Impact of nitrogen fertilisation on cactus pear mucilage functionality

AUTHORS:
Vuyelwa Nkoi[1]
Maryna de Wit[1]
Angeline van Biljon[2]
Johan van Niekerk[1]
Brandon van Rooyen[1]
Wilben Pretorius[1]

AFFILIATIONS:
[1]Department of Sustainable Food Systems and Development, University of the Free State, Bloemfontein, South Africa
[2]Department of Plant Sciences, University of the Free State, Bloemfontein, South Africa

CORRESPONDENCE TO:
Vuyelwa Nkoi

EMAIL:
NkoiVF@ufs.ac.za

DATES:
Received: 29 June 2024
Revised: 28 May 2025
Accepted: 16 June 2025
Published: 11 Aug. 2025

HOW TO CITE:
Nkoi V, de Wit M, van Biljon A, van Niekerk J, van Rooyen B, Pretorius W. Impact of nitrogen fertilisation on cactus pear mucilage functionality. S Afr J Sci. 2025;121(7/8), Art. #19012. https://doi.org/10.17159/sajs.2025/19012

ARTICLE INCLUDES:
☒ Peer review
☐ Supplementary material

DATA AVAILABILITY:
☐ Open data set
☒ All data included
☐ On request from author(s)
☐ Not available
☐ Not applicable

EDITORS:
Annchen Mielmann
Leslie Swartz

KEYWORDS:
cactus pear mucilage, nitrogen, functional ingredients, food production, sustainability

FUNDING:
University of the Free State (Central Research Fund)

© 2025. The Author(s). Published under a Creative Commons Attribution Licence.

The spineless cactus pear (*Opuntia ficus-indica*) mucilage is a physically slimy, green extract that is sourced from the cladodes (modified stems) of the crop. The mucilage can be freeze-dried into a powder, and this powder has shown potential to be considered as a novel functional food biopolymer because it exhibits good functional properties, especially with regard to emulsification. The main objective of this study was to investigate whether nitrogen fertilisation had an effect on the functionality of *O. ficus-indica* (L.) Mill 'Morado' mucilage. The functionality of the mucilage was attributed to its protein content. Nitrogen is one of the main elements in soil that makes up proteins. Nitrogen fertilisers from three nitrogen sources (urea, limestone ammonium nitrate, and ammonium sulfate) were applied at four application levels (0 kg/ha, 60 kg/ha, 120 kg/ha and 240 kg/ha). The functionality of mucilage was also compared to high-protein content commercial food biopolymers known to have good functional properties. Of the parameters tested, the oil-holding and oil-absorption capacities of the mucilage were significantly affected by nitrogen fertilisation. Scanning electron microscopy revealed that all freeze-dried mucilage samples resembled broken glass or showed a flake-like structure. Different nitrogen fertilisation sources and levels appeared to have no visible morphological implications on the different freeze-dried mucilage samples evaluated.

Significance:

- The study sheds light on the functionality of freeze-dried mucilage from spineless cactus pear. It highlights the impact of nitrogen fertilisation on mucilage properties.
- The mucilage exhibits promising functional properties, particularly in emulsification. This makes it a potential novel biopolymer for food applications.
- Understanding how nitrogen affects the mucilage functionality provides insights into crop management and sustainable food production.
- The ammonium-sulfate-treated mucilage performs similarly to high-protein commercial biopolymers, suggesting its practical use in food formulations.
- These findings contribute to food science knowledge and offer sustainable alternatives for food ingredient development.

Introduction

The spineless *Opuntia ficus-indica* (L.) Mill., commonly known as cactus pear, is a crop from the Cactaceae family. The family Cactaceae consists of approximately 145 accepted genera and 1519 accepted species. The genus *Opuntia* Mill., to which *O. ficus-indica* belongs, currently includes 152 accepted species.[1] The cultivar of interest for this study is 'Morado'. It is one of the most popular cultivars in the arid and semi-arid regions of southern Africa. 'Morado' produces a white-green fruit.[2] *Opuntia ficus-indica* cladodes (also called modified stems) contain insoluble fibre and soluble fibre. The insoluble fraction is mostly composed of cellulose, and the soluble fraction is composed of mucilage.[3]

Native mucilage has a unique, branched structure that contains various charged fractions.[4] Mucilage is seen as a hydrocolloid because it has a long-chain biopolymeric structure (composed of polysaccharides and proteins) that dissolves in aqueous substances for a viscous-inducing effect. There is a wide range of interest in exploring exciting new sources of hydrocolloids, mainly because of their usefulness in many industries, including the food industry.[5] Hydrocolloids have different applications in foods, including thickening, gelation, emulsification and stabilisation.[6]

Mucilage has shown potential to be considered as a novel functional food biopolymer because it exhibits good functional properties, especially with regard to emulsification. The functionality of the mucilage has been attributed to its protein fraction.[7] Proteins are known to have the ability to form and stabilise emulsions at the oil–water interface and to form and stabilise foams at the air–water interface, or the ability to solubilise (binding water and proteins). In addition, proteins have hydrodynamic properties based on intermolecular interactions, including gelling, texture and sensory (taste and odour) properties.[8]

Soy protein isolate (SPI) is a fine surfactant with extensive application in the food industry because of its high functionality in stabilising oil–water emulsions and air–water foams.[9] SPI has a high-protein content of 90%, achieved by alkaline extraction and isoelectric precipitation of soya bean meal.[10] The high-protein content in SPI, health benefits, and its protein's functionality make it a model functional ingredient to compare mucilage proteins. The protein content found in mucilage is low. However, it is important as it gives the mucilage the ability to form emulsions and foams.[11] Whey (a by-product of cheese making) and egg albumin are the most popular animal-derived globular proteins used in food processing. Like SPI, whey and egg albumin also have a high-protein content; attractive functional properties such as high-water solubility; and are excellent gelling, foaming and emulsification agents.[12]

A previous study investigated the effect of nitrogen (N) fertilisation on the protein content of *Opuntia* mucilage.[7] Three N sources were evaluated: urea, limestone ammonium nitrate (LAN), and ammonium sulfate (AmSul) (Omnia Holdings Ltd., Johannesburg, South Africa; Kynoch Fertilisers, Johannesburg, South Africa). Each of the N sources was applied at four levels (0 kg/ha, 60 kg/ha, 120 kg/ha and 240 kg/ha).[7] These N sources and levels were applied to the crops as part of the orchard management practices. Furthermore, the functionality of the mucilage was assessed to analyse whether a higher protein content would result in improved functionality (emulsification and foaming) of the *Opuntia* mucilage. According to LECO analysis, the protein content of the mucilage ranged from 1.5% to 7.0%. All the mucilage samples from the N fertilisation trial had higher protein contents than the control mucilage sample (0 kg N/ha). SPI and egg albumin, the high-protein content commercial biopolymers used in the study to compare the functionality of mucilage, recorded protein contents of 81.1% and 79.1%, respectively.[7] N fertilisation also improved the functionality of the mucilage in terms of foam capacity and emulsion capacity and stability, with all the mucilage samples from the N fertilisation trial possessing higher values for these functionality tests than the control mucilage sample (0 kg N/ha).[7] Although its foam capacity values were lower, the mucilage sample from the AmSul 60 kg/ha fertiliser application obtained similar foam stability and emulsion capacity and stability as SPI. Recently, Van Rooyen et al.[4] studied the effect of native mucilage precipitates from different *O. ficus-indica* cultivars on the mechanical and microstructural properties of blended pectin and alginate biopolymer films. Mucilage was also studied for its potential as a functional bio-based polymer to address some limitations associated with homopolymer–pectin and homopolymer–alginate films.

In the current study, the same N fertilisation sources and levels were evaluated to assess their effect on the oil-holding capacity (OHC), the water-holding capacity (WHC), and stability and microstructures of the mucilage. These three fertiliser sources were chosen for application in this study because they are the most popular fertilisers sold by South African retailers, and they are also most commonly used by South African farmers. At present, the recommended fertiliser application levels in South Africa for cactus pear fruit production are 60 kg N/ha for 2-year-old trees and 90 kg N/ha for 3-year-old trees. The recommended application levels for phosphorus (P) are 13 kg P/ha and 16 kg/ha for 2-year-old and 3-year-old trees, respectively. For potassium (K), it is 60 kg K/ha and 80 kg K/ha for 2-year-old and 3-year-old trees, respectively.[13]

Recent research has shown that agronomic factors, such as irrigation and fertilisation, can influence the yield and quality of *O. ficus-indica* mucilage. Luna-Zapién et al.[14] found that irrigation levels affect mucilage yield and composition, whilst Hasanzadeh et al.[15] reported that N and P fertilisation impacts plant productivity under water stress. Additionally, Quintero-García et al.[16] demonstrated that preprocessing methods alter mucilage's chemical and functional properties. These findings highlight the importance of exploring how N fertilisation affects mucilage functionality, particularly in relation to its protein content and structural characteristics.

In addition to food-related applications, mucilage and other hydrocolloid-rich materials from *O. ficus-indica* have gained increasing attention for their potential in environmental remediation. Recent studies have demonstrated that *Opuntia* mucilage and biomass can serve as effective, low-cost bio-adsorbents for removing dyes and other pollutants from wastewater, particularly in the textile industry. These findings underscore the versatility of *Opuntia* hydrocolloids and their relevance in both food and environmental applications.[17-19]

The effects of N fertilisation on the functional and structural properties of *O. ficus-indica* mucilage remain underexplored, particularly in relation to OHC, WHC, stability and microstructure. We aimed to address this gap by evaluating how different N sources and application levels influence these properties. The null hypothesis (H_0) is that N fertilisation will improve the functionality of *Opuntia* mucilage, whilst the alternative hypothesis (H_a) is that N fertilisation will have no effect.

Materials and methods

Sample preparation

Samples included freeze-dried mucilage powder from cladodes grown with different N fertiliser sources (urea, LAN and AmSul) at four application levels (0 kg/ha, 60 kg/ha, 120 kg/ha and 240 kg/ha), as previously described in Nkoi et al.[7] The orchard was established in 2016 at the University of the Free State, Bloemfontein, South Africa, and the N treatments were applied annually from the 2017/2018 to 2020/2021 seasons. Treatments were administered in October/December and February/April of each season, and all plants received recommended P and K supplements. Cladodes were harvested on 22 April and 12 May 2021, from 5-year-old plants, between 9:00 and 11:15, from the east side of the orchard at hip height, selecting cladodes that had not borne fruit. One cladode per treatment combination per replication was collected, totalling 30 samples.[7] SPI powder and egg albumin powder were also analysed as commercial high-protein standard products. The mucilage was extracted and freeze-dried into a powder according to the patented method described by Du Toit and De Wit[20] and Van Rooyen et al.[4]

Protein content

The protein content of the samples used in this study has been previously determined and reported.[7] The same batches were used in the current experiments. The N content of the freeze-dried mucilage samples was determined by thermal combustion. Approximately 0.1 g of each sample was placed in foil containers and analysed using a LECO Nitrogen Analyzer (FP-528, LECO Corporation). Crude protein content was automatically calculated by multiplying the measured N content by a factor of 6.25.

Scanning electron microscopy

Freeze-dried mucilage (from all N sources and N levels), SPI powder and egg albumin powder were subjected to scanning electron microscopy (SEM) imaging according to the methods described in Van Rooyen et al.[4]

Functional properties

Water-related properties of dried mucilage powder

Water-holding capacity

Traynham et al.[21], Ayadi et al.[22], López-Cervantes et al.[23] and Du Toit[2] described the WHC method that was used with minor modifications. Briefly, 1 g of freeze-dried mucilage was dissolved in 20 mL distilled water by first making a paste using only 2 mL of water and blending it using a Vortex Genie for 10 s. This procedure was followed in order to form a paste that could dissolve more easily without the formation of lumps in the larger amount of water. The rest of the distilled water (18 mL) was then added and vortexed again for 10 s. The mucilage was homogenised for 30 s using a Kenwood stick blender and allowed to stand for 1 h in order to dissolve fully. It was centrifuged using a PLC-024 centrifuge (KK centrifuge, Taiwan) for 10 min at 7155×g. The supernatant was decanted and weighed, and the centrifuge tube was inverted for 30 min. The same method was used for the SPI powder and egg albumin powder. The amount of water held by the powder was calculated using Equation 1:

$$WHC\ (g/g) = \frac{wet\ precipitate\ (g) - dried\ precipitate\ (g)}{dried\ precipitate\ (g)} \quad \text{Equation 1}$$

Water-absorption capacity

Water-absorption capacity (WAC) is the volume of water (in mL) retained by a powder after centrifugation. It is calculated as the difference between the initial volume of water added and the volume of water recovered after centrifugation. This value reflects the amount of water absorbed and held within the powder matrix. WAC is expressed in mL of water absorbed per gram of powder (mL/g) and was calculated using Equation 2[24,25]:

$$WAC\ (mL/g) = \frac{water\ initially\ added\ (mL) - supernatant\ (mL)}{dried\ precipitate\ (g)} \quad \text{Equation 2}$$

Oil-related properties of dried mucilage powder

Oil-holding capacity

The methods described by Ayadi et al.[22] and Du Toit[2] were slightly modified to determine the OHC. Freeze-dried mucilage, SPI powder and egg albumin powder (0.1 g) were each added to 2 mL sunflower oil (Pick n Pay Group) and shaken for 5 min using a Vortex Genie. The solution was centrifuged using a 5417C centrifuge (Eppendorf, Germany) for 30 min at 2090×g. The supernatant oil was separated carefully using a pipette and weighed, and the centrifuge tube was inverted for 12 h. The precipitate was weighed, and the amount of oil held by the powder was calculated using Equation 3:

$$OHC\ (g/g) = \frac{precipitate\ (g) - origional\ sample\ (g)}{origional\ sample\ (g)} \quad \text{Equation 3}$$

Oil-absorption capacity

Oil-absorption capacity (OAC) was determined by using the method described by Samia El-Safy[25] and Du Toit[2], with slight modifications. Freeze-dried mucilage, SPI powder and egg albumin powder (0.1 g) were each added to 2 mL sunflower oil (Pick n Pay Group) and shaken for 5 min using a Vortex Genie. The solution was centrifuged (5417C centrifuge) for 30 min at 2090×g. Similar to the method used for WAC, the amount of supernatant oil was deducted from the initial amount of oil added. This value was divided by the value of the original sample (g) added and expressed in mL/g:

$$OAC\ (mL/g) = \frac{oil\ initially\ added(mL) - supernatant(mL)}{sample\ (g)} \quad \text{Equation 4}$$

Statistical analysis

Data obtained for the functional properties of *O. ficus-indica* mucilage from different fertiliser sources and levels, along with SPI and egg albumin, were entered into a Microsoft Excel spreadsheet. The Excel package XLSTAT (2022) was used for statistical analysis. The means, standard deviations and coefficients of variation were calculated for each treatment. An analysis of variance was performed to detect significant differences ($p < 0.05$) between different fertiliser treatment means. A further post-hoc test, the Tukey's HSD (honestly significant difference), was conducted to determine the exact differences between the samples.

Results

Scanning electron microscopy

The microstructures of freeze-dried mucilage from 'Morado' cactus pear cladodes treated with different N sources and levels, along with those of the commercial powders – SPI and egg albumin – were observed using SEM, as shown in Figure 1. The results show that all freeze-dried mucilage samples resembled broken glass or showed a flake-like structure. The particle morphology had random sizes, shapes and orientations, and the particles randomly aggregated with one another. The freeze-dried mucilage was ground with a mortar and pestle, which

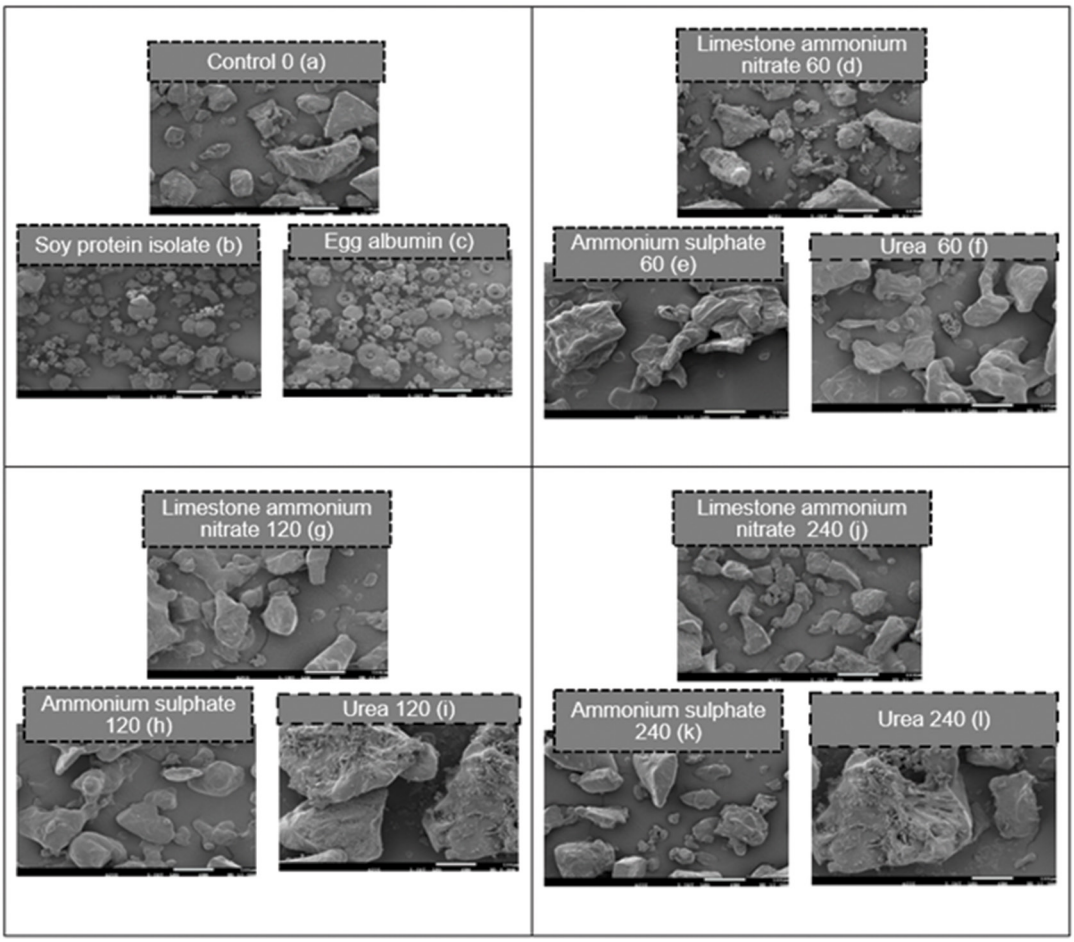


Figure 1: Scanning electron micrographs (×200 magnification) of freeze-dried mucilage from 'Morado' cactus pear cladodes treated with different nitrogen sources and application levels: control 0 kg/ha (a); commercial reference powders – soy protein isolate (b) and egg albumin (c); limestone ammonium nitrate 60 kg/ha (d), 120 kg/ha (g) and 240 kg/ha (j); ammonium sulfate 60 kg/ha (e), 120 kg/ha (h) and 240 kg/ha (k); and urea 60 kg/ha (f), 120 kg/ha (i) and 240 kg/ha (l).

resulted in varying degrees of fineness between the freeze-dried mucilage samples. Different N fertilisations had no visible morphological effects on the different freeze-dried mucilage samples evaluated. In contrast, the commercial SPI and albumin samples had consistent spherical-shaped particles of different sizes, with smooth surfaces and some dents. There was also aggregation of smaller particles into larger particles. The SPI and albumin particles were smaller than the freeze-dried mucilage particles.

Protein content

The protein content of the samples used in this study has been previously determined and reported[7], and the same batches were used in the current experiments. Briefly, the protein contents were: Control (0) – 1.5%, LAN 60 – 3.9%, LAN 120 – 7.0%, LAN 240 – 6.5%, Urea 60 – 1.9%, Urea 120 – 4.4%, Urea 240 – 5.0%, AmSul 60 – 2.4%, AmSul 120 – 5.4%, AmSul 240 – 3.2%, SPI – 81.1% and egg albumin – 79.1%.[7]

Functional properties

Water-related properties of dried mucilage powder

Water-holding capacity

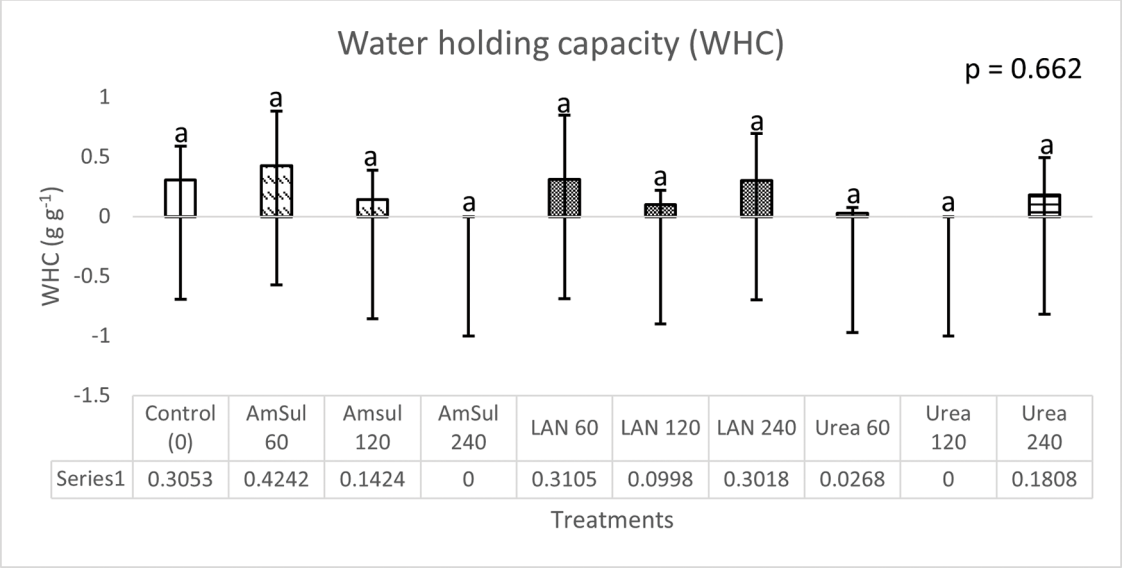
WHC is the amount of water that is held by the powder after the supernatant is decanted following centrifugation and the reaction tube inverted for 30 min.[2,17] WHC is an important protein–water interaction that occurs in various food systems. WHC represents the ability of a protein matrix to absorb and retain bound, hydrodynamic, capillary and physically entrapped water against gravity.[20] The WHC of the freeze-dried mucilage ranged from 0 g/g (AmSul 240 and Urea 120) to 0.4242 g/g (AmSul 60). WHC did not differ significantly ($p = 0.662$) among the different N sources and N levels (Figure 2). The LAN treatments and the control recorded similar WHC on average, and their WHC was higher than those of the AmSul and Urea treatments. An interesting phenomenon occurred, namely AmSul 240 and Urea 120 recorded a WHC of 0 g/g, although the freeze-dried mucilage samples looked wet when they were measured for WHC. This happened because, when the supernatant was decanted after centrifugation, a portion of the mucilage sample was dissolved in the supernatant, and this portion of mucilage was decanted along with the supernatant. Therefore, the wet precipitate left in the reaction tube had less mucilage powder than initially reacted with the water. Therefore, the WHC calculation method should be modified to account for the loss of powder in samples that contained a highly soluble fraction.

The WHC of the freeze-dried mucilage (all samples from N-fertilised plants) and albumin (Figure 3) differed significantly ($p < 0.0001$) from SPI as it recorded a WHC value (5.420 g/g) that is significantly higher than that of all the other samples. The hypothesis states that a higher protein content will result in a higher functionality of the sample.[7] Therefore, it was expected that SPI and albumin would perform much better than freeze-dried mucilage in terms of WHC. This hypothesis was true for SPI but not true for albumin. The low WHC of albumin may be because of its high solubility in water.[12] Albumin recorded a WHC of 0 g/g, as already explained with AmSul 240 and Urea 120, and when the supernatant of the albumin sample was decanted after centrifugation, a portion of the albumin powder was dissolved in the supernatant, and this portion of albumin powder was decanted along with the supernatant. Therefore, the wet precipitate left in the reaction tube had less albumin powder than what had initially reacted with the water.

Water-absorption capacity

WAC is the volume of water (in mL) retained by a powder after centrifugation. It is calculated as the difference between the initial volume of water added and the volume of water recovered after centrifugation.[2,25] WAC is related to the presence of hydrophilic constituents in the powder, such as proteins or polysaccharides.[2] The WAC of the freeze-dried mucilage ranged from 1.166 mL/g (AmSul 240) to 2.786 mL/g (AmSul 60). The WAC did not differ significantly ($p = 0.445$) among the different N sources and N levels (Figure 4). The LAN treatments recorded a constant WAC as the N levels increased, and the control also recorded similar WAC values. The AmSul and Urea treatments showed similar trends, with WAC values decreasing with an increase in N level. As with WHC, AmSul 60 was also the best N treatment for the highest WAC.

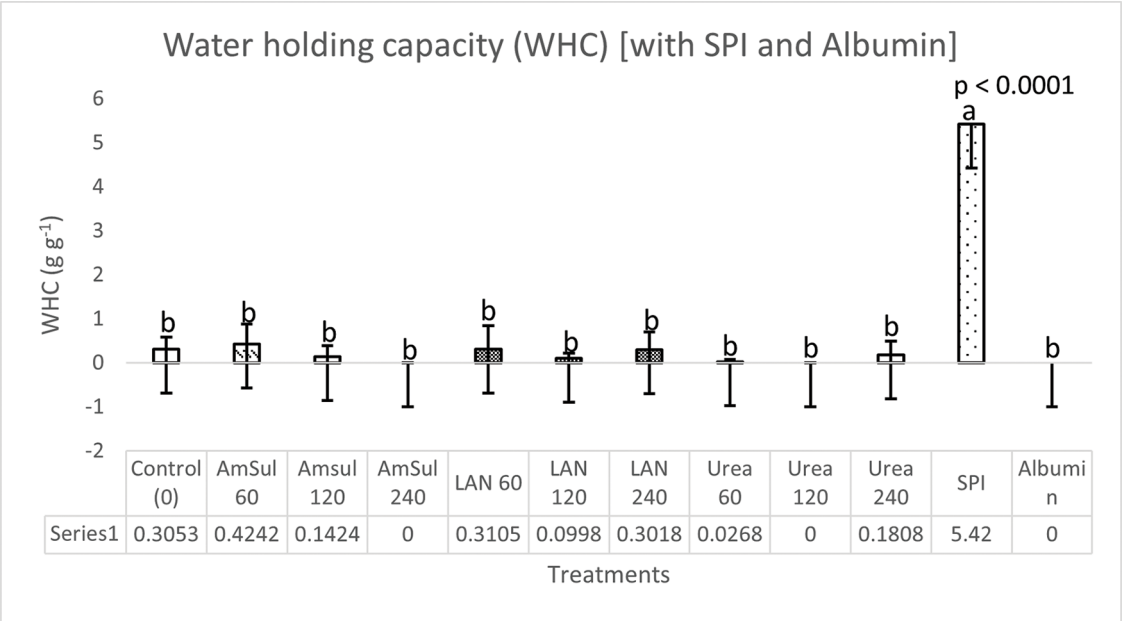
The WAC of the freeze-dried mucilage (all samples from N-fertilised plants) and albumin (Figure 5) differed significantly ($p < 0.0001$) from that of SPI (9.041 mL/g), which was significantly higher than that of all the other samples. The hypothesis states that a higher protein content will result in a higher functionality of the sample.[7] Therefore, it was expected that SPI and albumin would perform much better than freeze-dried mucilage in terms of WAC, but this hypothesis was true only for SPI. Of the freeze-dried mucilage samples, LAN 120 was expected to perform the best for WAC, but the hypothesis was not true for WAC. Urea 60 and the control were expected to perform the worst, but again this was not true for WAC. All the mucilage samples had a WAC similar to that of albumin. In essence, a high-protein content resulted in a high WAC for SPI only.[7]



Data are the mean ± SE of three replicates (n = 3).

[a,b,c]*Bars with different superscripts differ significantly.*

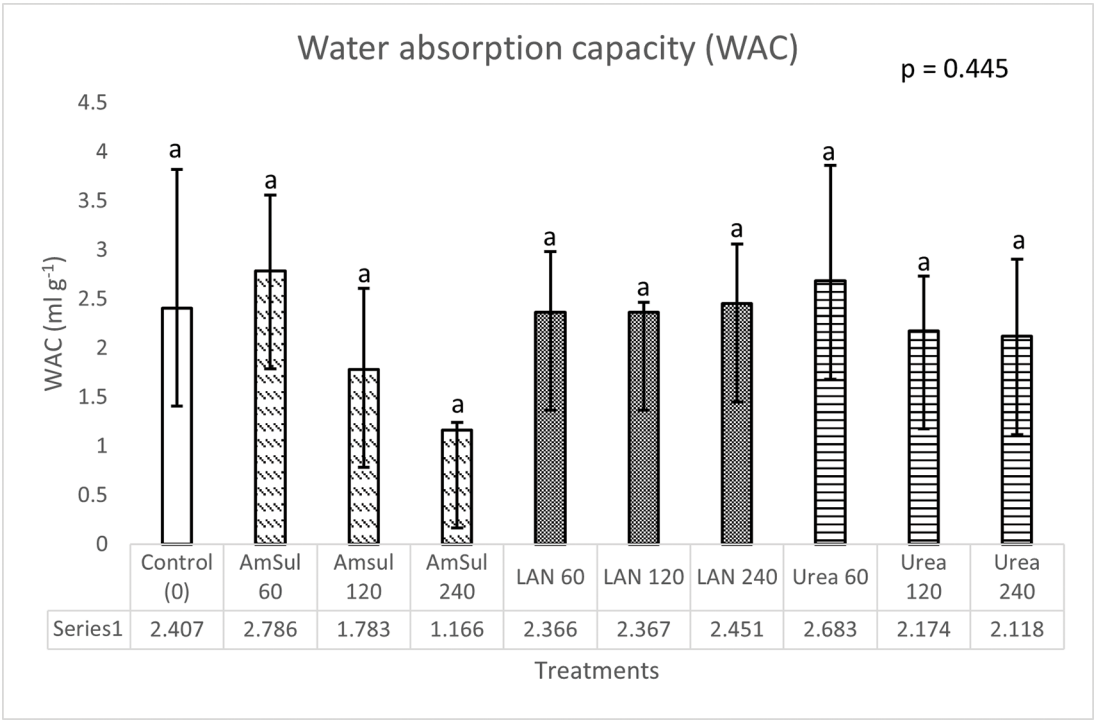
Figure 2: Effect of nitrogen sources (limestone ammonium nitrate [LAN], AmSul and Urea) and nitrogen levels (0 kg/ha, 60 kg/ha, 120 kg/ha and 240 kg/ha) on the water-holding capacity of reconstituted mucilage.



Data are the mean ± SE of three replicates (n = 3).

[a,b,c]*Bars with different superscripts differ significantly.*

Figure 3: Effect of nitrogen sources (LAN, AmSul and Urea) and nitrogen levels (0 kg/ha, 60 kg/ha, 120 kg/ha and 240 kg/ha) on the water-holding capacity of reconstituted mucilage, soy protein isolate (SPI) and albumin.



Data are the mean ± SE of three replicates (n = 3).

[a,b,c]*Bars with different superscripts differ significantly.*

Figure 4: Effect of nitrogen sources (limestone ammonium nitrate [LAN], AmSul and Urea) and nitrogen levels (0 kg/ha, 60 kg/ha, 120 kg/ha and 240 kg/ha) on the water-absorption capacity of reconstituted mucilage.

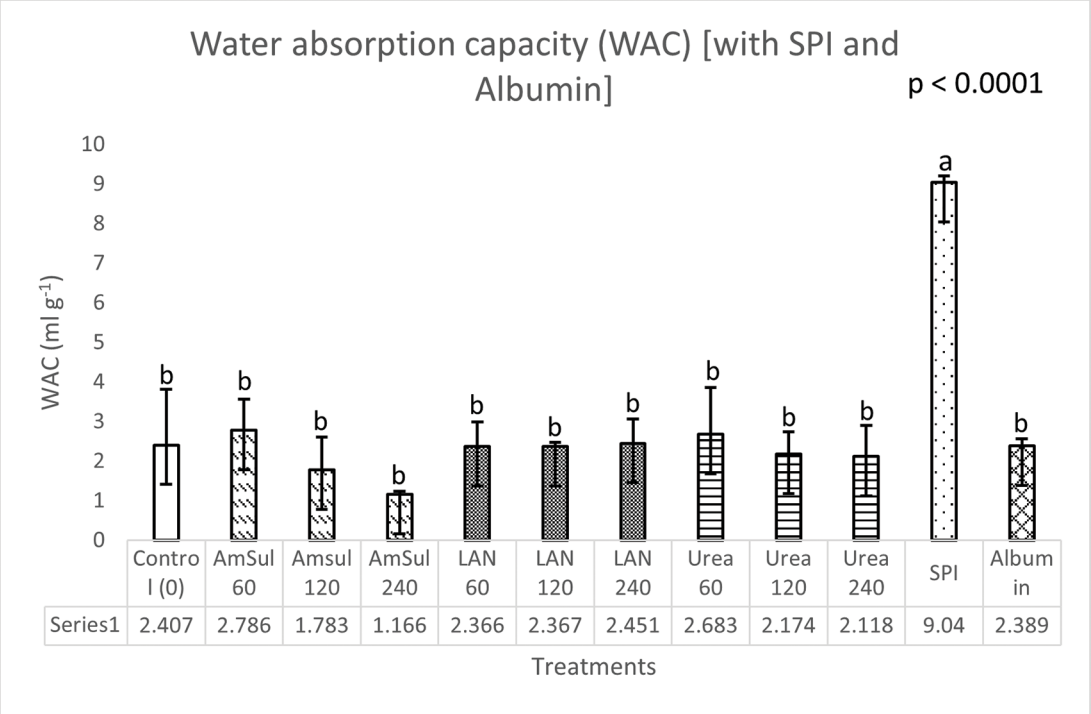
Oil-related properties of dried mucilage powder

Oil-holding capacity

The OHC is the amount of oil that is held by the freeze-dried mucilage sample after the supernatant is decanted following centrifugation and the reaction tube is inverted for 12 h.[2,23] The functional properties of the freeze-dried mucilage sample are normally linked to the interaction between the water/oil and sample. They are also associated with properties related to the protein structure and the compatibility with other food components.[26] The OHC of the freeze-dried mucilage ranged from 0.2961 g/g (LAN 60) to 1.383 g/g (Urea 240). These values simply translate to, after 12 h of the reaction tube being inverted, 1 g of freeze-dried mucilage that had

retained 0.2961 g or 29.61% and 1.383 g or 138.3% of oil for the LAN 60 and Urea 240 samples, respectively. The OHC differed significantly ($p = 0.009$) among the different N sources and N levels (Figure 6); specifically, the OHC of LAN 60 was significantly lower than that of Urea 240. The LAN treatments recorded the lowest OHCs, even lower than that of the control. The AmSul and Urea treatments recorded OHC values comparable to the control.

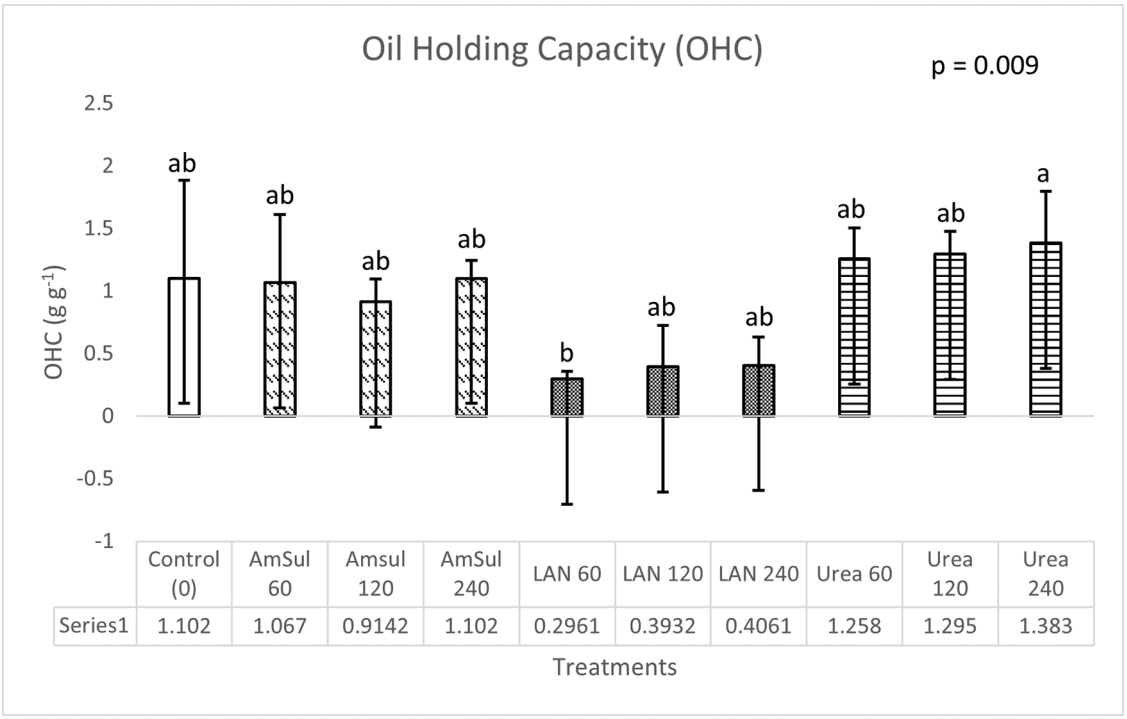
The OHC of the freeze-dried mucilage (all samples from N-fertilised plants), SPI and albumin (Figure 7) differed significantly ($p < 0.000$). SPI



Data are the mean ± SE of three replicates (n = 3).

[a,b,c]Bars with different superscripts differ significantly.

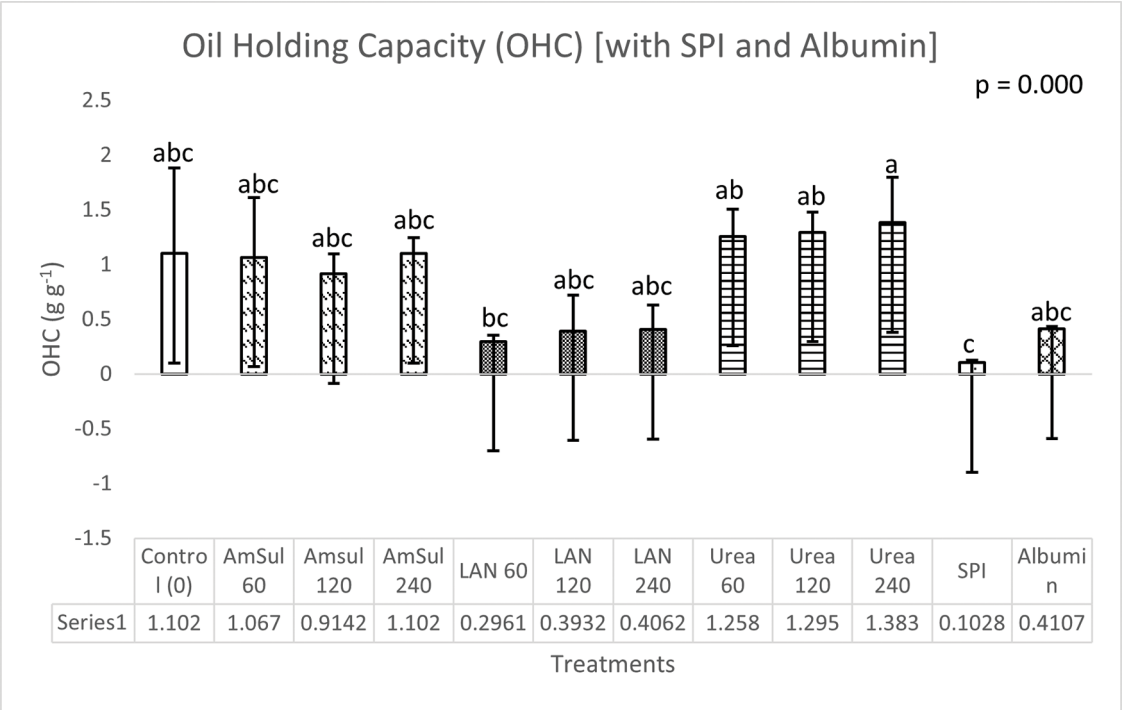
Figure 5: Effect of nitrogen sources (limestone ammonium nitrate [LAN], AmSul and Urea) and nitrogen levels (0 kg/ha, 60 kg/ha, 120 kg/ha and 240 kg/ha) on the water-absorption capacity (WAC) of reconstituted mucilage, soy protein isolate (SPI) and albumin.



Data are the mean ± SE of three replicates (n = 3).

[a,b,c]Bars with different superscripts differ significantly.

Figure 6: Effect of nitrogen sources (limestone ammonium nitrate [LAN], AmSul and Urea) and nitrogen levels (0 kg/ha, 60 kg/ha, 120 kg/ha and 240 kg/ha) on the oil-holding capacity (OHC) of the reconstituted mucilage.



Data are the mean ± SE of three replicates (n = *3).*

[a,b,c]*Bars with different superscripts differ significantly.*

Figure 7: Effect of nitrogen sources (limestone ammonium nitrate [LAN], AmSul and Urea) and nitrogen levels (0 kg/ha, 60 kg/ha, 120 kg/ha and 240 kg/ha) on the oil-holding capacity (OHC) of the reconstituted mucilage, soy protein isolate (SPI) and albumin.

(0.1028 g/g) recorded an OHC that was significantly lower than that of the Urea 240 (1.383 g/g) freeze-dried mucilage sample. SPI also recorded the lowest OHC value compared to all the other mucilage samples. Albumin (0.4107 g/g) recorded a low OHC value compared to the control, AmSul and Urea treatments. The hypothesis states that a higher protein content will result in more functionality of the sample.[7] Therefore, it was expected that SPI and albumin would perform much better than freeze-dried mucilage in terms of OHC, but this hypothesis was not true for OHC. Of the freeze-dried mucilage samples, LAN 120 was expected to perform the best for OHC, but the hypothesis was again not true for OHC for the LAN 120 samples. Urea 60 and the control were expected to perform the worst, although this was not observed for OHC of Urea 60 and the control. In essence, a high-protein content seems to not have had a positive effect on the OHC of SPI, albumin and freeze-dried mucilage.[7]

Oil-absorption capacity

The OAC is the volume of oil (in mL) retained by a powder after centrifugation. It is calculated as the difference between the initial volume of oil added and the volume of oil recovered after centrifugation.[2,25] The OAC of the freeze-dried mucilage ranged from 0.020 mL/g (LAN 120) to 4.279 mL/g (AmSul 240). These values simply translate to 1 g of freeze-dried mucilage that had absorbed from 0.020 mL (LAN 120) to 4.279 mL (AmSul 240) of oil, in relation to the original weight of the freeze-dried mucilage. Unlike OHC, OAC is immediately determined after centrifugation. OAC differed significantly among the different N sources and application levels ($p = 0.031$; Figure 8), indicating a potential overall effect of N fertilisation. However, post hoc comparisons (Tukey–Kramer) did not reveal statistically significant differences between specific treatment combinations. This suggests that, whilst there was some variation in OAC amongst treatments, the effect was not strong or consistent enough to attribute to any particular N source or level. The LAN group recorded the lowest mean OAC value, although this was not statistically distinct from the others.

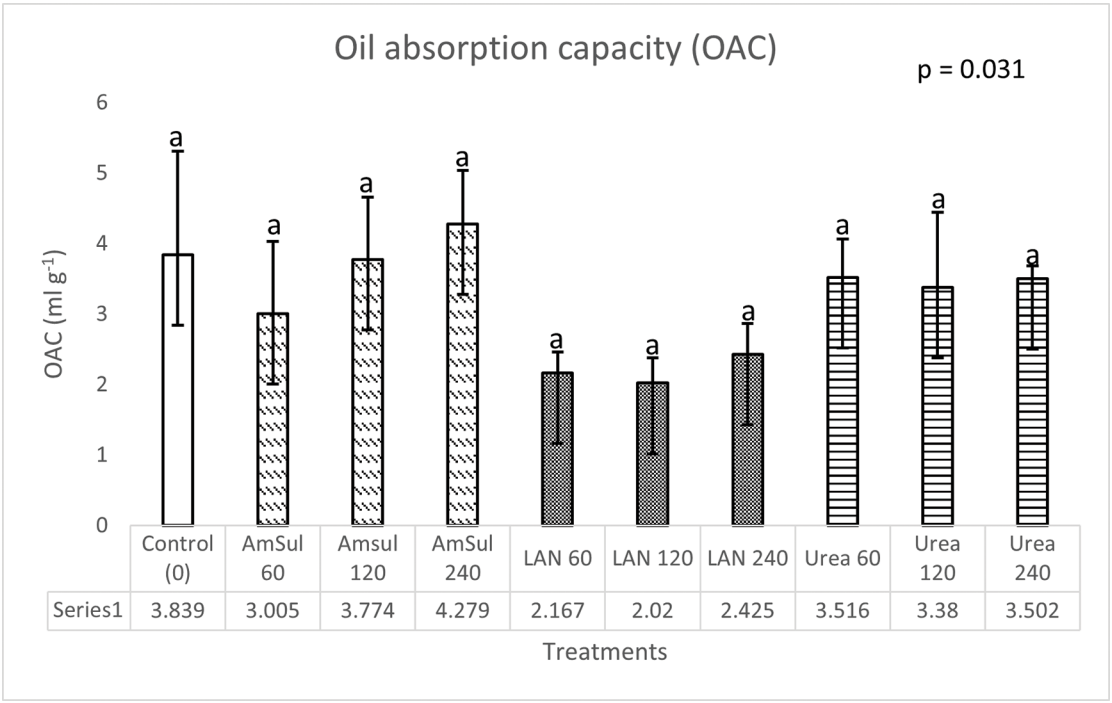
The OAC of the freeze-dried mucilage (all samples from N-fertilised treatments), SPI and albumin (Figure 9) differed significantly ($p = 0.013$). LAN 120 (2.020 mL/g) recorded an OAC that is significantly lower than that of AmSul 240's (4.279 mL/g) freeze-dried mucilage. SPI (2.383 mL/g) and albumin (2.777 mL/g) recorded lower OAC values compared to the control, AmSul and the Urea treatments.

Discussion

When comparing the influence that different N fertilisation sources and levels had on the morphology of the freeze-dried mucilage, no variation could be attributed to N fertilisation as all the mucilage samples had broken glass, flake-like microstructures. Similar broken glass, flake-like microstructures were observed for freeze-dried mucilage[2], freeze-dried egg albumin[27] and freeze-dried SPI[28]. Similar spherical-shaped particles with a smooth surface and some dents were observed for spray-dried egg albumin[27], spray-dried mucilage[29] and spray-dried SPI[28]. The results indicate that different drying methods were used to dry the mucilage and the commercial SPI and albumin powders. The spray-drying method created smaller atomised droplets with spherical shapes for SPI and albumin. However, the freeze-drying method (used in this study) lacked the forces that allow the splitting of the frozen native mucilage into spherical droplets during the vaporisation process. This method led to large freeze-dried mucilage particles with broken-glass-like microstructures.[27]

Although the freeze-drying method used in this study likely contributed to the formation of larger, irregularly shaped particles, it is also plausible that the observed differences in aggregate size and compactness were partially influenced by the protein content of the mucilage. Higher protein concentrations may promote intermolecular interactions, such as hydrogen bonding and hydrophobic associations, which could lead to the formation of more cohesive or denser aggregates during the drying process.[30,31] These structural differences may, in turn, influence the functional properties of the mucilage, particularly its WHC, OHC and stability. Therefore, whilst the drying method plays a significant role in determining the final morphology, the protein content of the mucilage may also contribute to the observed microstructural variations.

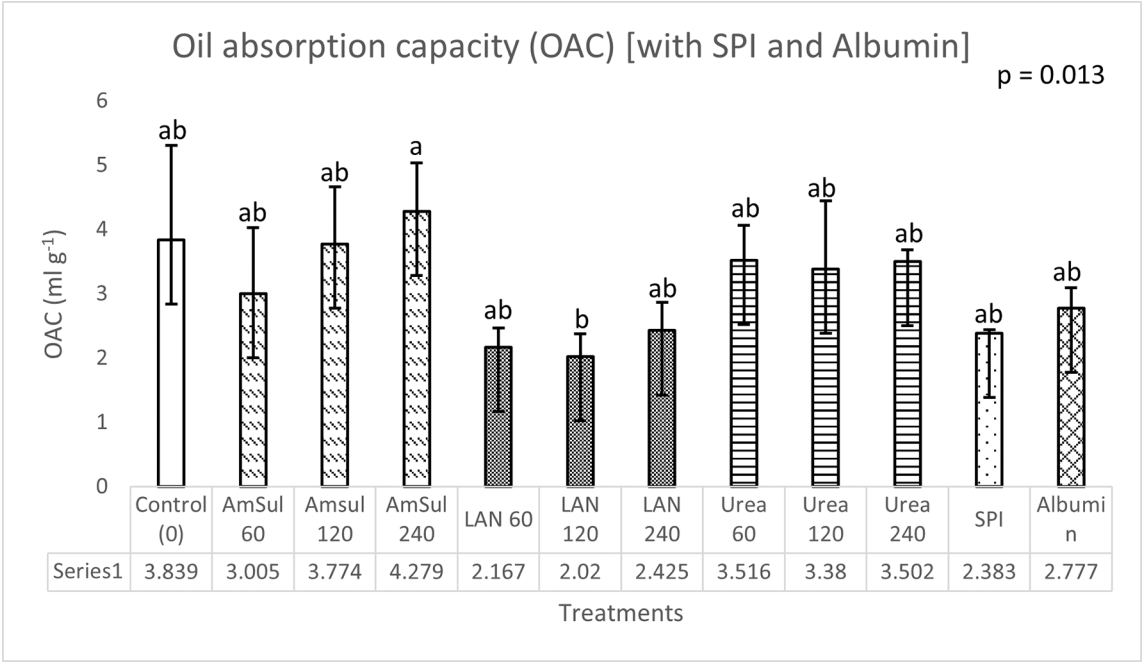
In addition to the effects of the drying method and protein content, the potential presence of calcium oxalate crystals, commonly referred to as druses, may also warrant consideration. These crystals are



Data are the mean ± SE of three replicates (n = 3).

[a,b,c]Bars with different superscripts differ significantly.

Figure 8: Effect of nitrogen sources (limestone ammonium nitrate [LAN], AmSul and Urea) and nitrogen levels (0 kg/ha, 60 kg/ha, 120 kg/ha and 240 kg/ha) on the oil-absorption capacity (OAC) of reconstituted mucilage.



Data are the mean ± SE of three replicates (n = *3).*

[a,b,c]Bars with different superscripts differ significantly.

Figure 9: Effect of nitrogen sources (limestone ammonium nitrate [LAN], AmSul and Urea) and nitrogen levels (0 kg/ha, 60 kg/ha, 120 kg/ha and 240 kg/ha) on the oil-absorption capacity (OAC) of reconstituted mucilage, soy protein isolate (SPI) and albumin.

frequently observed in the tissues of *Opuntia* species and are known to contribute to structural rigidity and a glass-like appearance under SEM. However, no calcium oxalate crystals were observed in the freeze-dried mucilage samples of previous studies, such as in Du Toit[2] and Sáenz et al.[32] It has been speculated that these crystals may not co-extract with the mucilage because of their low solubility and the predominantly soluble fibre nature of mucilage.[33] Additionally, they may be removed during filtration or lost during the drying process. Whilst their contribution to the observed morphology cannot be entirely ruled out, current evidence suggests that calcium oxalate druses are unlikely to be a major factor in the microstructure of freeze-dried mucilage.

WHC is related to the availability of polar amino groups of proteins for hydrogen bonding with water molecules.[8] Other factors affecting WHC are protein denaturation and unfolding, and the presence of non-protein components such as carbohydrates.[26] N fertilisation did not have a significant effect on the WHC of *O. ficus-indica* freeze-dried mucilage (Figure 2). SPI recorded a WHC value that was significantly higher than those of the albumin and mucilage samples (Figure 3). With reference to the protein content of the mucilage samples[7], LAN 120 was expected to perform the best for WHC, but the hypothesis was not true for WHC. Urea 60 and the control were expected to perform the worst, but this was also not true for WHC. In essence, a high-protein content had a positive effect on the WHC for SPI only. No scientific journal references on the effects of N fertilisation or any other form of fertilisation on the WHC of *O. ficus-indica* freeze-dried mucilage seem to exist. According to Du Toit[2], cultivar did not have a significant effect on the WHC of freeze-dried mucilage. The month of harvest had a significant effect on the WHC of freeze-dried mucilage, with June being the month when WHC values were the highest.[2] A WHC average of 0.89 mL/g was recorded for four cultivars, and 'Robusta' was the best cultivar for WHC.[2] The values obtained by Du Toit[2] for WHC are higher than the values found in the current study. The difference is probably because the harvesting dates in the two studies differed significantly. The WHC of powders added to food is an important functional property because it affects the sensory attributes, such as the taste, texture and juiciness of food, and it also affects the shelf life of food, particularly baked products.[26]

N fertilisation did not have a significant effect on the WAC of *O. ficus-indica* freeze-dried mucilage (Figure 4). As with the WHC, SPI recorded a WAC value that was significantly higher than those of the albumin and mucilage samples (Figure 5). There appear to be no research outputs on the effects of N fertilisation or any other form of fertilisation on the WAC of *O. ficus-indica* freeze-dried mucilage. According to Du Toit[2], cultivar did not have a significant effect on the WAC of freeze-dried mucilage. The month of harvest had a significant effect on the WAC of freeze-dried mucilage, with August being the month when WAC was best.[2] A WAC average of 8.04 g/g was recorded for four cultivars, and 'Morado' had the highest WAC value.[2] The values obtained by Du Toit[2] do not compare well with the values found in the current study, probably because the harvesting dates differ significantly. A WAC range of 2.45–4.60 mL/g was reported for okra mucilage.[24] The mean WAC recorded for the *Opuntia* mucilage samples (2.23 mL/g) in the current study is lower than the mean WAC recorded for okra mucilage (3.51 mL/g).[34]

The literature suggests a positive correlation between a high OHC and a protein structure consisting of many amino acids with nonpolar side chains.[35] N fertilisation had a significant effect on the OHC of freeze-dried mucilage (Figure 6). SPI and albumin recorded OHC values that were lower than those of the mucilage samples (Figure 7). To postulate, compared to SPI and albumin, mucilage may have a higher fraction of amino acids with non-polar side chains. No scientific journal references on the effects of N fertilisation or any other form of fertilisation on the OHC of *O. ficus-indica* freeze-dried mucilage could be found. According to Du Toit[2], cultivar did not have a significant effect on the OHC of freeze-dried mucilage. The month of harvest had a significant effect on the OHC of freeze-dried mucilage, with February being the month when OHC was best.[2] An OHC range of 1.10–1.32 g/g was recorded for four cultivars and 'Morado' recorded an average OHC of 1.23 g/g.[2] In the current study, Urea 240 was the best N source and level for an optimal OHC of freeze-dried mucilage from 'Morado'. Powders with high OHC are desirable for use in the cold meat industry, particularly for sausages, where the protein can bind the fat and water in these products.[26]

The OAC is commonly attributed to the physical entrapment of oil by the protein.[26] Oil-absorption properties are vital because they are associated with a positive mouth feel and an improved flavour perception of the food product into which they are incorporated.[25] N fertilisation significantly affected the OAC of freeze-dried mucilage (Figure 8). Whilst a statistically significant overall effect of N fertilisation on OAC was observed, the lack of significant pairwise differences between treatments limits the strength of this conclusion. The observed variation may suggest a trend, but further studies with larger sample sizes or more sensitive designs are needed to confirm specific treatment effects. SPI and albumin recorded lower OAC values than most of the mucilage samples (Figure 9). The hypothesis states that a higher protein content will result in more/higher functionality of the sample. Therefore, it was expected that SPI and albumin would perform much better than the freeze-dried mucilage samples in terms of OAC, but this hypothesis was not true. Of the freeze-dried mucilage samples, LAN 120 was expected to perform the best for OAC because of its protein value, but the hypothesis was not true. Urea 60 and the control samples were expected to perform the worst, and again this was not true for OAC. In essence, a high-protein content seems to not have had a positive effect on the OAC of SPI, albumin and freeze-dried mucilage samples. SPI and albumin may have recorded lower OAC values than the freeze-dried mucilage samples because they are more soluble in oil.

There appear to be no references on the effects of N fertilisation or any other forms of fertilisation on the OAC of *O. ficus-indica* freeze-dried mucilage. According to Du Toit[2], cultivar did not have a significant effect on the OAC of freeze-dried mucilage. The month of harvest had a significant effect on the OHC of freeze-dried mucilage, with February being the month when OAC was best.[2] An OAC range of 3.46–3.70 mL/g was recorded for four cultivars, and 'Morado' recorded an average OAC of 3.70 mL/g.[2] Kaur and Singh recommended that plant powders have OAC values equal to or higher than 2 mL/g to impart adequate emulsion capacities to the food in which the plant powders are incorporated. All the freeze-dried mucilage powders in the current study fall into this ideal range for OAC. Gemede et al.[24] recorded an OAC range of 2.02–3.64 mL/g for okra mucilage. The mean OAC recorded for the *Opuntia* mucilage samples (3.19 mL/g) in the current study is higher than the mean OAC recorded for okra mucilage (2.69 mL/g).[32]

Conclusion

We have demonstrated that N fertilisation significantly influences the OHC of freeze-dried mucilage, with statistically significant differences observed amongst treatments. Our findings also show an overall, although not treatment-specific, effect on OAC. These findings have direct implications for optimising food-processing techniques and enhancing ingredient functionality within sustainable food systems. By understanding the impact of N fertilisation, farmers can fine-tune crop management practices to improve functionality in mucilage. Processors stand to gain by incorporating N-fertilised mucilage into food products, thereby enhancing texture and stability. Consumers benefit from healthier, functional foods. Environmentally, utilising mucilage – often considered waste – aligns with sustainability goals by reducing waste and optimising resource use. Economically, creating value-added products and streamlining processes benefit all stakeholders.

Funding

This work was supported by the University of the Free State Central Research Fund. We acknowledge GrainSA and the South African National Research Foundation (NRF) for funding provided to V.N.

Data availability

All data are included.

Declarations

We have no competing interests to declare. We have no AI or LLM use to declare.

Authors' contributions

V.N.: Methodology, investigation, writing – original draft, writing – review and editing, project leadership. M.d.W.: Conceptualisation, methodology, validation, writing – review and editing, supervision, project administration, funding acquisition. A.v.B.: Methodology, validation, review and editing, supervision. J.v.N.: Supervision, funding acquisition. B.v.R.: Methodology, investigation, review and editing. W.P.: Formal analysis, data curation. All authors read and approved the final manuscript.

References

1. Royal Botanic Gardens, Kew. *Opuntia ficus-indica* (L.) Mill. Plants of the world online [data repository]. No date [cited 2025 May 23]. Available from: https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:30000028-2
2. Du Toit A, De Wit M. Patent PA153178P – A process for extracting mucilage from *Opuntia ficus-indica*, *Aloe barbadensis* and *Agave americana*. Bloemfontein: University of the Free State; 2011.
3. Saenz C, Yoong M, Figuerola F, Chiffelle I, Estevez AM. Cactus pear cladodes powders as a source of dietary fibre: Purification and properties. Int J Food Sci Nutr. 2012;63(3):283–289. https://doi.org/10.3109/09637486.2011.624492
4. Van Rooyen B, De Wit M, Osthoff G, Van Niekerk J, Hugo A. Effect of native mucilage on the mechanical properties of pectin-based and alginate-based polymeric films. Coatings. 2023;13, Art. #1611. https://doi.org/10.3390/coatings13091611
5. Du Toit A, De Wit M, Fouche H, Taljaard M, Venter SL, Hugo A. Mucilage powder from cactus pears as functional ingredient: Influence of cultivar and harvest month on the physicochemical and technological properties. J Food Sci Technol. 2019;56(5):2404–2416. https://doi.org/10.1007/s13197-019-03706-9
6. Saha D, Bhattacharya S. Hydrocolloids as thickening and gelling agents in food: A critical review. J Food Sci Technol. 2010;47(6):587–597. https://doi.org/10.1007/s13197-010-0162-6
7. Nkoi V, De Wit M, van Biljon A, van Niekerk JA. Comparison and integration of cactus mucilage protein and soy protein in functional food systems. Acta Hortic. 2022;1343:425–434. https://doi.org/10.17660/ActaHortic.2022.1343.54
8. Foegeding EA, Davis JP. Food protein functionality: A comprehensive approach. Food Hydrocoll. 2011;25:1853–1864. https://doi.org/10.1016/j.foodhyd.2011.05.008
9. Zhang X, Qi B, Xie F, Hu M, Sun Y, Han L, et al. Emulsion stability and dilatational rheological properties of soy/whey protein isolate complexes at the oil–water interface: Influence of pH. Food Hydrocoll. 2021;113, Art. #106391. https://doi.org/10.1016/j.foodhyd.2020.106391
10. Chatterjee C, Gleddie S, Xiao CW. Soybean bioactive peptides and their functional properties. Nutrients. 2018;10, Art. #1211. https://doi.org/10.3390/nu10091211
11. Du Toit A, De Wit M, Naudé S, Taljaard M, Fouche H, Hugo A, et al. Functional properties and sensory evaluation of mucilage from South African cactus pear cladodes. Acta Hortic. 2019;1247:251–260. https://doi.org/10.17660/ActaHortic.2019.1247.34
12. Sim SYJ, Srv A, Chiang JH, Henry CJ. Plant proteins for future foods: A roadmap. Foods. 2021;10(8), Art. #1967. https://doi.org/10.3390/foods10081967
13. Potgieter JP. Guidelines for the cultivation of spine-less cactus pears for fruit production. 2nd ed. Pretoria: Group 7 Trust Printers; 1997.
14. Luna-Zapién EA, Zegbe JA, Meza-Velázquez JA, Contreras-Esquivel JC, Morales-Martínez TK. Mucilage yield, composition, and physicochemical properties of cultivated cactus pear varieties as influenced by irrigation. Agronomy. 2023;13(2), Art. #419. https://doi.org/10.3390/agronomy13020419
15. Hasanzadeh H, Arba M, Bouasriya A. Effect of removing the spring flush and nitrogen and phosphorus fertilization on the late ripening of cactus pear (*Opuntia ficus-indica* (L.) Mill). World J Adv Sci Technol Sustain Dev. 2024;5(2):13–22. https://doi.org/10.53346/wjast.2024.5.2.0033
16. Quintero-García M, Gutiérrez-Cortez E, Bah M, Rojas-Molina A, Cornejo-Villegas MDLA, Del Real A, et al. Comparative analysis of the chemical composition and physicochemical properties of the mucilage extracted from fresh and dehydrated *Opuntia ficus-indica* cladodes. Foods. 2021;10(9), Art. #2137. https://doi.org/10.3390/foods10092137
17. Boumezough Y, Arris S, Carabineiro SA, Viscusi G. Optimized removal of methylene blue using chemically activated and thermally modified *Opuntia ficus-indica* bioadsorbent. Biomass Convers Biorefin. 2025;15:17427–17444. https://doi.org/10.1007/s13399-024-06370-y
18. Otálora MC, Wilches-Torres A, Lara CR, Cifuentes GR, Gómez Castaño JA. Use of *Opuntia ficus-indica* fruit peel as a novel source of mucilage with coagulant physicochemical/molecular characteristics. Polymers. 2022;14(18), Art. #3832. https://doi.org/10.3390/polym14183832
19. Das N, Ojha N, Mandal SK. Wastewater treatment using plant-derived bioflocculants: Green chemistry approach for safe environment. Water Sci Technol. 2021;83(8):1797–1812. https://doi.org/10.2166/wst.2021.100
20. Du Toit A. Selection, extraction, characterization and application of mucilage from cactus pear (*Opuntia ficus-indica* and *Opuntia robusta*) cladodes [PhD thesis]. Bloemfontein: University of the Free State; 2016.
21. Traynham TL, Myers DJ, Carriquiry AL, Johnson LA. Evaluation of water-holding capacity for wheat-soy flour blends. J Am Oil Chem Soc. 2007;84:151–155. https://doi.org/10.1007/s11746-006-1018-0
22. Ayadi MA, Maksoud W, Ennouri M, Attia H. Cladodes from *Opuntia ficus-indica* as a source of dietary fiber: Effect on dough characteristics and cake making. Ind Crops Prod. 2009;30:40–47. https://doi.org/10.1016/j.indcrop.2009.01.003
23. López-Cervantes J, Sánchez-Machado DI, Campas-Baypoli ON, Bueno-Solano C. Functional properties and proximate composition of cactus pear cladodes flours. Ciênc Tecnol Aliment. 2011;31:654–659. https://doi.org/10.1590/S0101-20612011000300016
24. Oladele AK, Aina JO. Chemical composition and functional properties of flour produced from two varieties of tiger nut (*Cyperus esculentus*). Afr J Biotechnol. 2007;6:2473–2476. http://doi.org/10.5897/AJB2007.000-2391
25. Samia El-Safy F. Evaluation and utilization of cladodes flour in formulating functional sponge cake. World Appl Sci J. 2013;27:512–523.
26. Nguyen DQ, Mounir S, Allaf K. Functional properties of water holding capacity, oil holding capacity, wettability, and sedimentation of swell-dried soybean powder. Sch J Eng Tech. 2015;3:402–412.
27. Zhao JH, Liu LS, Sablani SS, Peng Y, Xiao H, Bai J, et al. Comparison of the thermal transitions of spray-dried and freeze-dried egg whites by differential scanning calorimetry. Food Bioprocess Technol. 2020;13:1329–1341. https://doi.org/10.1007/s11947-020-02477-y
28. Bordón MB, Gabriela P, Maria B, Andrea C, María R, Pablo MM. Microencapsulation of chia seed oil (*Salvia hispanica* L.) in spray and freeze-dried whey protein concentrate/soy protein isolate/gum Arabic (WPC/SPI/GA) matrices. Proceedings. 2020;53(1), Art. #22. https://doi.org/10.3390/proceedings2020053022
29. León-Martínez FM, Méndez-Lagunas LL, Rodríguez-Ramírez J. Spray drying of nopal mucilage (*Opuntia ficus-indica*): Effects on powder properties and characterization. Carbohydr Polym. 2010;81(4):864–870. https://doi.org/10.1016/j.carbpol.2010.03.061
30. Harnkarnsujarit N, Kawai K, Suzuki T. Effects of freezing temperature and water activity on microstructure, color, and protein conformation of freeze-dried bluefin tuna (*Thunnus orientalis*). Food Bioprocess Technol. 2015;8:916–925. https://doi.org/10.1007/s11947-014-1460-1
31. Allmendinger A, Häuser C, Kumar L, Vollrath I. Formulation design for freeze-drying: Case studies of stabilization of proteins. In: Jameel F, editor. Principles and practices of lyophilization in product development and manufacturing. Cham: Springer; 2023. p. 83–101. https://doi.org/10.1007/978-3-031-12634-5_5
32. Sáenz C, Yoong M, Figuerola F, Chiffelle I, María Estevez A. Cactus pear cladodes powders as a source of dietary fibre: Purification and properties. Int J Food Sci Nutr. 2012;63:283–289. https://doi.org/10.3109/09637486.2011.624492
33. Rojas-Molina I, Gutiérrez-Cortez E, Bah M, Rojas-Molina A, Ibarra-Alvarado C, Rivera Muñoz E, et al. Characterization of calcium compounds in *Opuntia ficus-indica* as a source of calcium for human diet. J Chem. 2015;7, Art. #710328. https://doi.org/10.1155/2015/710328
34. Gemede HF, Haki GD, Beyene F, Rakshit SK, Woldegiorgis AZ. Indigenous Ethiopian okra (*Abelmoschus esculentus*) mucilage: A novel ingredient with functional and antioxidant properties. Food Sci Nutr. 2018;6:563–571. https://doi.org/10.1002/fsn3.596
35. Hopkins WG, Hüner NPA. Nitrogen assimilation. In: Witt K, Foxman J, Muriello L, editors. Introduction to plant physiology. Hoboken, NJ: John Wiley & Sons; 2008. p.