

Article

Cultivar and Harvest Month Influence the Nutrient Content of *Opuntia* spp. Cactus Pear Cladode Mucilage Extracts

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Abstract: Mucilage extracted from cactus pear cladodes is a hydrocolloid gum. It is a novel, natural, low-kilojoule, cost-effective texture-modifying ingredient in functional food products. Yet, the cultivar with the most optimal nutrient content and the preferred harvest times are as yet unknown. For this reason, mucilage from three *Opuntia ficus-indica* (Algerian, Morado and Gymno-Carpo) and one *Opuntia robusta* (Robusta) cultivar were investigated to determine their nutrient content over six months. Nutrients that contribute energy (10.2 kJ/g) were low. The mineral content was high (ash 17.7/100 g), particularly calcium (3.0 g/100 g) and phosphorous (109.5 mg/kg). Low insoluble acid-detergent fibre (1.4 g/kg) and neutral-detergent fibre (2.1 g/kg) values indicated that mucilage was mostly soluble fibre. Calcium oxalate crystals were not detected in dried mucilage. *Opuntia robusta* powders had higher protein, extractable fat and potassium content, while *Opuntia ficus-indica* mucilage powders had higher polyunsaturated (Linoleic and α -Linolenic acid) fat content. *O. robusta* Robusta mucilage, harvested after the fruit harvest (February) had the lowest energy content and the highest mineral and protein content. Mucilage powders were highly soluble, low-kilojoule and mineral-rich. This is a functional ingredient that is produced from an easily cultivated crop, as cactus pears grow in areas with poor soil, extremely high daytime temperatures and limited water supplies.

Keywords: prickly pear; nopal; *Opuntia ficus-indica*; *Opuntia robusta*; hydrocolloid gum; dietary fibre; calcium oxalate crystals; minerals and trace elements

1. Introduction

Food developers, with health-conscious consumers in mind, are interested in low-kilojoule, natural, cost-effective and texture-enhancing hydrocolloids [1]. Hydrocolloids are used to modify textural properties of food when ingredients such as gluten, fats, egg and dairy products are omitted in order to develop low-kilojoule or allergy-free functional food products [2]. Cactus pear cladodes contain a slimy substance (mucilage) that is a hydrocolloid and exhibits promising functional and rheological properties [2,3].

Mucilaginous molecules found in cladodes are long-chain acidic hetero-polysaccharides, which increase viscosity when dissolved [4]. The extracted mucilage is a non-Newtonian liquid, able to absorb and hold huge amounts of water in its structure [4]. Previous research has demonstrated its unique rheological properties, the occurrence of pseudoplastic (shear-thinning) behaviour, and the influence of temperature, pH and ionic strength fluctuations during processing, preparation and consumption of food products containing mucilage [5].

The mucilage consists mostly of soluble fibre, which is an important component of a healthy diet [1,6]. Additionally, cactus pear products have been proven to be effective in the treatment of ailments and has numerous therapeutic properties [7,8]. The development of mucilage-containing functional food products would not only benefit consumers by providing natural remedies and treatments for chronic diseases such as cancer, diabetes mellitus and cardiovascular diseases, but would also develop the cactus pear as a commercial crop [7].

Cactus pear plants have to retain water at all costs, in addition to obtaining CO₂, and are adapted to use crassulacean acid metabolism (CAM), which is an alternative to photosynthesis. The stomata are only open at night for CO₂ fixation when water-loss is limited [9]. CAM is more efficient during long, sunny summer days; thus, the cactus pear thrives in summer and struggles in cold frosty winters. The commercial cultivation and research of *Opuntia ficus-indica* and *Opuntia robusta* in South Africa is limited to the spineless Burbank varieties, which are susceptible to frost [10].

Of the cactus pear cultivars available for research, four were feasible for the commercial production of mucilage powders. These cultivars included *O. ficus-indica* cv Algerian (pink fruit) with low-viscosity and high-yield mucilage, *O. ficus-indica* cv Morado (green fruit) with high viscosity, yield and commercial fruit production use, *O. ficus-indica* cv Gymno-Carpo (orange fruit) with medium-high mucilage viscosity and yield and *O. robusta* Robusta (purple fruit), which is well known as animal fodder.

The nutrient content of freeze-dried mucilage may be influenced by cultivar, species, growth stage and weather at time of harvest. Therefore, the objective of this study was to determine the influence of cultivar and time of harvest on the nutrient content of freeze-dried mucilage. Knowledge of the nutrient components is necessary for recommendations in terms of the optimal cultivar and time of harvest to produce cactus pear mucilage.

2. Results and Discussion

2.1. Differences in the Nutrient Content Observed among the Four Cultivars

2.1.1. Protein Content

In the protein fingerprint determination SDS-PAGE electrophoretogram image (Figure 1), the most pronounced bands fell between 37 and 75 kD, which indicated the presence of prolamins (high in glutamine and proline). Other pronounced bands were observed at 10 kD (smallest proteins) in all samples except for Robusta (lanes 4, 8, 12 and 16), which indicated 2S albumin proteins present in the three *O. ficus-indica* cultivars, but not in *O. robusta*. 2S albumin functions as nutrient storage and as a protective protein in plants, but has been reported to be the cause of food allergies [11]. The prominence of glutamine [12,13], as well as albumin-like proteins [14], has been reported in cladodes.

The protein content of the three *O. ficus-indica* cultivars was not significantly different (2.7–3.2/100 g) (according to the statistical analysis $p < 0.001$) from each other, but was significantly lower than that of *O. robusta* (4.8/100 g; $p < 0.001$) [2]. The protein content of 6.8% reported [15] in mucilage was in the range of the mean protein content (3.4/100 g) determined in these mucilage samples [2]. The fraction of protein occurring in mucilage is small; however, it is consequential, as it grants mucilage the capacity to form emulsions by reducing the surface activity at the interphase. Mucilage has the ability to stabilize emulsions [16], act as an emulsifying agent [14], stabilizing agent [14], thickening agent [4,14] and serve as a fat replacement agent [17].

2.1.2. Total Extractable Fat Content (EFC) and Fatty Acid Analysis

In Table 1, Robusta mucilage had higher EFC content than Gymno-Carpo and Algerian ($p = 0.012$). The most abundant fatty acid (Table 2) was the polyunsaturated linoleic acid (C18:2c9,12 (n-6)). It is rare for linoleic acid (Omega-6) to be predominant in plant materials [18]. Linoleic acid (Omega-6)

is not synthesised in human cells, yet is indispensable in brain development, growth and cardiac health [19].

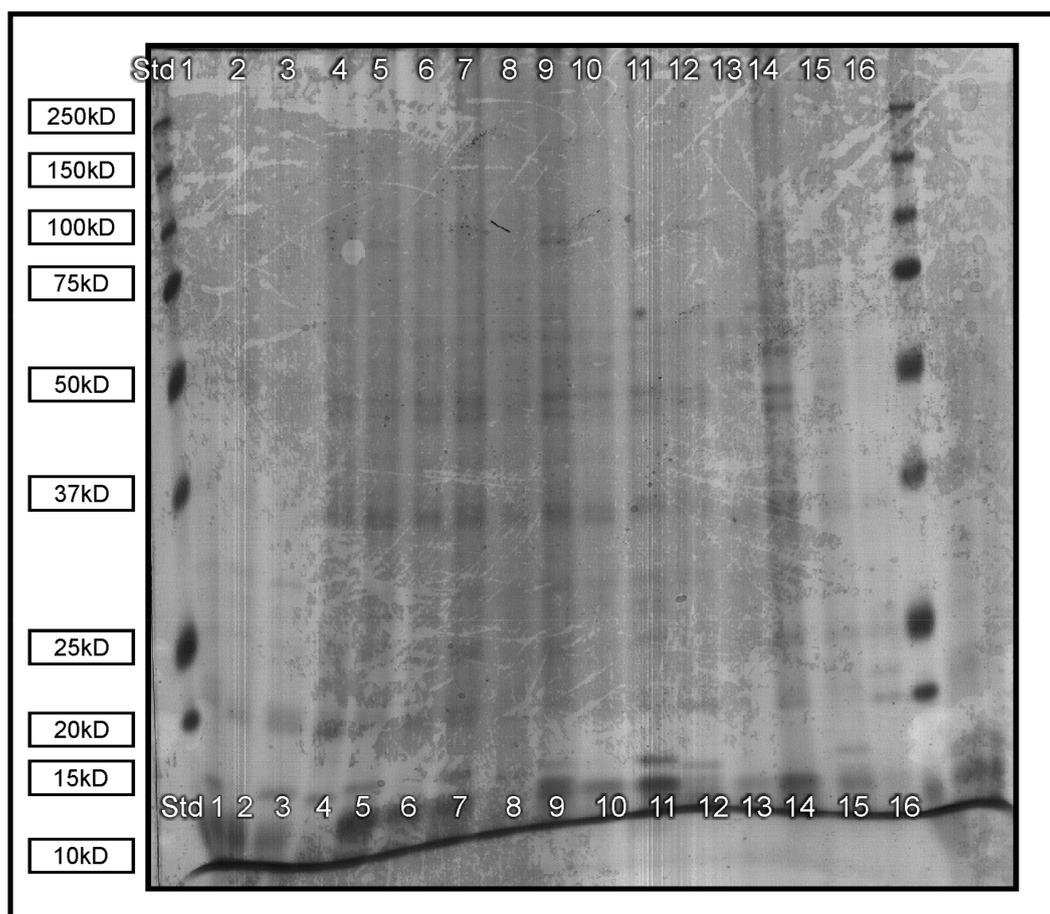


Figure 1. SDS-PAGE electrophoretogram image (February: 1 = Algerian, 2 = Morado, 3 = Gymno-Carpo, 4 = Robusta. May: 5 = Algerian, 6 = Morado, 7 = Gymno-Carpo, 8 = Robusta. June: 9 = Algerian, 10 = Morado, 11 = Gymno-Carpo, 12 = Robusta. August: 13 = Algerian, 14 = Morado, 15 = Gymno-Carpo, 16 = Robusta).

The other abundant fatty acid in mucilage powders was the monounsaturated oleic acid (C18:1c9) that is omnipresent in plant food sources and usually the most abundant fatty acid [18]. Together with saturated palmitic (C16:0) and stearic acid (C18:0), these four most abundant fatty acids made up 98.2% of the total fatty acids in the mucilage (Table 2). The SFA are deemed unhealthy, because the uncontrolled intake thereof is associated with high cholesterol, overweight, obesity and the potential cause of many chronic diseases [19,20]. Mucilage, however, contained less than 20% SFA. Foods rich in PUFA improve blood cholesterol levels, which can decrease the risk of cardiovascular diseases and diabetes [19]. The PUFA was 64.4% of the EFC (Table 2). The fat content of 0.4% reported in mucilage powders [15] was in the range of the mean EFC value (0.6/100 g) obtained in this study.

2.1.3. Indigestible Carbohydrates

Low insoluble acid-detergent fibre and neutral-detergent fibre values (Table 1) indicated that mucilage was mostly pure soluble fibre (mostly mucilage and possibly pectin). Robusta had significantly higher neutral-detergent fibres than Algerian and Morado (Table 1). Thus, *O. robusta* mucilage would be the better choice when more insoluble fibre is required for a food product. The crude

fibre value (0.1%) reported [15] in mucilage powders was lower than that obtained for *O. robusta* Robusta (0.7/100 g) and *O. ficus-indica* (0.2/100 g) mucilage in this study.

Table 1. Fat, gross energy, carbohydrate, starch and detergent fibre content of freeze-dried mucilage powders extracted from four cactus pear cultivars harvested from cladodes over six months.

		Extractable Fat Content	Gross Energy	Total Carbohydrates	Starch	Acid-Detergent Fibre	Neutral-Detergent Fibre
		g/100 g	kJ/g	g/100 g	g/100 g	g/kg	g/kg
Cultivar	Algerian	0.5 ± 0.2 ^a	9.8 ± 0.9	62.4 ± 3.5	5.7 ± 1.1	0.9 ± 0.8	0.7 ± 0.6 ^a
	Morado	0.6 ± 0.2 ^{ab}	10.3 ± 1.1	62.8 ± 3.5	5.5 ± 0.1	1.1 ± 0.1	1.4 ± 0.1 ^a
	Gymno-Carpo	0.4 ± 0.2 ^a	10.1 ± 0.1	62.6 ± 3.4	6.1 ± 0.9	1.5 ± 1.5	1.7 ± 2.1 ^{ab}
	Robusta	0.9 ± 0.3 ^b	10.1 ± 0.1	62.1 ± 5.2	6.5 ± 1.9	2.3 ± 1.9	4.5 ± 3.1 ^b
	Significance level	(<i>p</i> = 0.012)	(<i>p</i> = 0.332)	(<i>p</i> = 0.989)	(<i>p</i> < 0.586)	(<i>p</i> = 0.321)	(<i>p</i> = 0.014)
Month	February	0.5 ± 0.2	9.1 ± 0.3 ^a	59.1 ± 2.7 ^a	5.4 ± 0.3 ^{ab}	1.4 ± 1.1	1.6 ± 0.4
	April	0.4 ± 0.2	9.2 ± 0.6 ^a	56.6 ± 0.6 ^a	5.1 ± 1.1 ^a	0.5 ± 0.4	0.6 ± 0.5
	May	0.7 ± 0.2	10.3 ± 0.5 ^b	63.6 ± 0.9 ^b	5.5 ± 0.9 ^{ab}	1.8 ± 1.6	3.3 ± 3.1
	June	0.7 ± 0.2	10.6 ± 0.3 ^{bc}	65.8 ± 1.0 ^b	5.7 ± 0.8 ^{ab}	2.4 ± 0.3	2.0 ± 1.8
	July	0.6 ± 0.4	10.7 ± 0.6 ^{bc}	64.9 ± 0.6 ^b	6.2 ± 0.9 ^{ab}	1.2 ± 2.3	2.7 ± 4.5
	August	0.8 ± 0.4	11.6 ± 0.5 ^c	64.9 ± 1.9 ^b	7.7 ± 1.7 ^b	1.4 ± 1.4	2.4 ± 1.5
	Significance level	(<i>p</i> = 0.359)	(<i>p</i> < 0.001)	(<i>p</i> < 0.001)	(<i>p</i> = 0.032)	(<i>p</i> = 0.523)	(<i>p</i> = 0.694)
	Means	0.6 ± 0.2	10.2 ± 0.1	62.5 ± 3.7	5.6 ± 0.9	1.4 ± 0.7	2.1 ± 0.1

Opuntia ficus-indica (Algerian, Morado and Gymno-Carpo), *Opuntia robusta* (Robusta); Cultivar means with different superscripts ^{a, b, c} in the same column differ significantly (*p* < 0.05) and represent the means of data obtained over the six harvest months; Month means with different superscripts ^{a, b, c} in the same column differ significantly (*p* < 0.05) and represent the means of data obtained from the four cultivars.

2.1.4. Mineral Content

High levels of K (above 0.4 g/portion) are rare in many fresh foods [19], yet cactus pear cladodes have been reported to be a good natural source [21]. Robusta mucilage had significantly higher K content than Morado (*p* = 0.043), which was in the range of values reported [3] of between 1.6/100 g and 2.0/100 g in mucilage. Robusta had significantly lower Mg content (*p* < 0.001) than the *O. ficus-indica* cultivars (Table 3). The P levels detected in mucilage were unusually high for plant products (Table 3).

2.1.5. Calcium Oxalate Crystals

Calcium oxalate crystals occur in Opuntioideae and have a negative effect on nutrition, as it binds with essential minerals such as calcium and cannot be absorbed in the human digestive system and could cause harm to the kidneys [22]. It was observed in this study that the calcium oxalate crystal morphology was different among cultivars as the crystals were bigger and more abundant in the fresh cladode tissues of the three *O. ficus-indica* (Algerian, Morado and Gymno-Carpo) cultivars than in *O. robusta*. To demonstrate, a typical calcium oxalate crystal observed in the *O. ficus-indica* cultivars is observed in Figure 2a. The typical whewellite stellate druses observed in *O. ficus-indica* tissue were large (ranged from 30 to 100 µm) and in abundance throughout the tissue. Similar calcium oxalate crystals in the form of whewellite or weddelite crystals have been reported [23,24]. However, in *O. robusta* tissue, the crystals were smaller (ranging from 6 µm to 35 µm), rounder, very scarce and mostly observed close to the epidermis (Figure 2b). The sparser occurrence of calcium oxalate crystals may be beneficial to consumer health, influence the lower Ca content of *O. robusta* cladodes (Table 3) and have an influence on the viscosity of the mucilage [5]. Nevertheless, no calcium oxalate crystals were observed in any of the dried mucilage samples, possibly due to the low solubility of calcium oxalate and the relatively low concentration of calcium oxalate found in soluble fibre [25]. It may be deduced that the calcium oxalate crystals did not co-extract when the mucilage was separated from the solids during the extraction process.

Table 2. Fatty acid content (% of total extractable fat) and ratios of freeze-dried mucilage powders extracted from four cactus pear cultivars harvested from cladodes over six months (February, April, May, June, July and August).

	Fatty Acids	Abbreviation	Algerian	Morado	Gymno-Carpo	Robusta	Mean	Significance Level
% of Total Fatty Acids								
Individual fatty Acids:	Myristic	C14:0	0.1 ± 0.03	0.1 ± 0.01	0.1 ± 0.02	0.1 ± 0.01	0.1 ± 0.01	$p = 0.614$
	Palmitic	C16:0	14.1 ± 1.8	13.8 ± 1.2	12.8 ± 1.5	15.2 ± 1.0	14.0 ± 0.5	$p = 0.094$
	Palmitoleic	C16:1c9	0.7 ± 0.04	0.7 ± 0.1	0.6 ± 0.2	0.6 ± 0.1	0.7 ± 0.05	$p = 0.142$
	Margaric	C17:0	0.02 ± 0.001	0.02 ± 0.001	0.02 ± 0.001	0.02 ± 0.001	0.02 ± 0.001	$p = 0.103$
	Heptadecenoic	C17:1c10	0.04 ± 0.01	0.1 ± 0.04	0.1 ± 0.1	0.1 ± 0.02	0.1 ± 0.02	$p = 0.59$
	Stearic acid	C18:0	3.1 ± 0.1	2.9 ± 0.4	2.9 ± 0.3	2.9 ± 0.4	3.0 ± 0.4	$p = 0.823$
	Oleic	C18:1c9	15.6 ± 4.5 ^a	20.1 ± 4.1 ^{ab}	16.1 ± 5.4 ^{ab}	23.5 ± 4.2 ^b	19.1 ± 1.8	$p = 0.023$
	Linoleic	C18:2c9,12 (n-6)	65.3 ± 5.2 ^{ab}	60.6 ± 5.0 ^{ab}	66.5 ± 6.8 ^b	56.2 ± 5.2 ^a	62.1 ± 2.0	$p = 0.018$
	Arachidic	C20:0	0.1 ± 0.04 ^a	0.2 ± 0.1 ^a	0.1 ± 0.04 ^a	0.5 ± 0.12 ^b	0.2 ± 0.1	$p < 0.001$
	Eicosenoic	C20:1c11	0.2 ± 0.02	0.2 ± 0.02	0.2 ± 0.02	0.2 ± 0.03	0.2 ± 0.01	$p = 0.335$
	α-Linolenic	C18:3c9,12,15 (n-3)	0.2 ± 0.1 ^a	0.2 ± 0.1 ^a	0.1 ± 0.1 ^a	0.3 ± 0.04 ^b	0.2 ± 0.1	$p = 0.018$
	Behenic	C22:0	0.1 ± 0.03 ^a	0.2 ± 0.1 ^{ab}	0.2 ± 0.1 ^{ab}	0.2 ± 0.04 ^b	0.2 ± 0.02	$p = 0.041$
Eicosatrienoic	C20:3c8,11,14 (n-6)	0.1 ± 0.1 ^a	0.1 ± 0.1 ^a	0.1 ± 0.1 ^a	0.4 ± 0.2 ^b	0.2 ± 0.1	$p < 0.001$	
Lignoceric	C24:0	0.03 ± 0.02	0.04 ± 0.02	0.03 ± 0.02	0.1 ± 0.03	0.04 ± 0.02	$p = 0.163$	
Fatty Acid Ratios:	Saturated Fatty Acids		17.7 ± 2.7	17.3 ± 1.3	16.3 ± 1.5	18.8 ± 1.2	17.5 ± 0.5	$p = 0.117$
	Monounsaturated		16.8 ± 4.5 ^a	21.9 ± 4.1 ^{ab}	17.0 ± 5.4 ^a	24.3 ± 4.1 ^b	20.0 ± 1.2	$p = 0.023$
	Polyunsaturated		65.5 ± 5.1 ^{ab}	60.9 ± 4.5 ^{ab}	66.8 ± 6.7 ^b	56.9 ± 5.2 ^a	64.4 ± 1.9	$p = 0.022$
	PUFA:SFA Ratio		3.8 ± 0.8 ^{ab}	3.6 ± 0.6 ^{ab}	4.2 ± 0.7 ^b	3.0 ± 0.4 ^a	3.6 ± 0.2	$p = 0.044$

Opuntia ficus-indica (Algerian, Morado and Gymno-Carpo), *Opuntia robusta* (Robusta). Means with different superscripts ^{a, b, c} in the same row differ significantly $p < 0.05$. and represent the means of data obtained over the six harvest months.

Table 3. Micronutrient content of freeze-dried mucilage powders extracted from four cactus pear cultivars harvested from cladodes over six months.

		Ash	Ca	K	Mg	P	Na	Mn	Fe	Cu	Zn
		g/100 g	g/100 g	g/100 g	g/100 g	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Cultivar	Algerian	18.8 ± 2.5	3.3 ± 0.9	2.9 ± 0.7 ^{ab}	2.6 ± 0.3 ^b	113.8 ± 24.1	86.3 ± 34.6	190.9 ± 30.1	32.3 ± 25.3	6.2 ± 1.0	23.7 ± 4.6
	Morado	17.1 ± 2.0	3.0 ± 0.9	2.3 ± 0.5 ^a	2.6 ± 0.2 ^b	93.9 ± 11.3	129.3 ± 50.1	185.8 ± 118.7	18.3 ± 18.0	5.7 ± 0.7	25.7 ± 4.2
	Gymno-Carpo	18.1 ± 2.5	3.0 ± 0.8	2.6 ± 1.0 ^{ab}	2.7 ± 0.3 ^b	100.2 ± 26.7	114.3 ± 46.4	220.7 ± 88.1	15.7 ± 18.8	5.4 ± 1.1	24.3 ± 3.0
	Robusta	16.8 ± 3.4	2.3 ± 1.2	3.3 ± 0.1 ^b	2.0 ± 0.1 ^a	129.9 ± 132.0	143.2 ± 72.6	156.2 ± 84.1	22.2 ± 23.7	4.9 ± 1.2	24.5 ± 6.4
	Significance level	(<i>p</i> = 0.545)	(<i>p</i> = 0.854)	(<i>p</i> = 0.043)	(<i>p</i> < 0.001)	(<i>p</i> = 0.808)	(<i>p</i> = 0.311)	(<i>p</i> = 0.647)	(<i>p</i> = 0.572)	(<i>p</i> = 0.235)	(<i>p</i> = 0.904)
Month	February	21.0 ± 1.1 ^b	3.5 ± 0.9 ^{bc}	3.7 ± 0.8	2.8 ± 0.4	125.2 ± 26.3	170.0 ± 18.7 ^b	302.5 ± 82.1 ^b	31.6 ± 4.8 ^{ab}	7.1 ± 0.8 ^b	27.8 ± 3.3 ^b
	April	20.1 ± 1.1 ^b	4.6 ± 0.4 ^c	2.6 ± 1.0	2.4 ± 0.2	103.0 ± 18.1	133.1 ± 92.9 ^{ab}	136.9 ± 16.4 ^a	3.9 ± 1.3 ^a	5.6 ± 0.5 ^{ab}	26.5 ± 5.1 ^{ab}
	May	17.0 ± 0.7 ^a	2.1 ± 0.4 ^a	2.8 ± 0.5	2.5 ± 0.4	85.9 ± 14.5	79.8 ± 14.5 ^{ab}	136.4 ± 50.4 ^a	2.6 ± 1.3 ^a	5.0 ± 0.4 ^a	28.3 ± 4.3 ^b
	June	16.1 ± 1.5 ^a	2.5 ± 0.5 ^{ab}	2.4 ± 0.5	2.5 ± 0.4	166.2 ± 156.1	70.5 ± 20.5 ^a	129.9 ± 53.2 ^a	16.9 ± 28.1 ^{ab}	4.9 ± 1.4 ^a	22.3 ± 1.7 ^{ab}
	July	15.6 ± 1.2 ^a	2.6 ± 0.4 ^{ab}	2.6 ± 0.4	2.3 ± 0.3	88.7 ± 20.6	113.0 ± 37.9 ^{ab}	222.3 ± 65.0 ^{ab}	28.8 ± 20.9 ^{ab}	5.3 ± 0.7 ^a	20.5 ± 3.3 ^a
	August	15.6 ± 1.4 ^a	2.75 ± 0.5 ^{ab}	2.5 ± 0.2	2.3 ± 0.4	87.8 ± 16.8	143.3 ± 38.4 ^{ab}	202.4 ± 80.2 ^{ab}	48.9 ± 9.0 ^b	5.4 ± 0.9 ^{ab}	22.0 ± 3.0 ^{ab}
	Significance level	(<i>p</i> < 0.001)	(<i>p</i> < 0.001)	(<i>p</i> = 0.090)	(<i>p</i> = 0.463)	(<i>p</i> = 0.498)	(<i>p</i> = 0.034)	(<i>p</i> = 0.005)	(<i>p</i> = 0.002)	(<i>p</i> = 0.015)	(<i>p</i> = 0.023)
Means		17.7 ± 2.3	3.0 ± 0.9	2.8 ± 0.5	2.5 ± 0.2	109.5 ± 31.5	118.3 ± 38.3	188.4 ± 68.0	22.1 ± 17.8	5.5 ± 0.8	24.5 ± 3.34
% Ash		—	—	—	—	—	—	0.01	0.002	N/A	0.002

Opuntia ficus-indica (Algerian, Morado and Gymno-Carpo), *Opuntia robusta* (Robusta). Cultivar means with different superscripts ^{a, b, c} in the same column differ significantly (*p* < 0.05) and represent the means of data obtained over the six harvest months. Month means with different superscripts ^{a, b, c} in the same column differ significantly (*p* < 0.05) and represent the means of data obtained from the four cultivars.

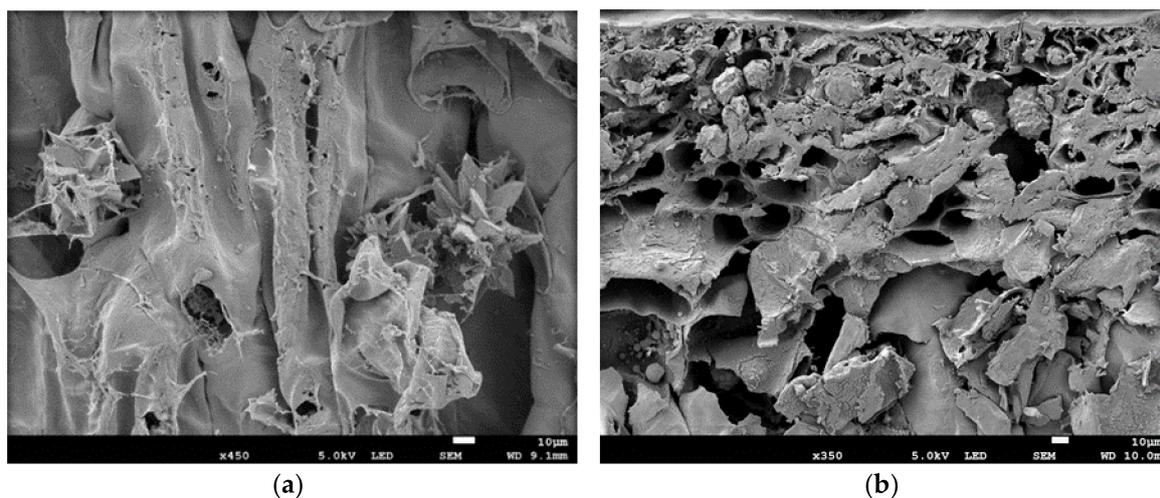


Figure 2. Fresh cladode parenchyma cells demonstrating different calcium oxalate crystal morphologies as (a) *Opuntia ficus-indica* (40–60 μm at $\times 450$): three large whewellite stellate druses; (b) *Opuntia robusta* (± 25 μm at $\times 550$): three smaller crystals close to the epidermis.

2.2. Differences in Nutrient Content Observed over the Six Months of Harvest

The cladode samples were harvested from the orchard situated in a semi-arid area where drastic changes in the climate takes place between summer and winter months. In summer (December to March), hot extreme daytime temperatures (above 30 $^{\circ}\text{C}$) with frequent rainfall (above 35 mm) prevail, while the winters (June to September) are dry (below 18 mm), cold (-5 to 5 $^{\circ}\text{C}$) and frost is severe [26]. Regarding the maintenance of the orchard, fertilization of the soil using 200 kg/ha KCl (50% K) took place at the end of April [27].

2.2.1. Gross Energy and Digestible Carbohydrates

Gross energy levels increased significantly from February to May and again to August (Table 1). This increase was attributed to the slight, yet cumulative increases that were observed in the EFC (Table 1) as well as the increases in the carbohydrate and starch (Table 1) content over the same period. In fact, February and April mucilage powders had significantly lower digestible carbohydrate content levels compared to the other months (Table 1). The starch content dropped from February to April, before increasing every month to $7.69/100$ g (significantly higher $p = 0.032$). Thus, the cladodes harnessed higher energy-containing components in late winter months. Lower gross energy content was reported in cladode flour (6.9 kJ/g) [28]. The mean digestible carbohydrate values obtained in this study of carbohydrates and starch was in the range of carbohydrates ($60/100$ g) and starch (7.63 – $13.9/100$ g) values reported [29] in cladodes.

2.2.2. Ash and Mineral Content

The ash values (Table 3) were significantly higher ($p < 0.001$) in February and April, compared to May to August. This decrease over months is the consequence of the decreases observed in most of the major and minor dietary elements (Table 3).

The values obtained in this study were lower than the ash values reported [15] (34.0%) for mucilage obtained from Ethiopia in February and March, which are warm (25 – 30 $^{\circ}\text{C}$) and dry months.

The Ca content in February and April dropped significantly ($p < 0.001$) in May and never fully recovered until August. Similar to the ash values, the Ca values were higher in February and April ($p < 0.001$). Values of $3.4/100$ g [21] and $3.6/100$ g [30] compared well with mean Ca values reported in this study. Ca is bioavailable as the oxalate: calcium ratio is ≥ 1 [25] in cladodes. The consumption of young cladodes (nopalitos) increase Ca intake in the diet [13].

The content of Na in February was significantly higher ($p = 0.034$) than June, although it recovered somewhat in August (Table 3). The Mn content decreased significantly ($p = 0.005$) from February to April and showed some recovery in July. These values compared well with Mn values reported [13] in cladodes (30 and 290 mg/kg).

The Fe content also decreased significantly from February to April; however, a recovery took place in June and continued with significantly high ($p = 0.002$) levels in August. The Cu content decreased significantly from February to May and showed a slight trend towards recovery by August. The Zn content differed significantly between months, but no trends were apparent. The mean Zn content was higher than 0.8 mg/kg [12]. The fluctuations seen in this study were attributed to changes in climate (temperature, rainfall and frost), rather than cladode maturity as suggested by [13].

3. Materials and Methods

3.1. Sample Collection

Cladodes were obtained at the Waterkloof experimental cactus pear orchard near Bloemfontein, Free State, South Africa (29°10'53" S, 25°58'38" E). The area is 1348 m above sea level and receives 556 mm average annual rainfall. It is a summer rainfall, semi-arid region with winter frost, where the mid-day temperature is 26 to 29 °C in summer (January to April) and cools down during May to night temperatures close to 0 °C in winter (June, July) [26].

The orchid hosts forty *Opuntia ficus-indica* cultivars and two *Opuntia robusta* cultivars laid out in a randomized complete block design, with two replications per cultivar.

The mature cladodes from the current growth season (approximately six months old) of three *O. ficus-indica* cultivars (Algerian, Morado and Gymno-Carpo) and *O. robusta* (Robusta) were harvested for mucilage extraction, drying and evaluation. One cladode from each of the five plants, from both replications, were harvested in the post fruit-harvest stage, between 9:00 and 11:15 on 25 February, 15 April, 20 May, 10 June, 15 July and 12 August 2015; thus, from peak summer to late winter. The sampling of fresh cladode tissue for scanning electron microscopy (SEM) was done in February 2016. In order to standardize samples, young good-quality cladodes were collected from the middle and north side of the plant, at 1 m height. They were labelled, packaged and transported to the laboratory where they were refrigerated. Extraction of mucilage proceeded according to a patented method [31] that involved slicing, microwave cooking for 4 min (800 W), macerating, centrifuging at 8000 rpm for 15 min and freeze-drying for 72 h in a Perano freeze-drier at -60 °C. In order to prevent moisture absorption, one g calcium citrate was added to 20 g of mucilage powder (5%), before being packaged in sealed containers [32] and frozen (-18 °C). The values resulting from subsequent analysis are presented on an "as is" basis (not "dry matter"), as it would be marketed and sold in its present form. Moisture content of freeze-dried powders ranged from 14.0 to 18.8/100 g (means 15.8/100 g) [33].

3.2. Methods

The Leco Automatic Calorimeter AC-500 series Oxygen Combustion Vessel (LECO Corporation, St. Joseph, MI, USA) was used to determine the gross energy as kJ/kg [34]. For Crude Protein (CP), nitrogen (N) was determined by thermal combustion using 0.6 g freeze-dried mucilage samples in a Leco Nitrogen analyser (FP-528, LECO Corporation, St. Joseph, MI, USA) [34]. Crude protein (CP/kg) was calculated by multiplying the N content by a factor of 6.25 [35].

For the separation of proteins, SDS-PAGE was performed on a C.B.S. DSG-200-02 instrument (C.B.S. Scientific Company, INC, Del Mar, CA, USA) at 16 °C. Freeze-dried mucilage samples of the four cultivars from February, May, June and August (0.2 g) were prepared [36]. February and August samples were used since it represented opposing growth stages and seasons of peak summer and late winter, while May and June samples represented the intermediary months. The samples (25 mL)

were loaded into a gel matrix with the Precision plus protein all blue standard (Bio-Rad catalogue # 161-0373).

Total lipid content was quantitatively extracted [37], using chloroform and methanol in a ratio of 2:1. Total extractable fat content (EFC) was determined gravimetrically and expressed as % fat (w/w) per 100 g mucilage. Fatty acid analysis (FAME) (% of total fatty acids) was carried out using 10 mg of the total lipid extraction [38]. The FAME samples were identified by comparing relative peak retention times with standards obtained from SIGMA (189–19). Fatty acids were expressed as a percentage of the total of all fatty acids present in the sample. The total saturated fatty acids (SFA), total monounsaturated fatty acids (MUFA), total polyunsaturated fatty acids (PUFA) and PUFA/SFA ratios were calculated using the fatty acid data.

The total carbohydrate (%) content for mucilage was determined by subtracting the total moisture, protein, fats and ash contents [39]. Neutral-detergent fibre (NDF) and acid-detergent fibre (ADF) were determined using 1 g of freeze-dried mucilage samples [40,41]. This was expressed as g/kg. Starch in freeze-dried mucilage was determined according to the UV-method assay by Boehringer Mannheim/R-Biopharm Enzymatic Bioanalysis/Food Analysis Starch UV Method Cat No.:10,207,748,035 [42]. The starch content $\mu\text{mol/g}$ was converted to g/100 g.

The mineral analysis and ash determinations [43] were performed according to standard methods [44], using two grams of the freeze-dried powder of each sample in duplicate. Exchangeable Ca, Mg, K and Na ($1 \text{ mol dm}^{-3} \text{ NH}_4\text{OAc}$ at pH 7) and extractable Cu, Fe, Mn and Zn (DTPA solution) were determined by atomic absorption. Phosphorous (P) was determined colorimetrically (430 nm) according to the Vanado-Molybdate method [43].

Scanning electron microscopy (SEM) was conducted on both fresh cladode tissue and freeze-dried samples. The fresh cladode tissue was obtained from the four cultivars in February 2016. The freeze-dried mucilage powder samples from the previous year (2015) (as previously described) from the first month (February 2015) and the last month (August 2015) were used as the samples represent the opposing growth stages and seasons of peak summer and late winter. Freshly cut samples were prepared according to a method described by [45]. All samples were mounted on a metal stub, and a sputter coater was used to coat it with gold [45]. The samples were inspected using a JEOL scanning electron microscope (JSM-7800F Extreme-resolution Analytical Field Emission JEOL FE-SEM, JEOL USA, Inc., Peabody, MA, USA).

3.3. Statistical Methods

Analysis of variance (ANOVA) [46] was used to determine the effect of month of harvest and cultivar on the nutrient composition of freeze-dried mucilage samples. The Tukey-Kramer multiple comparison test ($\alpha = 0.05$) was carried out to determine whether significant differences exist between treatment means [46].

4. Conclusions

The protein found in the mucilage powders may be too low to be nutritionally important, yet it provides mucilage with the ability to act as a functional ingredient. The change in climate from February to August significantly influenced the energy and mineral content. Mucilage extracted from cladodes in late winter months harnessed higher energy-containing components, while the Ca, Na, Mn, Fe and Cu contents dropped significantly after February. Optimal harvest time for mineral content was directly after the fruit harvest in February, as the energy content is the lowest and the dietary minerals the highest.

The protein fingerprint, EFC, fibre, K and Mg content and the shape and occurrence of calcium oxalate crystals were significantly different between the four cultivars. *O. robusta* powders had the highest content of crude protein, EFC and K, while the fresh cladode tissue had smaller and fewer calcium oxalate crystals. However, the three *O. ficus-indica* cultivars had the highest content of polyunsaturated fats (Linoleic and α -Linolenic acid). The selection of specific cultivars for their most

beneficial components is recommended, depending on the purpose of mucilage powder application in functional food products. However, *O. robusta* Robusta harvested directly after the fruit harvest in February is recommended for the production of mucilage powders.

Cactus pear mucilage is a promising hydrocolloid that could replace unwanted ingredients in functional food products. The highest-quality mucilage was obtained from cladodes harvested in hot and dry conditions; therefore, it may present a rare opportunity for the commercial cultivation of cactus pears in arid and semi-arid areas.

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Abbreviations

CAM	Crassulacean acid metabolism
EFC	Extractable fat content
MUFA	Monounsaturated fatty acids
PUFA	Polyunsaturated fatty acids
SFA	Saturated fatty acids

References

1. Li, J.M.; Nie, S.P. The functional and nutritional aspects of hydrocolloids in foods. *Food Hydrocoll.* **2014**, *53*, 46–61. [[CrossRef](#)]
2. Du Toit, A.; De Wit, M.; Naudé, S.; Taljaard, M.; Fouché, H.J.; Hugo, A.; Venter, S.L. Determination of the functional properties of cactus pear mucilage from cladodes of four South African cultivars. In Proceedings of the 9th International Congress on Cactus Pear and Cochineal, Coquimbo, Chile, 26–30 March 2017.
3. Sepúlveda, E.; Sáenz, C.; Aliaga, E.; Aceituno, C. Extraction and characterization of mucilage in *Opuntia* spp. *J. Arid Environ.* **2007**, *68*, 534–545. [[CrossRef](#)]
4. Cárdenas, A.; Higuera-Ciapara, I.; Goycoolea, F.M. Rheology and aggregation of cactus (*Opuntia ficus-indica*) mucilage in solution. *J. Prof. Assoc. Cactus Dev.* **1997**, *2*, 152–159.
5. Du Toit, A.; De Wit, M.; Seroto, K.D.; Fouché, H.J.; Hugo, A.; Venter, S.L. Rheological characterization of cactus pear mucilage for application in nutraceutical food products. In Proceedings of the 9th International Congress on Cactus Pear and Cochineal, Coquimbo, Chile, 26–30 March 2017.
6. Rocchetti, G.; Pellizzoni, M.; Montesano, D.; Lucini, L. Italian *Opuntia ficus-indica* cladodes as rich source of bioactive compounds with health-promoting properties. *Foods* **2018**, *7*, 24. [[CrossRef](#)] [[PubMed](#)]
7. Nazareno, M.A. New insights about medicinal uses and health-beneficial properties of cactus products. In Proceedings of the 7th International Congress on Cactus Pear and Cochineal, Agadir, Morocco, 17–22 October 2010.
8. Feugang, J.M.; Konarski, P.; Zou, C.; Stintzing, F.C. Nutritional and medicinal use of cactus pear (*Opuntia* spp.) cladodes and fruits. *Front. Biosci.* **2006**, *11*, 2574–2589. [[CrossRef](#)] [[PubMed](#)]
9. Salisbury, F.B.; Ross, C.W. *Plant Physiology*, 4th ed.; Wadsworth Publishing Company: Belmont, CA, USA, 1992, ISBN 100534151620.
10. Van Sittert, L. ‘Our irreplaceable fellow-colonist’: The biological invasion of prickly pear (*Opuntia ficus-indica*) in the Eastern Cape c.1890–c.1910. *J. Hist. Geogr.* **2002**, *28*, 397–419. [[CrossRef](#)]
11. Moreno, F.J.; Clemente, A. 2S Albumin Storage Proteins: What Makes them Food Allergens? *Open Biochem. J.* **2008**, *2*, 16–28. [[CrossRef](#)] [[PubMed](#)]

12. El-Mostafa, K.; El Kharrassi, Y.; Badreddine, A.; Andreoletti, P.; Vamecq, J.; El Kebbij, M.S.; Latruffe, N.; Lizard, G.; Nasser, B.; Cherkaoui-Malki, M. Nopal cactus (*Opuntia ficus-indica*) as a source of bioactive compounds for nutrition, health and disease. *Molecules* **2014**, *19*, 14879–14901. [[CrossRef](#)] [[PubMed](#)]
13. Hernández-Urbiola, M.I.; Contreras-Padilla, M.; Pérez-Torrero, E.; Hernández-Quevedo, G.; Rojas-Molina, J.I.; Cortes, M.E.; Rodríguez-García, M.E. Study of nutritional composition of nopal (*Opuntia ficus-indica* cv. Redonda) at different maturity stages. *Open Nutr. J.* **2010**, *4*, 11–16. [[CrossRef](#)]
14. Majdoub, H.; Roudesli, S.; Picton, L.; Le Cerf, D.; Muller, G.; Grisel, M. Prickly pear nopals pectin from *Opuntia ficus-indica* physico-chemical study in dilute and semi-dilute solutions. *Carbohydr. Polym.* **2001**, *46*, 69–79. [[CrossRef](#)]
15. Gebresamuel, N.; Gebre-Mariam, T. Comparative physico-chemical characterization of the mucilages of two cactus pears (*Opuntia* spp.) obtained from Mekelle, Northern Ethiopia. *J. Biomater. Nanobiotechnol.* **2012**, *3*, 79–86. [[CrossRef](#)]
16. Fenema, O.R. *Food Chemistry*, 3rd ed.; Fennema, O.R., Ed.; Marcel Dekker: Madison, WI, USA, 1996, ISBN 0824793463.
17. Akoh, C.C. Fat replacers. *Food Technol.* **1998**, *52*, 47–53.
18. Charley, H. *Food Science*, 2nd ed.; John Wiley and Sons: Etobicoke, ON, Canada, 1982.
19. Whitney, E.; Rolfes, S.R. *Understanding Nutrition*, 14th ed.; Wadsworth Publishing Company: Belmont, CA, USA, 2015, ISBN 101305396456.
20. Bennion, M. *Introductory Foods*, 8th ed.; Collier Macmillan Publishers: New York, NY, USA, 1985.
21. Sáenz, C. Cladodes: A source of dietary fiber. *J. Prof. Assoc. Cactus Dev.* **1997**, *2*, 117–123.
22. Franceschi, V.R.; Horner, H.J. Calcium oxalate crystals in plants. *Bot. Rev.* **1980**, *46*, 361–427. [[CrossRef](#)]
23. McConn, M.M.; Nakata, P.A. Oxalate reduces calcium availability in the pads of the prickly pear cactus through formation of calcium oxalate crystals. *J. Agric. Food Chem.* **2004**, *52*, 1371–1374. [[CrossRef](#)] [[PubMed](#)]
24. Contreras-Padilla, M.; Rivera-Muñoz, E.M.; Gutiérrez-Cortez, E.; del López, A.R.; Rodríguez-García, M.E. Characterization of crystalline structures in *Opuntia ficus-indica*. *J. Biol. Phys.* **2015**, *41*. [[CrossRef](#)] [[PubMed](#)]
25. Rojas-Molina, I.; Gutiérrez-Cortez, E.; Bah, M.; Rojas-Molina, A.; Ibarra-Alvarado, C.; Rivera-Muñoz, E.; Del Real, A.; Aguilera-Barreiro, M. Characterization of calcium compounds in *Opuntia ficus-indica* as a source of calcium for human diet. *J. Chem.* **2015**, *7*, 1–8. [[CrossRef](#)]
26. World Weather and Climate Information Bloemfontein, South Africa. Available online: <https://weather-and-climate.com/average-monthly-Rainfall-Temperature-Sunshine,bloemfontein,South-Africa> (accessed on 24 June 2016).
27. Potgieter, J.P. The Influence of Environmental Factors on Spineless Cactus Pear (*Opuntia* spp.) Fruit Yield in Limpopo Province, South Africa. Ph.D. Thesis, University of the Free State, Bloemfontein, South Africa, 2007.
28. Sáenz, C.; Pak, N.; Lecaros, M.; Sepulveda, E. Chemical and physical characterization of cactus cladode (*Opuntia ficus-indica*) powder. *Ital. J. Food Sci.* **2010**, *22*, 416–423.
29. Ayadi, M.A.; Abdelmaksoud, 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. [[CrossRef](#)]
30. Aguilera-Barreiro, M.; Rivera-Marquez, J.A.; Trujillo-Arriaga, H.M.; Orozco, J.A.T.; Barreira-Mercado, E.; Rodríguez-García, M.E. Intake of dehydrated nopal (*Opuntia ficus-indica*) improves bone mineral density and calciuria in adult Mexican woman. *Food Nutr. Res.* **2013**. [[CrossRef](#)] [[PubMed](#)]
31. Du Toit, A.; De Wit, M. A Process for Extracting Mucilage from *Opuntia ficus-indica* and *Aloe barbadensis* 2011. Patent No. PA153178/P, 12 May 2011.
32. Aeckerle, E. Process for Improving the Stability of Hygroscopic Substances 1941. U.S. Patent US2238149A, 15 April 1941.
33. Du Toit, A. Selection, Extraction, Characterization and Application of Mucilage from Cactus Pear (*Opuntia ficus-indica* and *Opuntia robusta*) Cladodes. Ph.D. Thesis, University of the Free State, Bloemfontein, South Africa, 2016.
34. *Leco FP-528 Protein/Nitrogen Determinator. FP-528 Instruction Manual*, version 1.2.; Leco Corporation: Saint Joseph, MI, USA, 2001.
35. Association of Official Analytical Chemists (AOAC). *Official Methods of Analysis of AOAC International*, 17th ed.; Horwitz, Ed.; AOAC: Washington, DC, USA, 2000; Volume 1.

36. Laemmli, U.K. Most commonly used discontinuous buffer systems for SDS electrophoresis. *Nature* **1970**, *277*, 680–685. [[CrossRef](#)]
37. Folch, J.; Lees, M.; Slaone-Stanley, G.H. A simple method for the isolation and purification of total lipids from animal tissue. *J. Biol. Chem.* **1957**, *226*, 497–509. [[PubMed](#)]
38. Park, P.W.; Goins, R.E. In situ preparation of fatty acid methyl esters for analysis of fatty acid composition in foods. *J. Food Sci.* **1994**, *59*, 1262–1266. [[CrossRef](#)]
39. Food and Agriculture Organization (FAO). Food and Agriculture Organization (FAO). Food energy—Methods of analysis and conversion factors. In *Food and Agriculture Organization of the United Nations Technical Workshop Report*; FAO: Rome, Italy, 2003; pp. 1–7, ISBN 92-5-105014-7.
40. Goering, H.K.; Van Soest, P.J. Forage fiber analyses (apparatus, reagents, procedures, and some applications). In *Agriculture Handbook*; U.S. Agricultural Research Service: New Orleans, LA, USA, 1970; p. 20.
41. Roberson, J.B.; Van Soest, P.J. The Detergent System of Analysis and its application to human foods. In *The Analysis of Dietary Fiber in Food*; James, W.P.T., Theader, O.D., Eds.; Marcel Dekker: New York, NY, USA, 1981; pp. 123–158.
42. Anonymous. *Methods of Biochemical Analysis and Food Analysis Using Test-Combinations*; Boehringer Mannheim GmbH: Mannheim, Germany, 1986.
43. Hesse, P.R. *A Textbook of Soil Chemical Analysis*; John Murray Ltd.: London, UK, 1971.
44. The Non-Affiliated Soil Analysis Work Committee. *Handbook of Standard Soil Testing Methods for Advisory Purposes*; Soil Science Society of South Africa: Pretoria, South Africa, 1990.
45. Glauert, A.M. (Ed.) *Practical Methods in Electron Microscopy*, 3rd ed.; North Holland Publishing Company: Amsterdam, The Netherlands, 1974.
46. NCSS Statistical Software. *Statistical Systems for Windows: Number Cruncher Statistical System*; NCSS: Kaysville, UT, USA, 2007.

Sample Availability: Samples of the compounds are not available from the authors.



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