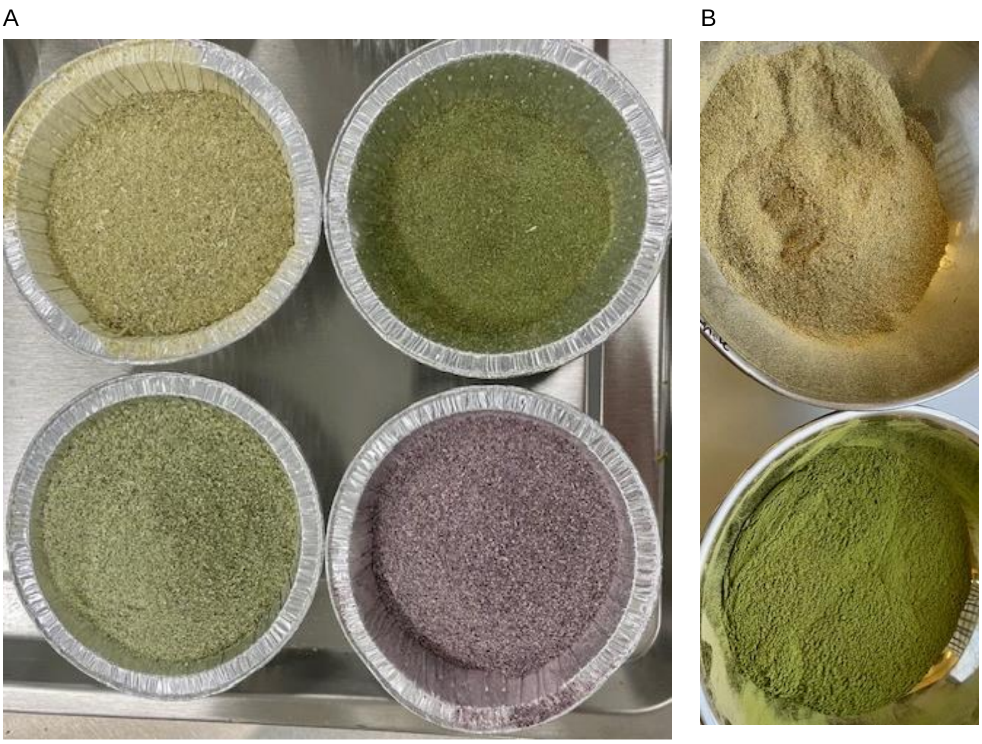

Figure 9. Dehydrated and powdered fibre-rich fractions.

A) Dried and blended (top left) lettuce, (top right) green cabbage, (bottom left) cauliflower, and (bottom right) red cabbage after blending, and B) lettuce and cabbage dried fibre fractions after stone milling to reduce particle size.

2.5 Nutritional, Protein and Fibre functionality tests

2.5.1 Nutritional analysis

A 200g batch of each of the fibre products were sent to an external laboratory (ALS global, Scoresby) for nutritional information panel analysis giving results on energy (kJ), moisture (%), protein (%), fat (and subtypes of fat – saturated, trans etc %), carbohydrates (%), sugars (%), total dietary fibre (%), ash (%) and sodium (mg/100g).

The efficacy of protein extractions was assessed by determining protein levels of precipitates and fractions using an Elementar nitrogen analyser, with aspartic acid as the standard and using a protein factor of N*6.25.

The total, soluble and insoluble fibre content of the dried fibre fractions produced during the project was measured using an ANKOM fibre analyser. Some technical difficulties in adjusting the values to account for ash weight (ashing furnace) and sample

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protein content meant that the results produced by the SARDI laboratory could only be considered approximate. Fibre levels were accurately characterised in the external nutritional panel analysis (see above).

2.5.2 Techno-functional properties

Some techno-functional properties of the dried fibre fractions (finely milled) were compared with two commercially available pectin samples (citrus and apple). The attributes measured were water holding capacity, least gelation concentration, and rapid viscoanalyser response.

2.5.2.1 *Water holding capacity*

The 0.5g sample of powder was mixed with 4.5g of distilled water for 30 min with intermittent vortexing at 5 min intervals and then centrifuged at 10,000 rpm for 15 min. Excess water on top of the pellet was drained by inverting the centrifuge tube for 10 min over a paper towel. The tube and wet pellet were reweighed, and the water retention capacity of the sample was then calculated.

2.5.2.2 Least gelation concentration

Samples of each fibre were mixed with distilled water to give dilutions of 4, 6, 8, 10, 12, 14, 16, 18 and 20% in a total volume of 10mL. They were vortex and allowed to absorb water for 1 hour. Duplicate 5mL samples were then pipetted into glass test tubes and put into a boiling water bath for 20 minutes. After cooling, the tubes were inverted and the response of the content recorded as liquid or gelled.

2.5.2.3 *Rapid viscoanalysis*

The rheological properties of each type of powder were determined with a Rapid Visco Analyser (Perten RVA 4800) using the Extrusion 1 protocol. The mix for the run was 5.0g of powder and 27.0g of RO water. Dry powder and water were hand mixed with the paddle as instructed for 10-15 min before the analysis. The RVA protocol was: 500rpm for 20 sec then constant 160rpm for the rest of the run (20 min). The temperature was 25ºC for 2 mins then ramping up to 95ºC at 10 mins then cooling to 25ºC by 16 mins and holding at 25ºC until 20 mins. Parameters recorded were pasting temperature, final viscosity and setback value.

2.6 Recipe development

An experienced food scientist used the dry and/or wet fibre factions produced from the extraction process to make a range of prototype food and drink products including:

- Crackers
- Bread
- Flatbread
- Pasta sauce
- Pasta noodles
- Gnocchi
- Beefburger patties
- Meatballs
- Juice
- Smoothie

The process of developing the prototype recipes will not be described in detail, but essentially the fibre ingredients were incorporated into basic recipes for each of the products by partial substitution of one or more other ingredients to maximise the inclusion of the green leaf ingredient but without negatively impacting on the appearance, functionality, taste and mouthfeel of the product (e.g. the cracker still had to have a brittle snap, the pasta needed to hold together during cooking and serving). Sensory feedback on the different recipe variations was sought from in-house tasters to improve and fine-tune the final recipe.

When prototype recipes were suitably well developed some of the products were tested on external parties, including Fight Food Waste CRC staff at the Waite campus, and offered at events such as EvokeAg 2022 in Adelaide and the Fight Food Waste CRC research conference in Sydney in 2023.

3. Results

This final report will not detail the results of every batch of extractions (20 from Woolworths and 10 from Perfection Fresh materials) as there was an evolving process of methodological improvements and efficiencies as the project progressed. Rather, the focus will be to describe the findings from the mature or optimised research achievements.

3.1 Yield of processing material

Each week the Woolworths store at Marraytville supplied between 12 and 16kg of green leaf material. The material was extremely fresh (nothing was wilted, or soft), very 'clean' (no contamination with non-leaf material) and mostly free of dried soil etc indicating that the internal Woolworths communication and training processes clearly worked extremely well for this trial. Most of the material provided was cauliflower stems and green cabbage outer leaves, with smaller amounts of red cabbage, lettuce and bok choy. After rinsing, sorting and trimming (particularly the heavy woody stalks of cauliflower) the actual yield of green leaves was 6 to 7kg, so approximately 45-50% of the material supplied.

The sprouted broccoli stem material delivered by Perfection Fresh was also in extremely fresh condition and was 98% stem material with just the occasional leaf attached. This was somewhat unexpected as the pre-project batch of sprouted broccoli 'waste' that was delivered to The Leaf Protein Co's collaborators at CSIRO had a much leafier composition. On raising this with the Perfection Fresh team it was decided to change the focus of this part of the project to work with the sprouted broccoli stems as they are heavier than the leaves and so contribute more to food 'waste'. All (100%) of the sprouted broccoli stem material that was delivered could be used by the researchers.

3.2 Yield and 'purity' of protein extractions

All of the biomass provided for this study was fresh, so the moisture content of the material was 85% (sprouted broccoli stem) to 94% (lettuce), so it is not surprising that levels and yields of protein on a wet weight basis are low (2-6%), but when expressed on a dry weight basis they are higher (27-51%; Table 1).

Table 1. The moisture and protein content of each of the green leaf materials.

Material	Moisture % (wet weight)	Protein % (wet weight)	Protein % (dry weight)
Cabbage (red and green)	88	4.5	37.5
Cauliflower	89	5.6	50.9
Lettuce and Bok choy	94	2.3	38.3
Sprouted broccoli stem	85	4.1	27.3

After the protein extraction process the protein level of the different products were as shown in Table 2.

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Table 2. Protein concentrations of green leaf products.

Material	Yield of Dry Protein fraction % (wet weight)	Protein level in Protein fraction % (dry weight)	Yield of Dry Fibre fraction % (wet weight)	Protein % in Fibre fraction (dry weight)
Cabbage (red and green)	1.5%	60%	25%	5% (red) 12% (green)
Cauliflower	2%	65%	20%	18%
Lettuce and Bok choy	1.5%	70%	15%	13%
Sprouted broccoli stem	0.5%	60%	30%	19%
Alfalfa fibre	-	-	-	21%

3.3 Functionality

- Proteins

Protein techno-functionality was not looked at in detail because the focus of the project moved towards the characterisation of the fibre-rich fractions. However, there were some obvious differences in the foaming capacity and stability of the different green leaf proteins that came from the Woolworths material (Figure 10).

A B

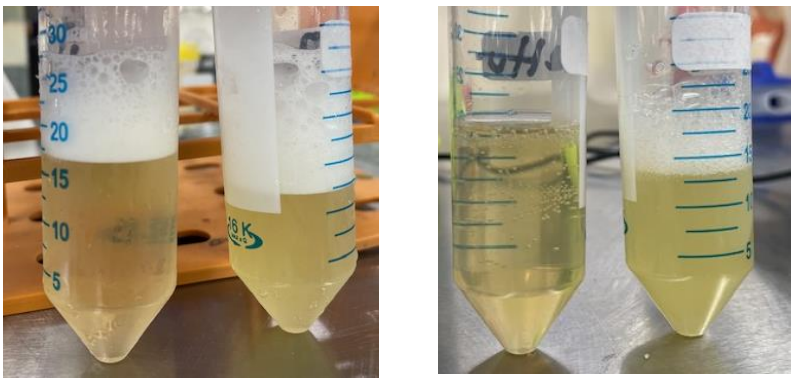

Figure 10. Foaming capacity and stability in green leaf protein isolate solutions.

A) freshly prepared solubilised protein from (left tube) sprouted broccoli stem and (right tube) lettuce leaf that were shaken to induce foaming, and B) the same tubes 16 hours later showing greater foam stability in the lettuce protein solution.

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- Fibre-rich fractions

Water holding capacity of the sprouted broccoli stem fibre and two commercial sources of pectin are shown in Table 3. The sprouted broccoli stem fibre absorbed 840% of its dry weight of water which was almost twice as much as the two pectins which were similar to each other.

Table 3. Water holding capacity of sprouted broccoli stem fibre and two commercial pectins.

	Sample (g)	Water (g)	Final pellet (g)	Water absorbed (g)	Water Holding Capacity %
Sprouted broccoli stem fibre	0.49	5.20	4.63	4.14	840
Pectin (citrus)	0.52	4.53	2.72	2.20	426
Pectin (apple)	0.52	4.54	2.89	2.38	459

Least gelation concentration of the sprouted broccoli stem fibre and two commercial sources of pectin are shown in Table 4. The sprouted broccoli stem fibre gelled at a dilution of 6% whereas the pectins from apples gelled at 12% and citrus at 14%.

Table 4. Least gelation concentrations of sprouted broccoli stem fibre and two commercial pectins.

	4%	6%	8%	10%	12%	14%	16%	18%	20%
Sprouted broccoli stem fibre	X	0	0	*	*	*	*	*	*
Pectin (citrus)	X	X	X	X	X	0	*	*	*
Pectin (apple)	X	X	X	X	0	0	*	*	*

X indicates no gelling
0 indicates gelling
* indicates sample had absorbed all of the water so not allowing the sample to be pipetted into the tube for boiling.

Rapid viscoanalysis results from the sprouted broccoli stem fibre and two commercial sources of pectin are shown in Table 5. The sprouted broccoli stem was much more viscous than the pectins and showed more pronounced responses to heating and cooling (Figure 11). To better understand what the RVA graphs show the x axis is time, the y axis shows the viscosity of the solution in cP (centipoise units), the red line shows the temperature changes in the mixing vessel and green is the mixing speed of the paddle that keeps the mixture in suspension but also measures the viscosity of that mixture. The blue line shows the viscosity of the suspension in the mixing vessel and the program identifies and labels several different stages of the typical process of hydration, gelation, retrogradation of the components (starch, protein) in the sample.

Table 5. Rapid viscoanalysis results of sprouted broccoli stem fibre and two commercial pectins.

	Peak 1	Trough 1	Break down	Final Viscosity	Setback	Peak Time (min)	Pasting Temperature
Sprouted broccoli stem fibre	3603	2202	1400	5016	2814	1.82	50.1°C
Pectin (citrus)	104	97	7	217	120	4.27	-
Pectin (apple)	199	175	24	574	399	7	-

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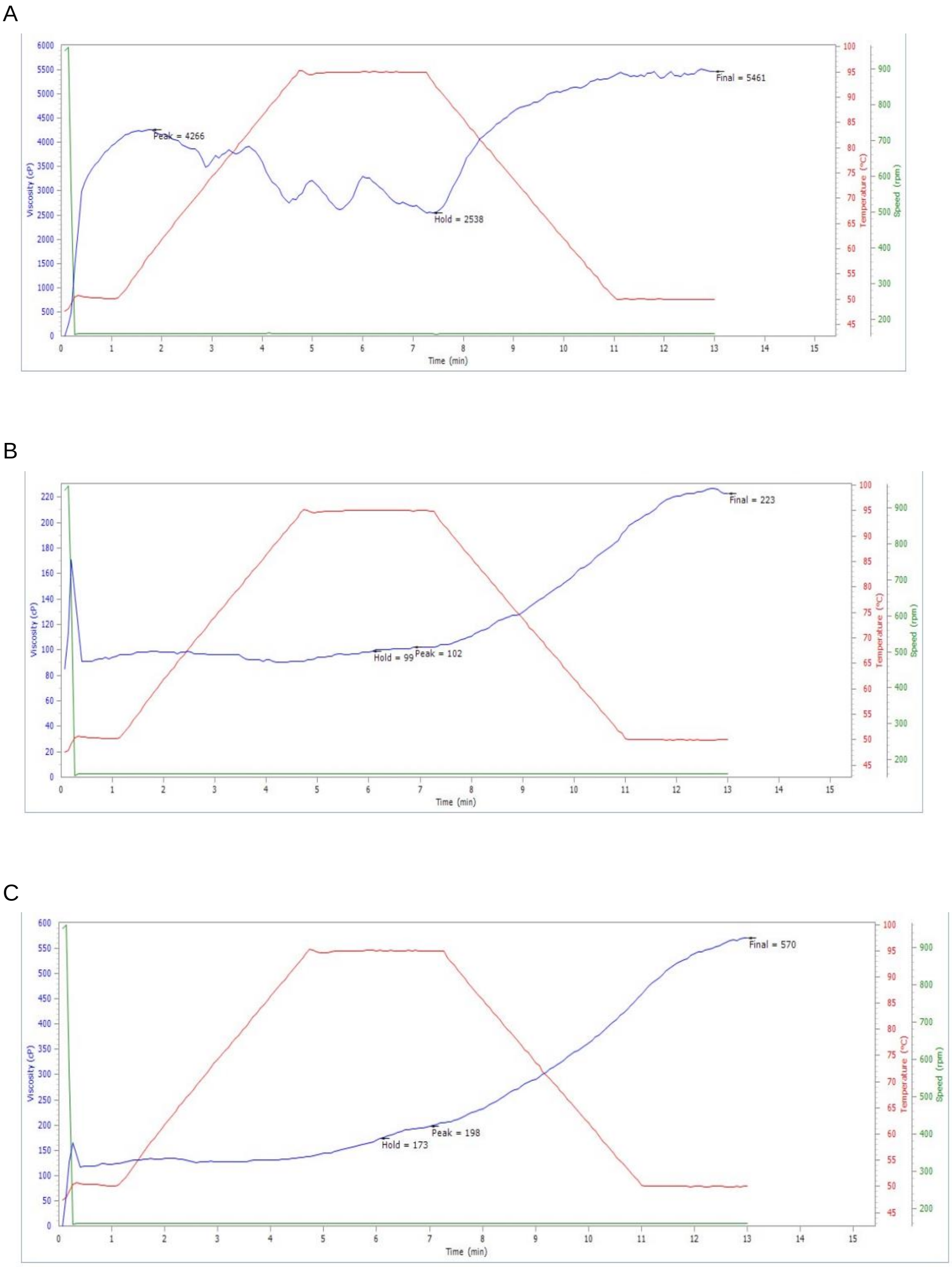

Figure 11. Rapid viscoanalyser results of typical runs of fibre-rich fractions.

A) sprouted broccoli stem fibre, B) pectin from citrus and C) pectin from apple.

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3.4 Nutritional Information Panels (NIPs)

The nutritional analysis showed generally similar levels of energy, protein, fat and fibre in the dried sprouted broccoli stem and fibre-rich fraction of The Leaf Protein Co. alfalfa process (Table 6). The main biochemical difference between the materials was the much higher level of sugars in the broccoli stem compared to the alfalfa.

Table 6. External nutritional information panel analysis of the two main fibre-rich fractions from this project.

	Dried Sprouted Broccoli Stem	Extracted and Dried Alfalfa Fibre
Energy kJ/100g	1104	1182
Protein %	19.4	20.1
Carbohydrate %	24.2	29.3
Total sugars %	18.3	1.8
Total Fat %	2.0	1.7
Total Dietary Fibre	36.1	34.9
Ash %	10.7	6.2
Moisture %	7.6	7.8
Sodium mg/100g	260	60

3.5 Use of fibre-rich fractions in prototype food and drink products

In general, the fibre fractions (wet or dried and powdered) could easily be incorporated into most product formats at levels that gave positive sensory attributes without impacting of the functionality of the product. The recipes for some of these products are provided below as guides for future work. A summary of how much of the different sources (green leaf or sprouted broccoli stem) in the dry and/or wet format could be successfully added to each of the product types is shown in Table 7, and images of the products in Figures 12-14.

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Table 7. Maximum percentages of fibre-rich ingredients in prototype food recipes which gave a satisfactory functional, taste and mouthfeel outcome.

Format	Cabbage and Cauliflower blend	Sprouted broccoli stem
Crackers	25% (dry; with-gluten and gluten-free recipes)	25-33% (dry; depending on recipe)
Bread	5% (dry)	7% (dry)
Flatbread	15% (dry)	15% (dry)
Pasta (linguini)	-	5% (dry)
Pasta sauce	-	20% (wet)
Beef pattie	-	30% (wet) 1mm sliced
Meatballs	-	40% (wet) 2mm sliced

Veggie Crackers (25% Sprouted broccoli stems)	
Ingredient	**Amount (g)**
Sprouted broccoli stem powder (dry)	25
Almond flour	20
Purple sweet potato	42
Soaked chia seeds	10
Salt	3

Put dough through a pasta roller until it is 2mm thick. Cooking time 150ºC x 23min

Tzatziki type dip (24% Sprouted broccoli stems)

Ingredient	Amount (g)
Greek yogurt	125
Sliced sprouted broccoli stems (wet)	44.26
Lemon juice	7.81
Garlic	1.93
Salt	1.42
Pepper	0.18
Dill	0.29
Olive oil	2.50

Note – Sprouted broccoli stems were boiled for 10 minutes.

Bread (7% dried green leaf material in a 700g loaf)

Ingredient	Amount (g)
Laucke crusty white loaf pre-mix	390
Yeast	7
Green leaf or Sprouted broccoli stem powder (dry)	50
Water	350

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Pasta (6.7% Sprouted broccoli stems (dry))

Ingredient	Amount (g)
Semolina (durum)	250
Salt	1.25
Sprouted broccoli stem powder (dry)	25
Water	87.5
Olive Oil	6.8 mL

Creamy Pasta Sauce (48% Sprouted broccoli stems (wet))

Ingredient	Amount (g)
Finely sliced sprouted broccoli stems (wet)	150
Cream cheese	75
Water	80
Salt	2.70
Pepper	0.9

Note – Sprouted broccoli stems require boiling for up to 10 minutes or until tender.

Beefburger patties (30% of initial quantity of meat is sprouted broccoli stem (wet))

Ingredients	30%
Beef	159g
Sprouted broccoli stems (wet) (3 x 2mm finely sliced)	68g
Salt	1.5g
Black pepper	0.2g
Onion Powder	0.7g
Garlic Powder	0.7g
Smoked paprika	1.0g
Dried Thyme	0.25g
Dried Sage	0.1g
Eggs	10g

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Meatballs (40% of initial quantity of meat is sprouted broccoli stem (wet))

Ingredients	40%
Beef Mince	75g
Pork Mince	75g
Sprouted broccoli stems (wet) (2 x 2mm, 1 x 1mm finely sliced)	100g
Italian Seasoning	1.25g
Salt	2.5g
Black pepper	0.2g
Egg	25g
Parmesan Cheese	25g
GF Breadcrumbs	38g
Parsley	2g
Milk	30g
Olive Oil	5g
Onion	15g
Garlic	3g

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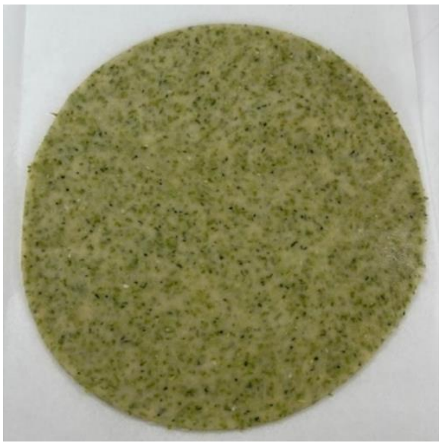
Thinly rolled cracker dough with sprouted broccoli powder (dry)

Baked crackers with (left) cabbage and (right) sprouted broccoli powder (both dry)



Flatbread make with sprouted broccoli powder (left) before and (right) after pan cooking

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White bread containing 10% cabbage and cauliflower fibre fraction (dry) showing normal crumb structure and acceptable colour

Linguini style fresh pasta (cooked) made with 6.7% sprouted broccoli stem finely milled powder (dry)

Figure 12. Some of the wheat-based products containing green leaf fibre-rich fractions developed in this project.

Figure 13. Before and after photographs of the meatballs made with the wet sprouted broccoli stem prepared during this project.

It should be noted that not all of the recipes developed with the fibre-rich ingredients in this project were successful. Despite sprouted broccoli stem juice being acceptable at between 10-15% of a mixture with apple and orange juice it was too fibrous even

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at 5% of the mixture when used in a blended smoothie. Further, dried sprouted broccoli stem fibre at 10% in gnocchi was not acceptable to tasters as it made the texture of the pasta tough and chewy. Alfalfa extract powders were not acceptable to the test panel in any food or drink format due to the strong grassy taste.

3.5.1.1 Product sensory testing

The dried and powdered fibre fractions of cabbage/cauliflower and sprouted broccoli stems had generally favourable sensory acceptability. Descriptors used included: mild, herby, leafy. Most people were surprised to find out the source was cruciferous vegetables because they did not discern any typical cooked cabbage flavour. In most product formats the green leaf fibre material was the only flavouring ingredient (apart from salt and pepper) so it was not disguised by other flavours. In the situations where the sprouted broccoli stem was incorporated into a product with other flavoured ingredients (e.g. the beefburger pattie) and/or served with a flavoured sauce (e.g. the meatballs had a passata-style tomato sauce) the flavour of the sprouted broccoli stem complemented the product as if it was a mixture of dried herbs (e.g. oregano, thyme, basil, parsley).

Samples of cracker products trialled at events such as evokeAg and the CRC conference were very well received with many people indicating that they enjoyed the subtle and interesting flavours and would buy them if they were commercially available.

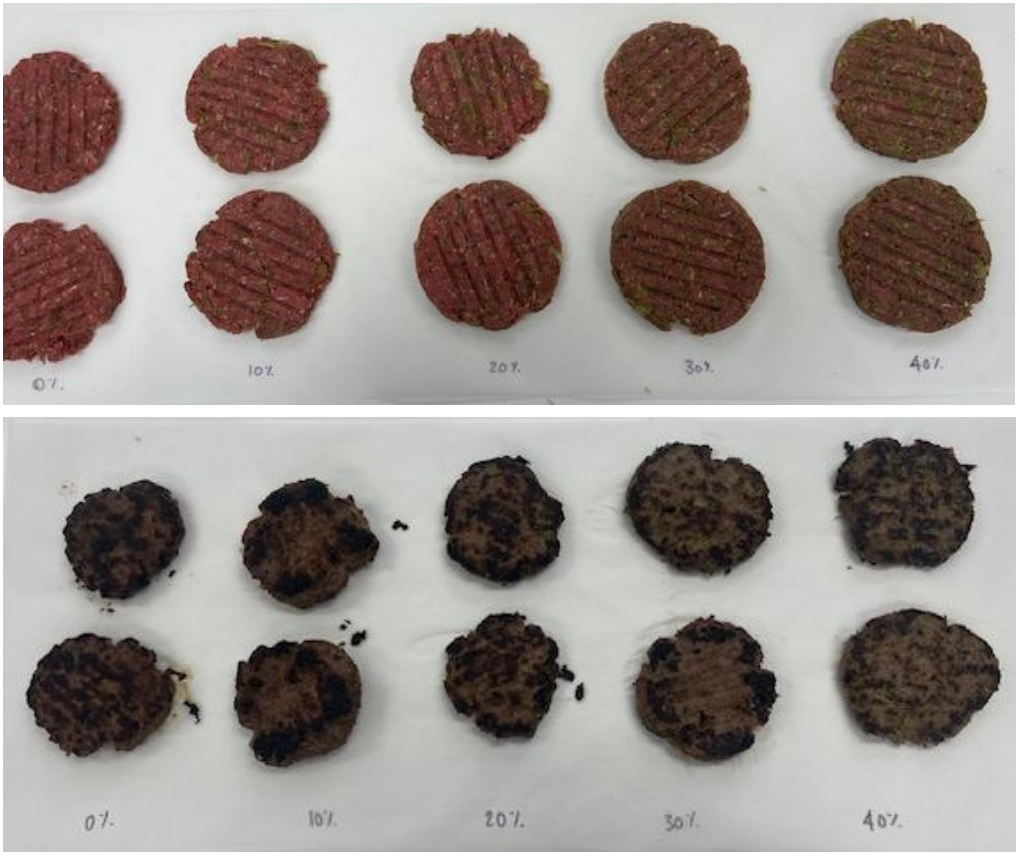

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Figure 14. Before and after photographs of the beefburger patties that were made with the wet sprouted broccoli stem prepared during this project.

4. Discussion

The original focus for this project was to determine whether green leaf material collected from Woolworths stores and sprouted broccoli processing 'waste' could be processed to yield high quality functional protein using the proprietary methodology of The Leaf Protein Co. Positive outcomes regarding this aim was that the project proved that high quality leaf protein could be extracted from the Woolworths green leaf material. Unfortunately, with about 50% extraction efficiency the overall yield of protein from such leafy biomass, using the methods described, was too low to be financially viable. Furthermore, the sprouted broccoli stem material supplied by the processor had very low initial levels of protein and wasn't the target leafy biomass material for the extraction process developed by The Leaf Protein Co. Further, it was considered that the practicalities and costs of creating chilled storage areas for daily accumulation of green leaves behind the scenes at supermarkets, the logistics and costs of operating a refrigerated vehicle to make daily green leaf pickups at all stores within a metropolitan area, the fact that the sprouted broccoli stem would need to be bought from the processor, packed and transported to a Leaf Protein Co extraction facility, and finally, finding a suitable site close to each metropolitan area where The Leaf Protein Co could build a facility to process the input biomass into the protein and fibre fractions were significant hurdles to the commercialisation of this approach. The other matter that became apparent very quickly during the project was that the protein extraction process only valorises a very small percentage of the green leaf biomass – in excess of 90% of the material was the 'fibre-rich' fraction that was not valorised by the extraction process. For these reasons the majority of the research effort in the project aimed towards determining whether the fibre-rich material remaining after protein extraction could be utilised in food (or other high value) applications, and if this were possible whether the benefits of having two product streams (protein and fibre fractions) that utilised 100% of the input material would help to address the logistical and practical hurdles outlined above.

In this project the green leaf material collected from the supermarket store had sizeable woody stalks of cauliflower in the biomass. This needed to be excluded from the process used by the SARDI researchers because the cutting and blending equipment available was not sufficiently robust enough to accommodate it and that was why the actual useable biomass was only 50%. However, if a commercial facility were built the installed equipment would almost certainly be robust enough so the biomass yield would be close to 100%, but about 50% would have a very woody fibrous composition and so it might need to be diverted to a third processing stream to provide an input to make something like compostable packaging material, animal feed or compost.

The protein extracts from the cabbage, cauliflower and lettuce leaves were of reasonably good quality but further refinement of the centrifugation protocol (rpm and time) to reduce the amount of starch and fine fibre in the extract before the acidification step would undoubtedly increase the protein level in the final powder. The protein-rich solutions (after centrifugation but before acidification) clearly had interesting differences in foaming capacity and foam stability. For example, the foaming capacity of lettuce protein extract was two-fold higher than the cabbage or cauliflower (which were similar to each other), and the foam on the lettuce extract was still present after 18 hours. This suggests there might be some opportunity to make protein extracts that have different techno-functional characteristics, but that would involve the tedious task of having to separate the different types of green leaves from the collected co-mingled biomass. It is easy to see how such a process could be automated using image analysis technology integrated into robotic 'pickers' on the input line.

Following the pivot from leaf protein as the main focus to the "fibre-rich" or "fibre fraction" of the green leaf material, the first issue was what to call it. The nutritional analyses (initial internal protein and fibre determination carried out by SARDI, and the subsequent comprehensive external accredited food laboratory testing) clearly show that the material was not just fibre – indeed total dietary fibre was 'only' 35-40% of the material, with 20% protein, ~30% carbohydrates (with most of this being sugars), only 2-

4% fat and ash and moisture. Given this, the most accurate description of the material is that it is the ‘fibre-rich’ fraction. From a nutritional perspective, the high fibre, moderately high protein and low-fat levels would be viewed positively. The relatively high level of sugar could be perceived negatively, but natural sugars in a complex matrix would be slowly digested and would still provide some taste of sweetness reducing the need for additional (refined) sugar to be added to the product. There would be no nutritional reason not to consider including it as an ingredient in a healthy food product.

One key question relating to whether the fibre fractions could be valorised as food ingredients is what do they taste like? The answer, fortunately, is that the dried and shelf-stable fibre fraction of the green leaf material collected from the supermarket store has a very acceptable mild, herby flavour that works well both as the only favour in a bland product (e.g. a wheat-based cracker), or as a complementing flavour in a more complex food (e.g. a meatball). In this regard it could substitute for some more traditional and familiar dried herb products. Similarly, the sprouted broccoli stem material in both the wet and dry format had a very palatable flavour, and certainly milder than most people would have anticipated knowing that the source was broccoli. The intensity of the grassy flavour in the alfalfa material was too strong for the tasting panels and this would limit its use in food products without flavour masking ingredients.

Another question that was posed was whether the fibre-rich fraction could contribute to allow a health or content claim to be made on a food product. The most obvious property of the fraction that could fulfill a FSANZ food standards health or content claim would be the 35% dietary fibre content. The various levels of FSANZ standard claim for dietary fibre are that if a serving of food has at least 2 g of dietary fibre then it can be labelled “contains dietary fibre”. This would be achieved by having 5.7g of dry sprouted broccoli stem in the serving size (assuming there is no other source of fibre in the recipe). If there is >4g of dietary fibre per serve (equivalent to 11.4g of dry sprouted broccoli stem) this would qualify the product to be labelled “a good source of fibre”. The “excellent source” claim would require >7g of fibre per serve and this would be 20g of dried sprouted broccoli stem per serve (given that most foods have a serving size of 50-120g this would be improbable). The only health claim permitted for fibre is that it “contributes to regular laxation” which, whilst very important, is not particularly marketable to most consumers. However, the current global mega-trend for improving intestinal health (and thereby indirectly contribute to better overall health) via foods that are pre-biotic (e.g. fermented products, have soluble fibre and/or resistant starch) that support a better gut microbiome and the promotion of probiotic foods and supplements is growing and is likely to become more visible in Australia (Farias et al., (2019). This would mean an increasing opportunity for fibre-rich ingredients to be used in manufactured foods to support such claims and associations. Clean label (i.e. not using highly purified and modified ingredients that have an ”E” number) fibre-rich ingredients such as these green leaf fractions could easily find a role in meeting that market.

Regarding marketing, one angle that food and supplement manufacturers look to promote is whether the product contains any ‘superfood’ ingredients e.g. acai, blueberries, kale etc. The concept of ‘superfoods’ is very polarising because most nutritionists would look at the nature of the evidence supporting such claims and consider if it is independent, peer-reviewed, published, placebo-controlled; whether the work was done in a test tube, in cell lines, in animal models or in people, how many people were involved and for what duration, and if it were an actual trial or a correlational study based on self-reported food consumption. Very few food trials fulfill all of these exacting requirements, so most nutritionists are sceptical about ingredients being labelled as ‘superfoods’ and the term is not recognised by food regulatory bodies such as FSANZ. On the other hand, media and marketing executives are not so exacting and so the descriptor ‘superfood’ is bandied about regularly, aided by the fact that the term ‘superfood’ is not specifically defined, so it can mean anything to anyone (Guillén and Yanes, 2022; Fernández-Ríos et al., 2023)

There are published papers on the nutritional benefits of cruciferous leafy greens and the compounds found in them (e.g. Syed et al., 2023). Certainly, leafy greens are widely considered as a healthy and nutritious food when consumed as part of a mixed and varied diet. However, most of the beneficial micronutrients found in cruciferous vegetables are present at similar concentrations in other vegetables and fruits, so there is no “unique” or “highest nutritional source of” proposition that marketers would be looking for.

Importantly, it is accepted that there are some compounds in the green leaves of these plants that have specific and important roles in certain biological structures and functions (e.g. lutein and zeaxanthin are especially important in the macula of the eye, protecting it from irreversible macular degeneration; Mrowicka et al., 2022). However, it remains to be determined whether such compounds persist during the temperature and maceration stages of an ingredient and/or food manufacturing process. It is also highly debatable whether at intake levels higher than what is recommended for each compound, such as is commonly suggested by marketers, there is any additional benefit of a higher intake, and recognising that, in some cases, 'more of a good thing' actually becomes harmful (e.g. iron; Lönnerdal 2017).

Many popular and heavily promoted health foods such as "green powders" contain blends of many different ingredients including broccoli, kale, spinach and alfalfa powders. It is easy to see that sprouted broccoli stem, cabbage and cauliflower leaf powders could be substituted for those other ingredients if the ingredient manufacturing and supply chains were set up. However, the new ingredients would probably have to offer price, flavour, sourcing (e.g. product of Australia), process efficiency and/or nutritional benefits over and above those of the existing inputs and that might not always be possible (e.g. organic certification). There's also the issue that sprouted broccoli stems and cauliflower leaves are not normally considered to be the most 'edible' part of the vegetable, so this may be a small barrier to some customers consuming something different than what they are used to.

One interesting property of the dried fibre fractions produced during this project was their very high water holding capacity. This was especially noted when the dried powders were introduced into basic recipes for crackers and breads and the powder competed with the wheat or other flours for access to the water, such that those recipes required an increase in water volumes to make them function (e.g. make a dough). This property to soak up and hold on to water is potentially useful as a cheaper and/or clean-label (not an E number) humectant in food manufacturing, where compounds such as inulin, pectin, microcrystalline cellulose (E460) and carboxymethylcellulose (E466) are used for this (and other) functions. In a comparison of techno-functional water holding and binding characteristics, the sprouted broccoli stem performed better (at the same concentration) than the pectin and inulin, but not as well as the chemically modified wood-pulp derived compounds. The only negative aspect of this was that the sprouted broccoli stem powder is green whereas the other compounds are white or colourless in solution. Further work may help to address this issue/opportunity.

The fundamental question that all enterprises have to address is whether the costs of procuring, transporting, processing, packaging, distributing and marketing the product are more or less than what customers are prepared to pay for it. From the other perspective it needs to be considered why a potential customer would want to buy a particular product instead of a different product or getting it from a different manufacturer (if one exists). The decision to purchase is based on many factors but key aspects are unique properties of an ingredient that deliver manufacturing efficiency to the customer or improve the quality and/or marketability of the product to the customer and some of these factors (e.g. clean label) have been discussed above. But, in a time of economic pressure, reducing the cost of ingredients used to make products is critical to them finding customers. So, how would sprouted broccoli stem or cauliflower/cabbage green leaf ingredients be priced in the marketplace? There would be a substantial cost to creating (buying/leasing a site, installing the necessary equipment) and operating (energy, water, labour) a facility (or multiple facilities, one in each metropolitan area) that could process fresh green leaf material into protein and/or dried fibre-rich fractions. Confidential calculations performed with the industry partners as part of this project have suggested the cost of shredding, drying and powdering sprouted broccoli stems would be broadly similar to the price ingredient suppliers pay for established products with some of the techno-functional attributes of the fibre-rich fraction. Thus, it seems possible that this could be a viable value-adding opportunity.

The costs of manufacturing wet outputs (chilled or frozen fibre-rich fractions) from the green leaf materials would be reduced since there is no need to install and operate drying equipment, but the lower cost comes with the disadvantage that the chilled product has a short shelf-life so it would need to be produced close to large and consistent customers that can take it on a just-in-time basis

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(e.g. to incorporate into burger patties, meatballs) that can then be sold chilled or frozen. But that begs the question of why would they want to sell those products with green leaf content and what benefit would it give the customer? Is it about increasing the intake of vegetables, increasing the intake of prebiotic foods to improve the gut microbiome, reducing the cost of the product by replacing some of the meat with the cheaper vegetable filler, or is it a sustainability angle (reducing meat intake and reducing food waste)? Those questions would need to be explored by those food manufacturers and that was not explored in this project.

5. Conclusions and Recommendations

There is no doubt that high quality protein can be extracted from fresh green leaf material collected from Woolworths stores. The different types of leaf (cabbage, cauliflower, lettuce) do appear to have some differences in protein functionality. There is less of a difference in the functionality of the fibre fractions but they offer different properties to existing commercial fibre products. However, the logistical and practical complexities of sourcing and consolidating the fresh green leaf material for processing to occur cannot be overlooked. Separation of the various types of leaf from the co-mingled collection would be extremely difficult and so a blended input is more likely to work but still the material would need to be collected and consolidated in a hygienic and timely manner which would be an expensive undertaking for green leaf collection points (supermarkets) and the transporter taking it to the consolidation facility. For these reasons it would not seem to be a viable exercise.

The sprouted broccoli stem does not readily release its protein content to The Leaf Protein Co extraction protocol, almost certainly because the stem does not do very much photosynthesis and the protocol has been optimised to extract the photosynthetic RuBisCo proteins found in leafy biomass. The sprouted broccoli stem material can be used in several wet (chilled and/or from frozen) food recipe formats with positive sensory feedback. Dried sprouted broccoli stem and fibre-rich fractions from the Woolworths green leaf materials can also be incorporates into a wide range of foods that are highly accepted by a wide variety of consumers. The economics of drying large quantities of green leaf fibre-rich fractions would be determined on the supply of heat/power being used by the manufacturing facility (e.g. do they utilise wind or solar renewable energy), but preliminary estimates of costs would suggest that a dry sprouted broccoli stem powder would be comparable in cost to similar fibres from citrus and apple pomace. Fibre-rich fractions that would be a by-product from alfalfa leaf protein extraction activities would be challenging to incorporate into most ‘bland’ foods at any appreciable level due to the strong grassy flavour. There may be opportunity for use in products that normally have a strong flavour, or by use of flavour-masking ingredients.

The fibre-rich fractions from Woolworth and Perfection Fresh inputs trialled during this project have good nutritional properties, being high in fibre (mostly insoluble) and still retaining up to 20% protein. There are no attributes that could easily translate to provide a marketing ‘superfood’ narrative until the mega-trend of prebiotic foods and the health benefits of a better gut microbiota composition breaks in Australia. The nutritional benefit of ingesting an extra 5-10g of dietary fibre each day by eating 50g of these ingredients would be substantial for most consumers (Fayet-Moore et al., 2018). Australian food marketing people need to reflect the global mega-trend on gut health and make prebiotic foods a big focus. Locally made, clean label ingredients have an opportunity to play a role in delivering that for food manufacturers.

The dried fibre-rich fractions have interesting water-holding and gelation properties that are different from more ‘pure’ preparations of fibre (such as pectin), so they could substitute for pectins (or other natural humectants/thickeners) in certain applications. The only potential negative is that the fibre-rich fractions are still a green colour whereas the other products are colourless or white, so it is more obvious in the final product.

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6. Impact and Ongoing Monitoring

This project has revealed a great deal about the practical, logistical, economic and marketing barriers related to upcycling of food wastes. This project found that it is feasible to upcycle close to 100% of these types of green leaf biomass and transform them into value-added food ingredients. The costs of doing so, however, cannot be covered by the value of the protein isolates alone, a viable market for the fibre-rich fractions is needed.

Discussions are ongoing between Industry Partners and potential market offtakes. Impact of the project will be updated as commercial discussions progress at annual impact follow-up meetings or as needed directly between Project Parties and End Food Waste CRC.

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