### REPUBLIC OF SOUTH AFRICA PATENTS ACT, 1978

## COMPLETE SPECIFICATION

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## **BACKGROUND TO THE INVENTION**

This invention relates to a process for extracting mucilage from succulent plants. In particular, this invention relates to a process for extracting mucilage from *Opuntia ficus-indica* (cactus pear), *Aloe barbadensis* (aloe vera) and *Agave Americana* (century plant).

The *Opuntia* genus belongs to the Cactacease family and originates from South America (Mexico), whilst the *Aloe barbadensis* originates from Northern Africa. Both plants are succulent plants and as a result are characterised by their production of slime that mainly consists of mucilage. Mucilage is found in the thick slimy fluid when cutting through a cladode (cactus leaf) or aloe vera leaf. This slimy material is commonly referred to as "nopal dribble" and exhibits the property to retain water. Mucilage is a complex polysaccharide carbohydrate, with a highly branched structure called a gum or hydrocolloid. Hydrocolloids are water-soluble dietary fibres that can be used as a healthy additive for commercial food products.

Hydrocolloids or gums are not true colloids, but polymers of colloidal size that remain suspended in water and form molecular solutions in most cases. Mucilage can be classified as a hydrocolloid as it is a long-chain polymer that dissolves in water to give a thickening or viscosity producing effect. Mucilage from *Opuntia ficus indica*, *Aloe barbadenis* and *Agave Americana* has the capacity to absorb huge amounts of water, therefore forming a viscous colloid with interesting rheological properties and has potential uses as an additive for several industrial products.

In addition to being natural, healthy and inexpensive, mucilage has the potential to be used as a fat mimic and a commercial emulsifier in food products. It is also known for its nutritional value and medicinal properties.

Mucilage is a very important source of soluble fibre and products using the whole cladode contain large amounts of both insoluble and soluble fibre. The advantage to the digestive system is that the insoluble fibre binds to toxins and the soluble fibre increases stool bulk. Mucilage found in cladodes is not hydrolyzed nor absorbed by the human digestive system, but they can make up the greater part of the alimentary fibre.

Mucilage is a soluble dietary fibre, which is associated with decreasing cholesterol levels and control of glucose in the blood. It diminishes the risk of cancer in more than one way. Firstly, it reduces the risk of cancer such as colon cancer because of the capacity to hold water that insures stool bulk. The fermentation of soluble fibre during digestion also produces rapid intestinal transit. Secondly, due to the presence of lignin, dietary fibre also has anti-oxidation properties through the prevention of free radical formation. Other medicinal uses of mucilage include, improving utilization of glucose at a cellular level, preventing the replication of DNA and RNA viruses, possibly acting as a prebiotic to promote the growth of probiotic bacteria, the prevention of stomach ulcers, acting as an anti-inflammatory agent and in the treatment of wounds.

Given the significance and vast uses of mucilage, what is important are the processes used to extract mucilage from succulent plants.

According to Sepúlveda *et al.*, Journal of Arid Environments 68 (2007) the three most important factors in mucilage extraction is the consideration of variables, such as, the relationships between pad/water, the temperature conditions and the type of alcohol used for precipitation.

Prior art teaches the extraction of mucilage by homogenizing it with water and then precipitating with ethanol. Medina -Torres et al., Food

Hydrocolloids 14 (2000) used acetone to precipitate the mucilage, washed it with 2-propanol and finally dried it. Majoub *et al.*, (2001) used petroleum ether and freeze-dried or purified by ultra-filtration and produced an end product that had no salts and no compounds of low molecular weight.

The extraction method proposed by Goygoolea & Cárdenas (2003), Pectins from *Opuntia spp.*: A Short Review. *Journal of the Professional Association of Cactus Development* 5: 17-29, is depicted in Figure 1.

Sepúlveda *et al.*, (2007) found that the best yields of mucilage were obtained with pad/water ratio at 1:7, a temperature of 40+2 °C and at 4 hours of extraction. They also claim that there are no significant differences in terms of yield between the alcohol type and the alcohol/water ratio. The moisture content was between 5.6 and 6.2 %. The ash content was at average 37.3 %. The nitrogen content was 1.2 %, calcium 8.3 % and potassium was 1.2 %. The results of the study showed that average yield after drying was 1.48 % based on fresh weight and 19.4 % based on dry weight. It was pointed out that yield is very dependent on climatic conditions, such as cold and rain due to the ability of the mucilage to absorb water as a defence against stress conditions.

Cárdenas *et al.*, (1997) freeze-dried the filtrate and found a dry mucilage yield of approximately 0.7 g/kg of cladodes.

In a study done by Peňa Valdivia et al., (2006) to evaluate variability of pectin and mucilage in 13 varieties, the average mucilage content of napolitos was 6.35 %, which contrasts sharply with the amount found in fruit (0.91 %). They also found that boiling the napolitos in 150ml distilled water for 15 minutes partly modified the nopalito composition therefore releasing a significant portion of the unavailable carbohydrates, like mucilage and pectin.

The content of dry matter from the cladodes ranged between 9 % and 19 %. However, it is very important to note that mucilage content increases

during acclimation to low temperatures and to drought. It also depends on the climatic conditions at the time of the pad's collection such as cold or rain. The mucilage obtained during very dry climatic conditions is more viscous. (Sepúlveda *et al.*, 2007).

Conventional solvent extraction methods do not produce the yields necessary for commercial viability. It is accordingly an object of the present invention to provide for a simple and effective process for the extraction of mucilage from succulent plants, namely, *Opuntia ficus-indica*, *Aloe barbadensis and Agave Americana*.

#### SUMMARY OF THE INVENTION

According to the present invention, there is provided a process for extracting mucilage from succulent plants, in particular, the cladodes of the *Opuntia ficus-indica* or the leaves of the *Aloe barbadensis* or *Agave Americana* the process comprising the steps of:

- a) providing a source of cladodes or leaves;
- b) cooking the cladodes or leaves using microwave energy; and
- c) centrifuging the mucilage pulp to separate it from the solids and obtain the mucilage itself.

It is preferable to carry out a maceration step after the cooking step b). Cooking the cladodes or leaves first, makes it softer and easier to mince. Alternatively, the maceration step may be performed prior to the cooking step b). The method of first mincing, then cooking before centrifuging is the preferred method of extracting mucilage. It is also preferable to slice the cladodes or leaves into a size that is easy to handle, prior to the cooking step b).

The cladodes or leaves should be peeled to give a better yield. It is important to cut away all the hard and unnecessary fibres that do not yield

any mucilage. Further, the addition of water to the cooking process helps to separate the mucilage from the solids as it dissolves in the water. However, the water dilutes the mucilage and the viscosity of the final mucilage is low. Adding no water produces a more viscous product, which is advantageous for emulsifying and fat-mimicking properties.

The cooking is carried out in a microwave oven having a power in the range of between 300W to 3000W, preferably 800W to 1700W. It is preferable that the cooking is done in a 900W microwave oven at 100% power for 4 minutes. The cladodes or leaves may be fresh or dried.

Water may be added during the maceration stage. The maceration may be carried out using a blender, extractor or mincer. When no water is added during the maceration step, then it is preferable to use a mincer. Alternatively, finely cutting the cladodes is also possible.

According to a further aspect of the invention, the mucilage obtained by the method described above may be used as a functional ingredient in food processing. In particular, the mucilage is used as an emulsifier in mayonnaise. Further, the mucilage obtained by the method of the invention can also be used to replace fat, in particular, pork fat in meat emulsions, or vegetable oil in mayonnaise, as a fat replacer in baked products, as a gelling agent in candy (Turkish Delight), or as a foaming agent in marshmallow.

According to a further embodiment of the invention, there is provided a meat emulsion comprising the mucilage as obtained by the method of the present invention. There is also provided a mayonnaise composition comprising the mucilage obtained by the method of the invention. There is further provided a baked product, candy, in particular Turkish Delight or marshmallow comprising the mucilage obtained by the process above.

## BRIEF DESCRIPTION OF THE FIGURES

The invention will now be described in more detail, by way of example only, with reference to the accompanying figures in which:

- Figure 1 is a schematic representation of prior art laboratory -scale extraction protocols for the isolation and purification of pectins from *Opuntia* cladodes, namely a gelling extract (GE) and a neutral non-gelling mucilage extract (NE);
- Figure 2 is a graph indicating the results from testing the three different maceration methods for the optimization of mucilage extraction;
- Figure 3 is a graph showing the % yield of mucilage from macerated pulp;
- Figure 4 is a graph showing the viscosity of mucilage before and after ageing;
- Figure 5 is a graph showing the relationship between viscosity and yield.
- Figure 6 is a representation of the variance quantitative descriptive analysis (QDA) test for the carrot cake.
- Figure 7 is a representation of the variance quantitative descriptive analysis (QDA) test for the turkish delight.

### **DETAILED DESCRIPTION OF THE INVENTION**

The most effective method of extracting mucilage from cladodes as well as producing the best possible yield and viscosity is as follows:

### Step 1: Peeling:

It is important to cut away all the hard and unnecessary fibres that do not yield any mucilage. The green part of the cladode as well as the hard fibres inside the green peel must be cut away. Only the light green slimy inside part of the cladode should remain.

### Step 2: Slicing

The inside part of the cladodes into a size that is easy to handle.

### Step 3: Cooking

The cladodes pieces are cooked for the purpose of softening it before the mincing process and for the cladodes to release more mucilage. Cooking is done in the microwave oven at 100% power for 4 minutes or until the cladode pieces are soft.

#### Step 4: Maceration:

Finely mincing or cutting the cladodes is the most effective way of macerating the cladodes. A Milex 4-in-1 multi-purpose Mean Juice Machine model MMJ004 was used. It has a fast moving rotating blade that macerates the cladode pieces without the addition of water. The cooked cubes are easier than the raw pieces to macerate. Cooked pieces are softer and therefore take less time to finely macerate. More cooked than raw pieces can be macerated at a time.

### Step 5: Centrifuge:

The mucilage pulp is divided into the centrifuge containers and centrifuged at 8000 rpm for 15 minutes to separate it from the solids and obtain the mucilage itself. A rotohead setting of 18 and temperature of 4°C was used.

The invention will now be described in more detail with reference to the following examples.

## 1. Evaluation of the maceration method on mucilage extraction

A fresh cladode was washed and the spines were removed. It was divided into three 100g samples. A Milex 4-in-1 multi-purpose Mean Juice Machine, model MMJ004 was used. This machine has three different applications that could be used to macerate the samples; the extractor, the mincer and the liquidizer. Three different methods were used to macerate the unpeeled samples: (a) the juice extractor, (b) the blender (liquidizer) and (c) the mincer.

During the process, 50 ml water was added to the (b) blending process in order to bring the cladodes in contact with the blades. The macerated pulp was then centrifuged at 8000 rpm, 4° C, for 15 minutes.

The results from testing the three different maceration methods are depicted in Figure 2. Figure 2 confirms the following:

Extractor. The mucilage did not extract during centrifugation. The product was too thick and slimy and did not separate during centrifugation.

Blender with added water. The supernatant was very watery and thin. The addition of water during maceration dilutes the mucilage and the viscosity is too low.

*Mincer:* The texture and yields were very good considering that no water was added during maceration; the supernatant was viscous but separated readily from the solids during centrifugation.

## 2. Evaluation of ageing on mucilage extraction

To determine the effect of ageing on the yield and viscosity of mucilage from minced pulp the following experiments were done:

Sample 1: Raw: cladodes were washed, peeled, minced, centrifuged and measured the same day.

Sample 2: Raw: cladodes were washed, peeled, minced, centrifuged and aged for 5 days before measuring.

Sample 3: Cooked: cladodes were washed, peeled, minced, cooked, centrifuged and measured the same day.

Sample 4: Cooked: cladodes were washed, peeled, minced, cooked, aged for 5 days and then centrifuged before measuring.

A Milex 4-in-1 multi-purpose Mean Juice Machine, model MMJ004 mincer was used to macerate the peeled cladodes. Samples 2 and 4 were aged at 4 °C for five days in order to age the mucilage pulp. The macerated pulp was then centrifuged at 8000 rpm, 4 °C, for 15 minutes. The yields for each different mucilage extraction method are depicted in table 1 below.

Sample	Treatment	Yield: mucilage from minced pulp
Sample 1	Raw: cladodes were washed, peeled, minced, centrifuged, measured same day.	46.95 %
Sample 2	Raw: cladodes were washed, peeled, minced, centrifuged and aged 5 days before measuring	46.14 %
Sample 3	Cooked: cladodes were washed, peeled, minced, cooked, centrifuged and measured same day.	54.67 %
Sample 4	Cooked: cladodes were washed, peeled, minced, cooked, aged 5 days and then centrifuged before measuring.	62.73

Table 1

- In raw mucilage ageing caused a slightly lower yield, with cooked mucilage ageing caused a higher yield.
- It was observed that the viscosity of aged mucilage was higher.
- It was evident that using recently extracted or aged mucilage had no significant effect on the yield produced in the extraction process, and therefore should not be included in the final extraction procedure.

## 3. Evaluation of the effect of heat on optimal mucilage extraction

The experiments in point 2 above were done to compare the yield from raw and cooked minced pulp. In samples 3 and 4, the minced pulp was cooked without adding water, by placing in the microwave for 4 minutes at 100 % power, stirring after 2 minutes. The internal temperature reached 85°C. The minced pulp was then centrifuged at 8000 rpm, 4 °C, for 15 minutes.

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Sample 3 was measured on the same day, and sample 4 was aged at

4 °C for five days. When sample 4 was examined after ageing, the

weight and volume was similar to that of the day of extraction.

Based on the table above, it is evident that cooking produces a

greater yield as both cooked samples produced significantly greater

vields.

4. Evaluation of the combined elements for optimal extraction

procedures

Nine individual extraction procedures were carried out on nine

cladodes to determine the highest yield together with the most

viscous mucilage:

raw or cooked

minced before or after cooking

fresh or allowed to stand for a few days

cooking by steaming or in a microwave oven.

Fresh, raw cladodes were washed, peeled and sliced. A Milex 4-in-1

multi-purpose Mean Juice Machine, model MMJ004 mincer was used

to macerate the cladodes into pulp. Cooking was done in a 900-Watt

microwave at full power for 4 minutes or steaming over boiling water

for 4 minutes. Refrigeration took place at 4°C.

Centrifugation: Rotorhead: 18

Speed: 8000 rpm

Temp: 4°C

Time: 15 min

Trial layout was as follows:

- Sample 1. A peeled cladode was minced and centrifuged to obtain mucilage. The raw mucilage was weighed and the viscosity was measured on the day of extraction.
- Sample 2. A Peeled cladode was cooked by steaming before being minced and centrifuged to obtain mucilage. The cooked mucilage was weighed and the viscosity measured on the day of extraction.
- Sample 3. A peeled cladode was minced before being cooked in the microwave oven, and centrifuged to obtain mucilage. The cooked mucilage was weighed and the viscosity measured on the day of extraction.
- Sample 4. A peeled cladode was minced and refrigerated for 5 days and centrifuged to obtain mucilage. The raw mucilage was weighed and the viscosity measured on the 5<sup>th</sup> day after extraction.
- Sample 5. A peeled cladode was minced and centrifuged to obtain mucilage and refrigerated for 5 days. The raw mucilage was weighed and the viscosity measured on the day of extraction as well as the 5<sup>th</sup> day.
- Sample 6. A peeled cladode was cooked by steaming, before being minced, centrifuged and refrigerated for 5 days. The cooked mucilage was weighed and the viscosity measured on the day of extraction as well as the 5<sup>th</sup> day.
- Sample 7. A peeled cladode was cooked in the microwave oven before being minced, refrigerated for 5 days and centrifuged. The cooked mucilage was weighed and the viscosity measured on the 5<sup>th</sup> day after extraction.

Sample 8. A peeled cladode was minced before being cooked in the microwave oven, centrifuged, and refrigerated for 5 days.

The cooked mucilage was weighed and the viscosity measured on the day of extraction as well as the 5<sup>th</sup> day.

Sample 9. A peeled cladode was minced before being cooked in the microwave oven, refrigerated for 5 days and centrifuged. The cooked mucilage was weighed and the viscosity measured on the 5<sup>th</sup> day after extraction.

The mucilage was weighed and viscosity was tested to obtain individual results, then the results for viscosity and yield were combined and compared. Samples 1, 2, 3, 5, 6 and 8 were not aged, and tested first. Samples 4, 7 and 9 were aged before being tested.

### 4.1. Yield determination

The cladodes were weighed before being peeled, cooked and minced according to the experiment. The mucilage and pulp were both individually weighed and the volume of the mucilage measured. The results are set out in Table 2 below.

Sample	Whole cladodes	Peeled cladodes	Peeled cladodes after cooking	Minced Pulp, ready to centrifuge	Mucilage	Yield of mucilage from pulp	Yield of mucilage from whole cladodes
	g	g	g	g	g	%	%
1	975	341.25	-	273	136.5	50	14.0
2	805.25	281.84	225.47	223.21	125	56	15.5
3	840.01	294.00	235.20	232.85	144.6	62.1	17.2
4	1009.52	353.33	-	282.67	127.2	45	12.6
5	918.81	321.58	-	257.27	118.6	46.1	12.9
6	839.32	293.76	235.01	232.66	91.9	39.5	10.9
7	919.50	321.82	257.46	254.88	143.5	56.3	15.6
8	909.63	318.37	254.70	252.15	134.9	53.5	14.8
9	869.94	304.48	243.58	241.15	151.2	62.7	17.4
Average	898.55	314.49	251.59	249.98	130.38	52.36	14.56

Table 2

In experiments 3, 8 and 9, the mucilage was first minced before cooking in the microwave oven. This gave very high yields, as is evident in the table above. Experiments 1, 4 and 5 that were raw gave low yields. Ageing of the mucilage for 5 days does not give meaningful differences in yield amounts, as there was no significant difference between experiment 3 (not aged) and 9 (aged).

The average yield in this experiment from macerated pulp to mucilage was 52.23% (as can be seen in table 2 and figure 3). The average yield from whole claddodes to mucilage was 14.56%.

This data confirms that the yield of mucilage from this extraction process is higher than the yields from processes used by Cárdenas *et al.* (1997), Medina -Torres *et al.* (2000), Majoub *et al.* (2001), Goygoolea & Cárdenas (2003), Del-Valle *et al.* (2005), Peňa Valdivia *et al.* (2006), Sepúlveda *et al.* (2007) and Iturriaga *et al.* (2009).

Sepúlveda et al., (2007) got 1.48 % based on fresh weight and 19.4 % based on dry weight and in a study done by Peňa Valdivia *et al.*, (2006) the average mucilage content of napolitos were 6.35 %.

### 4.2. Viscosity determination

Viscosity tests were done with the Brookfield Viscometer in CPS with a number three spindle. It was done according to the experiment on the first day of centrifuge and again on the 5th day. Experiments 4, 7 and 9 were only done on the fifth day as they were refrigerated before centrifuging.

Two of the experiments (5 and 6) had a significantly higher viscosity after standing for 5 days, in one sample (8), the viscosity decreased slightly. The results showing the viscosity of mucilage before and after ageing can be seen in table 3 below and figure 4.

SAMPLE	VISCOSITY Day 1	VISCOSITY Day 5
:	cps (centipoise)	cps (centipoise)
1	650	Not tested
2	330	Not tested
3	250	Not tested
4	Not tested	380
5	300	470
6	670	940
7	Not tested	280
8	780	710
9	Not tested	730

Table 3

The relationship between yield and viscosity can be seen in Figure 5.

It was observed that when the yield of mucilage was high, the viscosity was generally lower, when the yield was lower; the viscosity was higher, as could be seen in samples 2, 3 and 7 in Figure 5. In samples 8 and 9 the yield as well as the viscosity was high. Therefore the method of first mincing, then cooking before centrifuging seems to be the best method of extracting mucilage. This result is not used in the final extraction process, since cooking the cladodes first makes it softer and easier to mince. There was no meaningful difference whether time elapsed before or after centrifugation but ageing did make a very significant difference in the viscosity of the mucilage as can be seen when comparing sample 3 (only day 1) (raw) and 9 (only day 5) (cooked) and 5 and 6 (viscosity determined on 1st and 5th day); in Figure 5, but not in the yield, as seen in Figure 5.

### 4.3. Determination of water activity

A Novasina thermoconstanter was used to measure water activity. The water activity for all nine experiments tested between 97.1 and 97.6%. This indicates a highly spoilable product on which mould, yeast and bacteria could flourish.

## 4.4. Determination of protein content

The Kjeldahl method according to AOAC 2000 was used to determine the protein content of the mucilage.

The protein content of mucilage was determined to be 0.23% (AOAC, 2000). This result indicates that mucilage does contain protein but the level of protein is very low. This may indicate that mucilage is a true emulsifier. When Iturriaga *et al.*, (2009) studied the use of cactus mucilage in emulsions; they also found a protein fraction in mucilage. It is known that xanthan and guar gum also contains protein fractions (1-2%). It is thus possible that this protein fraction could provide mucilage with the amphyphilic features needed for acting as a stabilizing agent.

# 5. Application of extracted mucilage as a functional ingredient in food processing.

#### 5.1. Sample collection

When the prickly pear cacti were pruned in May, cladodes were stored for the purposes of this study. Three cladodes from Algerian, Robusta, Nepgen and Ficus Indice were used in order to determine % yield for each cultivar. Cladodes were stored in bags at 4°C. In September when the study commenced, the cladodes were in a very good condition. The cladodes were

firm, dark green and only very few cladodes had evidence of mould growth.

### 5.2. Method of mucilage extraction

The most effective method of mucilage extraction had already been established in the earlier parts of this study.

### Preparation:

- Washed and spines removed.
- Peeled: All green outer part was removed, leaving only the white inner part of the cladode.
- · Cooked: Microwave oven without added water.
- Minced: Milex 4-in-1 multi-purpose Mean Juice Machine, model MMJ004 mincer
- Centrifuge: Speed: 8000 rpm, Time: 15 min, temperature: 4°C. The supernatant was then decanted and weighed.

## 5.3. Yield of mucilage used for mayonnaise and meat emulsions

The mucilage was extracted using the above effective method determined in the earlier study. Three cladodes from four cultivar were used. The yield was determined by weighing each of the whole cladodes, again after it was peeled, cooked and minced and yet again after the mucilage was extracted in the centrifuge. The results of the yield from cladodes to the final mucilage product are depicted in Table 4 below.

From the fresh whole cladodes to the final mucilage product, the yield was approximately 14% for Algerian. Algerian proved to provide the most mucilage (13.98%). Algerian was therefore used to extract the mucilage in mayonnaise and polony manufacture described later in the study.

Cultivar	Weight of whole cladode	Peeled cladode	Weight of peeled cladode after cooking	Weight of minced pulp	Mucilage	Peeled cladode from whole cladode	Minced pulp from peeled cladode	Minced pulp from whole cladode	Mucilage from minced pulp	Mucilage from whole cladode
	g	g	g	g	g	%	%	%	%	%
Algerian										
Algerian 1	1087.00	438.00	399.25	375.98	148.04	40.29	85.84	34.59	39.37	13.62
Algerian 2	1123.00	423.98	356.36	350.10	192.43	37.75	82.57	31.18	54.96	17.14
Algerian 3	900.00	357.55	286.08	285.26	100.77	39.73	79.78	31.70	35.33	11.20
Average	1036.67	406.51	347.23	337.11	147.08	39.26	82.73	32.49	43.22	13.98
Robusta								<u> </u>		
Robusta 1	1002.00	298.63	213.55	199.41	57.68	29.80	66.77	19.90	28.93	5.76
Robusta 2	715.00	251.80	202.70	202.99	65.41	35.22	80.62	28.39	32.22	9.15
Robusta 3	1075.00	359.30	299.48	294.48	119.14	33.42	81.96	27.39	40.46	11.08
Average	930.67	303.24	238.58	232.29	80.74	32.81	76.45	25.23	33.87	8.66
Nepgen										
Nepgen 1	719.00	227.00	160.57	159.57	49.77	31.57	70.30	22.19	31.19	6.92
Nepgen 2	1007.00	355.99	303.09	289.53	39.94	35.35	81.33	28.75	13.79	3.97
Nepgen 3	788.20	228.32	167.17	160.99	17.95	28.97	70.51	20.43	11.15	2.28
Average	838.07	270.44	210.28	203.36	35.89	31.96	74.05	23.79	18.71	4.39
							1			
Ficus Indice										
Ficus Indice 1	518.00	213.42	149.14	147.98	57.58	41.20	69.34	28.57	38.91	11.12
Ficus Indice 2	700.00	224.80	171.25	169.94	74.28	32.11	75.60	24.28	43.71	10.61
Ficus Indice 3	720.00	211.52	172.46	170.85	74.54	29.38	80.77	23.73	43.63	10.35
Average	646.00	216.58	164.28	162.92	68.80	34.23	75.24	25.52	42.08	10.69

Table 4

## 5.4. Mayonnaise formulation

Different formulations for home-made mayonnaise were tested and the following formulation was decided upon:

Ingredients	ml	g	%
Egg yolks, large	40	34	15.93
White vinegar	12	12	5.62
Lemon juice	12	12	5.62
Sugar, white, granulated	2	2.5	1.17
Salt, ionized	2	2.5	1.17
Cayenne pepper	0.5	0.25	0.12
White pepper	0.5	0.25	0.12
Oil, canola	150	150	70.25
TOTAL	219	213.5	100
	1	1	I

Table 5

### Method:

- 1. Mix the egg, vinegar and seasoning.
- 2. Using an electric hand beater, beat while adding oil a drop at a time, and later on in a very thin steady stream.
- When all the oil has been added, the mixture should be a light creamy colour, thick and creamy.

Oil: 70.25%

Egg: 15.93%

Vinegar and lemon juice: 11.24%

# Determination of the emulsifying properties of mucilage in mayonnaise

The emulsifying properties of mucilage were determined by replacing the emulsifying ingredient (egg yolk) with mucilage as seen in table 6 below.

Ingredien	Control	Α	В	С	D
ts					
	Egg yolk				
	Replaceme	Replaceme	Replaceme	Replaceme	Replaceme
	nt	nt	nt	nt	nt
	0%	25%	50%	75%	100%
	ml	ml	ml	ml	ml
Egg yolks,	4	30	20	10	0
large					
Mucilage	0	10	20	30	40
White	12	12	12	12	12
vinegar					
Lemon	12	12	12	12	12
juice					
Sugar,	2	2	2	2	2
white,					
granulate					
d					
Salt,	2	2	2	2	2
ionized					-
Cayenne	0.5	0.5	0.5	0.5l	0.5
pepper				2	
White	0.5	0.5	0.5	0.5	0.5
pepper					
Oil,	150	150	150	150	150
canola					

Table 6

The results and discussions for each sample can be seen in table 7 below. Table 7 shows the percentage mucilage replacement in mayonnaise and the properties of the finished product. The slight amount of separated oil is evident in sample D.

Experiment	Mucilage replacement	Properties of finished mayonnaise
Control	40 ml egg yolk 0 ml mucilage	The texture is smooth and thick. The viscosity is thick and is "resistant to running", it is semi-solid and mouldable.  The mayonnaise has an acceptable taste, acid is evident but not overpowering.  The mayonnaise forms a stable emulsion After 24 hours, emulsification is stable.
A: 25%	30 ml egg yolk 10 ml mucilage	Product has lower viscosity than control Consistency thick enough to make the ribbon Taste is more acidic Emulsification is stable and permanent After 24 hours, emulsion is stable.
B: 50%	20 ml egg yolk 20 ml mucilage	The viscosity is lower than sample A, it is like thick cream.  Taste more acidic than in sample A Emulsion is stable and permanent After 24 hours, emulsion is stable
C: 75%	10 ml egg yolk 30 ml mucilage	The viscosity is lower than sample B, it is like cream It tastes more acidic than sample B Emulsion is stable and permanent After 24 hours emulsion is stable
D: 100 %	40 ml mucilage	The viscosity is lower than sample C. It is like salad cream It tastes very acidic, more than sample C Emulsion is stable and permanent After 12 hours, 1/2 cm separated at bottom, after 24 hours 1 cm separated fluid at bottom. After 3 and 5 days, the separation did not increase any further.

Table 7

# Determination of the fat-mimicking properties of mucilage in mayonnaise.

The fat mimicking properties of mucilage was determined by replacing one third of the canola oil in the mayonnaise formulation with mucilage as seen in table 8. Further, determine the effect of the addition of a commercial additive (xanthan gum) to restore the consistency and flavour of the mayonnaise.

A: No oil replacement

B: Replace 33.3% (50 ml) of canola oil with mucilage.

C: Replace 33.3% (50 ml) of canola oil with mucilage, add 1% Xanthan gum.

Ingredients	Control	Α	В
	Full Fat	Fat Replacement	Fat Replacement
		33.3 %	33.3 % + xanthan
	ml	ml	ml
Egg yolks, large	40	40	40
White vinegar	12	12	12
Lemon juice	12	12	12
Sugar, white, granulated	2	2	2
Salt, ionized	2	2	2
Cayenne pepper	0.5	0.5	0.5
White pepper	0.5	0.5	0.5
Oil, canola	150	100	100
Mucilage	0	50	50
Xanthan gum	0	0	1

Table 8

The results and discussions for this experiment are seen in table 9 below.

Experimen	Experiment % of mucilage replacement		Properties of finished mayonnaise
A: Control	150 ml cañola oil		The texture is smooth and thick. The viscosity is thick and is "resistant to running" it is semi-solid and mouldable. The mayonnaise has an acceptable taste, acid is evident but not overpowering.  The mayonnaise forms a stable emulsion.  After 24 hours, emulsion is stable.
B: mucilage	33.3%	50 ml mucilage 100 ml canola oil	As soon as mucilage is added, the viscosity decreases considerably.  The emulsion has the consistency of thin

C: 33.3%	50 ml mucilage	cream and is very thin. The texture is smooth and creamy. Emulsion is good and stable. Taste is very acidic, unacceptable strong taste of vinegar. After 24 hours: The volume decreased with 2 cm. Bubbles formed on top. Viscosity decreased to a thin cream like evaporated milk. The cayenne pepper formed sediment. Viscosity is thick and semi-solid
mucilage & 1 ml Xanthan gum	100 ml canola oil 1 ml xanthan gum	consistency of the control experiment. The emulsion is thick and smooth. The sour taste is not prominent. It is acceptable not overpowering. Overall, it has a good taste. The mouth feel is consistent with the control. After 24 hours: Emulsion is stable, flavours, volume and texture is the same as the previous day

Table 9

# 5.5. Determination of fat- mimicking properties of mucilage in meat emulsions

The fat-mimicking properties of mucilage were determined by replacing 25, 50, 75 and 100 % pork fat with mucilage in the meat emulsion formulation.

## Meat emulsion formulation

Table 10 below indicates the ingredients and amounts used in the making of meat emulsions.

Ingredients:	kg
Lean Beef	2.13
Soya Protein Isolate	0.12
Ice Water	1.8585
Back fat (Pork)	0.60
Mucilage	0
Spice Mixture	0.20
Salt Mixture	0.09
Erythrosine (colour)	0.0015
Total	5.00

Table 10

#### Method:

- 1. Mince beef through a 3 mm plate.
- 2. Chop the beef, soya protein isolate, salt and sodium nitrite in the bowl cutter for three rounds at slow speed.
- 3. Add half the ice water and chop at high speed until a strong binding is achieved and a temperature of 8°C is reached.
- 4. Add half the remaining ice water, spices and back fat/mucilage and chop until a temperature of 8.5°C is reached.
- 5. Add the rest of the ice water and chop until a temperature of 12.5°C is reached.
- 6. Fill in French polony casing of desired diameter.
- 7. Cook at 75°C until an internal temperature of 68.5°C is reached.
- 8. Cool product.

### Experiment:

In the meat emulsion formulation, fat is replaced with mucilage by 25%, 50%, 75% and 100%. The percentage of mucilage replacement in the meat emulsion formulation is shown in Table 11 below.

Ingredients:	A (control)	В	С	D	E
(kg)	Full Fat	Fat Replacement 25%	Fat Replacement 50%	Fat Replacement 75%	Fat Replacement 100%
Lean Beef	2.13	2.13	2.13	2.13	2.13
Soya Protein Isolate	0.12	0.12	0.12	0.12	0.12
Ice Water	1.8585	1.8585	1.8585	1.8585	1.8585
Back fat (Pork)	0.60	0.45	0.30	0.15	0
Mucilage	0	0.15	0.30	0.45	0.60
Spice Mixture	0.20	0.20	0.20	0.20	0.20

Table 11

# Determination of the fat mimicking properties of mucilage in meat emulsions:

The results and discussion from replacing fat in meat emulsions with mucilage is discussed in table 12 below.

Experiment	Mucilage replacement	Properties of finished meat emulsion product
A(control):	0.6 kg pork fat 0 mucilage	As seen in Sample A, the texture is firm, set and emulsified.  The flavour is acceptable and very tasty.  Appearance: The colour is consistent and the consistency is firm. The meat emulsion has a good cutting edge.
B:	0.45 kg pork fat 0.15 kg mucilage	As seen in Sample B the texture, flavour and appearance is consistent with sample A.
C:	0.30 kg pork fat 0.30 kg mucilage	As seen in Sample C the texture, flavour and appearance is consistent with sample A.
D:	0.15 kg pork fat 0.45 kg mucilage	As seen in Sample D, the texture, flavour and appearance is consistent with sample A but a very faint taste difference is detected.
E:	0 kg pork fat 0.60 kg mucilage	As seen in Sample E, the texture, flavour and appearance consistent with sample A but a slight taste difference is detected.

Table 12

### Sensory testing

Sensory testing is a scientific method to analyze, interpret and measure how food smells, tastes and feels (Campbell *et al.*, 1980). It depends on human judgement, which is subjective, but the general human reaction to food products will determine whether or not consumers will buy a product containing mucilage or not. This will tell the manufacturer if the product is similar to other food products and therefore it dictates the quality of food that is produced and sold (McWilliams, 1989).

A naïve consumer panel of 25 inexperienced people participated in a tasting test without any previous information regarding the product. Members of the panel did not have an opportunity to interact with each other in the separate tasting booths. Panel members had to indicate the acceptability of the meat emulsion product containing mucilage in the preference testing method by scoring, using a prepared scorecard with a hedonic scale. The hedonic scale is a nine point verbal scale

describing the like or dislike experienced by the panel member. It is regarded as the most used evaluation technique for measuring food preferences (McWilliams, 1989).

The ages of the judges were between 21 and 65. 15 were female and 6 were male. Two judges failed to mention their gender. The judges scored the meat emulsion according to taste, texture and general impression.

After the data was collected, a variant analysis test (ANOVA) as well as a multiple comparison test (Fisher LSD) were done.

The results of the sensory testing from the Fisher LSD test are seen in table 13:

Product	Α	В	С	D	E	Sign. Level
Taste	7.32 <sup>b</sup>	6.72 <sup>ab</sup>	5.92 <sup>a</sup>	5.60 <sup>a</sup>	5.88ª	p < 0.05
Texture	7.20	7.16	6.00	6.00	6.32	NS
General	7.24 <sup>b</sup>	7.08 <sup>ab</sup>	6.00 <sup>a</sup>	6.04 <sup>a</sup>	6.00 <sup>a</sup>	p < 0.05
Impression		Ì				,
Overall	21.70°	20.96 <sup>bc</sup>	17.92 <sup>ab</sup>	17.64 <sup>a</sup>	18.20 <sup>ab</sup>	p < 0.05

Table 13

Means with different superscripts in the same row differ significantly.

## NS = Not significant

In the above table, it is evident that the panel was unable to detect a difference in the texture. In taste and general impression, the panel chose the control and sample B (25 % fat replacement) above samples C to E (50% to 100% fat replacement).

### Determination of microbiological activity in meat emulsions

The tests were done according to the methods described by Harrington (1998) to determine the coliform count, E. coli

presence, yeast and mould count as well as the total bacterial count. Sample A (no mucilage), B (50% mucilage) and E (100% mucilage) were tested.

Microbiological tests were done on the meat emulsion according to the methods described by Harrington, W. F (1998). In the microbiological tests, sample A (with no mucilage), sample C (50% mucilage) and sample E (100% mucilage) was tested for coliform, mould and yeast count, E. coli presence and total bacteria count. The results can be found in tables 14 and 15 below.

Analysis parameter		Sample	
Analysis parameter	Α	С	E
Total bacterial count (cfu/g)	80	30	10
Coliform count(cfu/g)	<10	<10	<10
E. coli presence	Absent	Absent	Absent
Yeast count (cfu/g)	Absent	Absent	Absent
Mould count (cfu/g)	Absent	Absent	Absent

Table 14

Parameter	Standard	
Total bacteria count	< 100 000 cfu/g	
Coliform count	< 5 000 cfu/g	
E. coli presence	Absent in 10 g	
Yeast count	< 100 cfu/g	
Mould count	< 100 cfu/g	
Salmonella	Absent in 25 g	

Table 15

It is evident in the tables above that the meat emulsion samples are safe for consumption as there are no significant amounts of bacteria, moulds or yeast present.

### 5.6 Fat replacer in baked products (carrot cake)

## Extraction of mucilage

The mucilage was extracted from freshly harvested cladodes according to the following method (De Wit and Du Toit, 2011):

The cladodes were peeled so that only the light green slimy inside part remained. The inside part of the cladodes are cut into small sizes and cooked in a microwave oven at 100% for four minutes until the cladodes are soft. A Milex 4 in 1 multi purpose Mean Juice Machine model MMJ004 was used to mince the cladodes. The mucilage was centrifuged at 8000rpm at 4°C for 15 minutes to separate it from the solids and obtain the mucilage itself.

### Standardization of the carrot cake recipe

The carrot cake was prepared according the following recipe (Pienaar, 2007):

### Ingredients:

2 large eggs

85 ml light brown sugar

62.5 ml cooking oil

156.3 ml cake flour

- 2.5 ml baking powder
- 1. 5 ml bicarbonate of soda
- 2.5 ml ground cinnamon
- 1.5 ml all spice
- 0.5 ml salt

156.3 ml grated carrots

62.5 ml chopped nuts

#### Method:

- 1. Whisk the eggs and sugar. Add the oil and beat until light fluffy.
- Sift the cake flour, baking powder, bicarbonate of soda, ground cinnamon, all spice and salt all together and fold into the egg mixture
- 3. Add the carrots and nuts. Mix well. Turn into a greased 23cm loose based round pan.
- 4. Bake in preheated oven at 180°C for 30 minutes. Cool slightly in the pan before turning out onto a wire rack to cool completely.

### Formulations of different mucilage: oil ratios

Five different formulations were used to evaluate the effectiveness of the mucilage to replace oil as indicated in Table 16 below.

Formulation	Mucilage (ml)	Oil (ml)
Control	0	63
25%	17.5	45
50%	30	32.5
75%	75	17.5
100%	62.5	0

Table 16

Five different mucilage: oil ratios were used to evaluate the suitability of mucilage to replace oil.

#### Results and discussion

### Carrot cake processing

Five cakes were made with different levels mucilage incorporation. There was an indicatation that not much change in quality with the incorporation of the mucilage took place. The crust appears to be more or less the same

for all the cakes: the color did not change nor do they have surface cracks on all the different concentrations. The volume of the loaves however was influenced by insufficient or excessive beating and not because of the mucilage. The peaks and the tunnel differences may be due to overdevelopment of gluten and loss of carbon dioxide.

## Physical and chemical analysis:

Sample	Muc 0%	Muc 25%	Muc 50%	Muc 75%	Muc 100%	Significance Level
Wateractivity	0.46 ± 0.01	0.47 ± 0.01	0.48 ± 0.01	0.48 ± 0.01	0.47 ± 0.01	p = 0.0671
Volume	$346.67 \pm 2.89^{ab}$	336.33 ± 27.21 <sup>a</sup>	$370.00 \pm 10.00^{bc}$	$371.67 \pm 2.89^{c}$	$360.67 \pm 9.29^{ac}$	p = 0.0424
Firm-cone	129.00 ± 0.04 <sup>b</sup>	137.00 ± 3.00°	$148.67 \pm 0.58^{d}$	146.00 ± 1.00 <sup>d</sup>	$99.00 \pm 0.05^{a}$	p < 0.0001
Firm-flat	$12.50 \pm 0.50^{a}$	20.50 ± 0.50 <sup>b</sup>	16.50 ± 2.50 <sup>b</sup>	$10.57 \pm 0.06^{a}$	10.40 ± 0.10 <sup>a</sup>	p < 0.001

Table 17 Analysis of Variance (ANOVA) of Physical Data

Means with different superscripts in the same row differ significantly.

Regarding the water activity of the control cake increased from 0.46 to 0.48 at 75% replacement. The water activity of foods is a very important aspect of food preservation. The growth of the various microorganisms stops at a given level of water activity. Water activity also affects other aspects such as quality or organoleptic properties which are also very important, but safety in food is the first and most significant criterion, and this means the control of microbial growth. The lowest limit for growth in foods or any other item is around a<sub>w</sub> 0.6. In the narrow range between a<sub>w</sub> 1 and a<sub>w</sub> 0.6 a large number of microorganisms can grow which are potentially dangerous to food. From our results we can see that the carrot cake of mucilage replacement has the required water activity to inhibit microorganisms.

While the volume of the control cake was on average 346 ml and increased to 360ml with 100% replacement water activity. The volume of the loaves is influenced by insufficient or excessive beating. And the peaks and tunnel differences are due to overdevelopment of gluten and loss of carbon dioxide. The ability of the mucilage to bind water greatly helps with the gelling properties of forming a complex network entrapping air and water.

The texture measured by both the flat attachment and cone attachment showed a decrease in values from 129 to 99 in the cone attachment and 12.50 to 10.40 in the firm attachment, with the replacement of the mucilage and the texture became harder. This is because mucilage is generally soluble and it is able to produce colloidal solutions with high viscosity and prevent dehydration.

### Sensory analysis

The results regarding the sensory analysis of the mucilage replaced cakes are indicated in Table 18.

- A Alberta .		25 %	50 % 7	75 %	100 %	Significance
Treatment	Control	Mucilage	Mucilage	Mucilage	Mucilage	Level
General Impression	6.70 ± 1.30 <sup>b</sup>	6.70 ± 1.79 <sup>b</sup>	6.60 ± 1.34 <sup>b</sup>	6.22 ± 1.66 <sup>b</sup>	5.22 ± 1.96 <sup>a</sup>	p < 0.001
Taste	6.70 ± 1.42 <sup>b</sup>	6.50 ± 2.00 <sup>b</sup>	6.60 ± 1.46 <sup>b</sup>	$6.08 \pm 1.78^{b}$	$5.00 \pm 2.04^a$	p < 0.001
Texture	6.50 ± 1.68 <sup>bc</sup>	6.78 ± 1.57 <sup>bc</sup>	6.90 ± 1.22°	6.16 ± 1.88 <sup>ab</sup>	$5.64 \pm 1.78^{a}$	p < 0.001
Appearance	6.52 ± 1.54	6.86 ± 1.68	6.98 ± 1.08	6.66 ± 1.62	6.42 ± 1.44	p < 0.3043

Table 18 Analysis of Variance (ANOVA) of Consumer Panel Data:

Means with different supertscripts in the same row differ significantly.

The sensory analysis results above are represented in Figure 6.

Regarding the general impression, no statistically significant difference were observed between the control, 25%, 50% and 75% mucilage replacement, however the 100% mucilage replacement showed a significant difference from the other samples. The general impression remained somewhat constant from  $6.70 \pm 1.30^{b}$  -  $6.22 \pm 1.66^{b}$  and the 100% mucilage replacement had  $5.22 \pm 1.96^{a}$ . This means the cakes were perceived as normal from the first impression, till after the tasting with the exemption of the 100%.

Regarding the taste of the cakes, only the 100% mucilage replacement differed significantly from the other samples. The texture of the 75% and

100% replacement were significantly different from the control, 25% and 50%. The 25%, 50% and 75% replacement had the highest values for appearance they were very high when compared to the control and the 100% replacement.

The cake attributes; of the general impression, taste, appearance and texture are all represented in Figure 6. For each attribute the relative intensity increases as it moves farther away from the center point. Therefore, the control, 25%, 50%, 75% and 100% mucilage replacement are all above 3.0 cm scale. There is no significant difference between the control, 25%, 50%, 75% and 100% in the appearance. The only significant difference is with the 100% mucilage replacement in the general impression, taste and texture.

## 5.7 Mucilage as gelling agent in Turkish delight candy

## Extraction procedures for mucilage

The mucilage was extracted from cladodes of *Opuntia ficus-indica* cactus pear. Method of extracting mucilage from cladodes as well as producing the best possible yield and viscosity is discussed below. According to De Wit and Toit, (2011) all the green part and hard fibers inside the green peel that do not yield mucilage we cut from fresh cladodes. Only the light green slimy inside part of the cladode remained. The inside part of cladodes was slices into a size that was easy to handle. Cladodes pieces were cooked in the microwave oven at 100% power for 4 minutes until the cladode pieces were soft. The mincer from a Milex 4-in-1 multi-purpose Mean Juice Machine model MMJ004 was used to finely mincing or cutting the cladodes in the most effective way of macerating the cladodes. More cooked pieces were macerated at a time. The mucilage pulp was divided into the centrifuge containers and centrifuged at 8000 rpm for 15 minutes to separate it from the solids and obtain the mucilage itself. A rotohead setting of 18 and temperature of 4 degrees was used.

## Formulation and preparation of Turkish delight: Standardization of recipe

It was decided to use a home-made Turkish delight rather than a commercial one, as to be familiar with the ingredients at all time. The formulation of a commercial brand would not be available and an unknown ingredient could have a major influence on the sensory attributes of the final product. The two main ingredients were gelatin and mucilage and commercial rose water flavor was used instead of 15 ml lemon juice because was very strong. The following recipe was used as a control for the study: (Jager, 1991).

Table 19 shows the list of ingredients for the making of Turkish delight. The gelatin was soaked in 50 ml water. Sugar, salt, Maizena and citric acid were mixed together, 200 ml water was added and the mixture was stirred over low heat until the sugar has dissolved. The mixture was boiled for 7 minutes over low heat at temperature of 98 °C, and then the lemon juice and gelatin were added. Two and a half ml of rose flavor was used instead of 15 ml lemon juice and 25 drops of pink food coloring to one were added and stirred into the mixture. The mixture was poured into a greased pan 120 by 220 mm to a thickness of 20 mm. Allow to cool overnight and cut into 20-mm squares. Roll in a mixture of equal parts of maizen and icing sugar. Cubes of Turkish delight were store in airtight container between layers of well-dusted with the icing sugar/Maizena mixture (Jager, 1991).

Ingredient	Quantity (ml/g )
Gelatin	20 g
Water	50 ml
Sugar	400 g
Pinch of salt	1 ml
Maizena	35 g
Citric acid	1 ml
Water	200 ml
Rose water flavor	2.5 ml
Corn flour (for coating)	50 g
Icing sugar (for coating)	50 g
Pink food coloring	25 drops

Table 19. List of ingredients of home-made Turkish delight (control) (Jager, 1991).

## Replacement of gelatin with mucilage

Five different formulations of Turkish delight with varying mucilage: gelatin ratios were prepared as in Table 20.

Experiment	Mucilage substitution
Control	20 g gelatin
1: 25%	15 g gelatin and 5 g mucilage
2: 50%	10 g gelatin and 10 g mucilage
3:75%	5 g gelatin and 15 g mucilage
4:100%	20 g mucilage

Table 20. Quantities of mucilage used in Turkish delight to determine jelling properties.

### Results and discussion

## Sensory analysis/ evaluation

Demographic Profile of Consumer Panel: Table 21 (Heymann, 1990).

Gender:	% of Total	Age:	% of Total
Female	74	< 20	4
Male	26	20-29	37
		30-39	24
		40-49	12
<del></del>		50-59	16
		>60	7

Table 21

Analysis of Variance (ANOVA) of Consumer Panel Data: Table 22 (Heymann, 1990).

Treatment	Beacon Turkish Delight		Turkish Delight With Gelatine		Turkish Delight With 28 Mucilag	5 %	Turkish Delight With 50 % Mucilage		Significance Level
General Preference	7.76 1.13 <sup>b</sup>	±	5.81 1.80 <sup>a</sup>	±	5.31 1.91 <sup>a</sup>	±	5.32 1.85ª	±	p < 0.001
Taste	8.05 1.18 <sup>b</sup>	±	5.80 1.77 <sup>a</sup>	±	5.25 2.01 <sup>a</sup>	±	5.24 2.08 <sup>a</sup>	±	p < 0.001
Texture	7.22 1.64 <sup>b</sup>	±	6.05 1.84 <sup>a</sup>	±	5.22 2.03 <sup>a</sup>	土	5.44 1.77 <sup>a</sup>	±	p < 0.001
Appearance	7.68 1.18 <sup>b</sup>	±	6.15 1.71 <sup>a</sup>	±	5.73 1.72 <sup>a</sup>	#	5.78 1.78 <sup>a</sup>	±	p < 0.001

Table 22

Means with different superscripts in the same row differ significantly.

## Statistical analysis:

#### Consumer panel:

From Table 22 it can be deducted that the liking for the different Turkish delight samples differed significantly (P < 0.001).

The most liked general preference for Turkish delight sample was an experimental Beacon Turkish Delight with the highest mean value of 7.76 ± 1.13<sup>b</sup>, which corresponds to between "like moderately" on the hedonic scale. There was no significantly differences between of general preference for Turkish Delight with Gelatine, Turkish Delight With 25 % Mucilage and Turkish Delight With 50 % Mucilage as shown in the Table 22 which is categorized as "Neither like nor dislike" (Heymann, 1990).

The most liked taste was for an experimental Beacon Turkish Delight with the highest mean value of  $8.05 \pm 1.18^b$  which corresponds to "Like very much" on the hedonic scale. There was no significantly difference for taste between Turkish Delight with Gelatine, Turkish Delight With 25 % Mucilage and Turkish Delight With 50 % Mucilage as shown in the Table 22 which is categorized as "Neither like nor dislike" (Heymann, 1990).

The most liked texture was for an experimental Beacon Turkish Delight with the highest mean value of  $7.22 \pm 1.64^{b}$  which corresponds to "Like moderately" on the hedonic scale. There was no significantly difference for

texture between Turkish Delight with Gelatine, Turkish Delight With 25 % Mucilage and Turkish Delight With 50 % Mucilage as shown in the Table 22 which is categorized between "Neither like nor dislike" and "Like slightly" (Heymann, 1990).

The most liked appearance was for an experimental Beacon Turkish Delight with the highest mean value of 7.22 ± 1.64<sup>b</sup> which corresponds to "Like moderately" on the hedonic scale. There was no significantly difference for appearance between Turkish Delight with Gelatine, Turkish Delight With 25 % Mucilage and Turkish Delight With 50 % Mucilage as shown in the Table 22 which is categorized between "Neither like nor dislike" and "Like slightly" (Heymann, 1990).

It can also be concluded that the commercially available Turkish delight did very well. Most of consumers nowadays don't want to eat home-made Turkish delight, but when we compare our results for control Turkish delight with all Turkish delight with mucilage, the was no significantly difference for all attributes which very good (Heymann, 1990).

# Physical evaluation (viscosity and texture) Analysis of Variance (ANOVA) of Physical Data: Table 23 (Heymann, 1990).

Treatment	Control		25	%	50	%	75 %	100 %	Significance
			Mucilage	•	Mucilage		Mucilage	Mucilage	level
Viscosity	4093.33	±	7380.00	±	7460.00	1+	10566.67 ±	6933.33 ±	p < 0.001
	847.90 <sup>a</sup>		1958.47 <sup>l</sup>	b	321.87 <sup>b</sup>		723.42°	550.76 <sup>b</sup>	
Penetrometer:	44.67	±	44.00	±	54.33	Ħ	ND	ND	p = 0.0044
Flat	4.04 <sup>a</sup>		1.00 <sup>a</sup>		1.53 <sup>b</sup>				(p<0.01)
Penetrometer:	233.00	±	230.33	±	230.67	±	ND	ND	p = 0.9062
Cone	4.36		8.39		10.02				
Line-spread	7.20	±	8.70	±	9.93	±	9.20 ± 0.66	7.10 ±	: P = 0.3979
	1.91		0.17		2.11			3.48	
% Sag	11.15	±	8.90	±	20.00	±	50.67 ±	87.45 ±	: p < 0.001
	8.03 <sup>a</sup>		8.15 <sup>a</sup>		0.01 <sup>a</sup>		8.33 <sup>b</sup>	0.95°	

Table 23

ND = Not Determined

Means with different superscripts in the same row differ significantly.

#### Viscosity (viscometer)

As shown in Table 23, at p < 0.001 the control sample shows there was a significantly difference for the viscosity. The viscosity for 25 % Mucilage, 50 % Mucilage and 100 % Mucilage, which shows that there was no significantly difference for viscosity but not over 75 % Mucilage which was the highest (Heymann, 1990).

## Viscosity (Line spread)

As shown in Table 23 for line spread viscosity there was no significantly difference between a control, 25% mucilage, 50% mucilage, 75% and 100% mucilage at P = 0.3979 level. But if more tests were done it was going to show differences (Heymann, 1990).

#### Texture (% sag)

For percentage sag, as shown in Table 23 there was no significantly difference between a control, 25% mucilage and 50% mucilage but there was a difference over 75% and 100% mucilage at p < 0.001 level (Heymann, 1990).

#### **Texture (penetrometer)**

There was no significantly difference for texture by Penetrometer (Flat) between a control and 25% mucilage but was difference over 50% mucilage at p = 0.0044 (p<0.01) level as shown in the Table 23 For 75% and 100% mucilage it was not determined, it means with different superscripts in the same row differ significantly. For the Penetrometer (cone) there was no significantly difference for texture between controls, 25% and 50% mucilage at p = 0.9062. For 75% and 100% mucilage was not determined at same level (Heymann, 1990).

QDA test was done to provide a visual presentation of product similarities and differences sensory attributes. From Figure 7 it is understandable that the relative intensity increases as it moves farther away from the centre (Sidel & Stone, 1983).

For general preference for as shown in Table 23 Turkish delight sample, an experimental Beacon Turkish Delight was the highest scored at 8 cm line scale and Turkish delight with 50% mucilage was scored lowest at 5 cm

line scale. The 25% mucilage was not scored according to the table for general appearance (Heymann, 1990).

For taste, an experimental Beacon Turkish Delight was the highest scored at 8 cm line scale, followed by Turkish delight with gelatin at 6 cm line scale and Turkish delight with 50% mucilage was scored lowest at 5 cm line scale as shown in Figure 7 (Heymann, 1990).

As shown in Figure 7 for texture attribute, Beacon Turkish Delight was the highest scored at 7 cm line scale, followed by Turkish delight with gelatin at 6 cm line scale, than 50% mucilage at 5 cm and Turkish delight with 25% mucilage was scored lowest at 4 cm line scale (Heymann, 1990).

Looking at results in Figure 7 for appearance, Beacon Turkish Delight was the highest scored at 7 cm line scale, followed by Turkish delight with gelatin at 5 cm line scale, than 50% mucilage at 4 cm and Turkish delight with 25% mucilage was not scored (Heymann, 1990).

#### 5.8 Mucilage as foaming agent in marshmallows

#### Extraction of mucilage

The mucilage was extracted from freshly harvested cladodes according to the following method (De Wit and Du Toit, 2011):

The cladodes were peeled so that only the light green slimy inside part remained. The inside part of the cladodes are cut into small sizes and cooked in a microwave oven at 100% for four minutes until the cladodes are soft. A Milex 4 in 1 multi purpose Mean Juice Machine model MMJ004 was used to mince the cladodes. The mucilage was centrifuged at 8000rpm at 4°C for 15 minutes to separate it from the solids and obtain the mucilage itself.

# Standardization of marshmallow recipe

Marshmallows were made according to the following recipe: 400g sugar(500ml)
Salt (pinch)
12,5ml golden syrup

250ml water
20g gelatin (30ml), soaked in
100ml cold water
2 egg whites
5ml essence

#### Formulations of different mucilage: gelatin ratios

Five different formulations were used to evaluate the effectiveness of the mucilage to replace gelatin in marshmallows. These include the control (no mucilage) (138,56 g gelatin), 25 % replacement of gelatin (35,11 g mucilage), 50 % inclusion of mucilage (70,82 g mucilage), 75 % inclusion (103,92 g mucilage) and 100 % replacement of gelatin (mucilage = 137,68 g).

#### Results and discussion

## Mucilage 100 % after 5 weeks storage.

After 5 weeks of storage, the foam became unstable: the egg white formed a layer on top, while the mucilage formed a layer in the middle. The sugar precipitated in clumps at the bottom of the container.

Mucilage was found to have excellent beating properties. The foam formed in marshmallows, however, was not stable after a few weeks of storage. It could not act to replace gelatin in foam formation. It is suggested that it could, however, be used as a foaming/beating aid to be used in temporary foams.

# 6. Method for extraction of mucilage from the Aloe Barbadensis

Aloe vera leaves were used using the same procedure of extraction as mentioned above, i.e. peeling, slicing, cooking, maceration and centrifuging. The aloe vera extraction showed the same properties as the cactus pear cladodes but not at the same degree. The final extraction fluid was slightly slimy and to a lesser degree behaved as

an elastic solid as the viscosity was thinner than that of the cactus plant. The slimy watery extraction of the Aloe leaves showed the same properties as the cactus cladodes, only to a lesser degree. Typically the extraction would be prone to mould growth if left standing at room temperature. This can, however, be prevented by the addition of preservatives such as 0.1% methyl paraben, 0.01% propyl paraben and 0.1% potassium sorbate.

#### 7. Method for extraction of mucilage from the Agave Americana

The leaf was cut into cubes (unpeeled) and microwaved until cooked. Cooking was done in a 900-Watt microwave oven at full power for 4-6 minutes until the internal temperature reached 95°C. A Multi-purpose Mean Juice Machine, model MMJ004 mincer was used to macerate the cubed Agave into pulp. The pulp was centrifuged at a speed of 8000 rpm, at 4°C for 15 minutes. The yield of mucilage from a 588 g cleaned agave leaf was 174.44 g, a 29.6% yield from the whole leaf and 37.6% from macerated pulp. The mucilage from Agave has no viscosity and is a water- like liquid.

This yield is more than was stated in research done by Yahia et al (2009). They extracted mucilage from Maguey (Agave salmiana) using ethanol and found that the yield was low (0.4 o 1.7%).

#### Claims

- A process for extracting mucilage from the cladodes of the Opuntia ficus-indica; or from the leaves of the Aloe barbadensis or Agave americana, the process including the steps of:
  - a) providing a source of cladodes or leaves;
  - b) cooking the cladodes or leaves using microwave energy; and
  - c) centrifuging the product from step b) to obtain mucilage.
- 2. The process according to claim 1, further comprising the step of macerating the cladodes or leaves after step b).
- 3. The process according to claim 1, further comprising the step of macerating the cladodes or leaves prior to step b).
- 4. The process according to claim 2 or 3, wherein the maceration includes the step of finely mincing, cutting, blending or extracting the cladodes or leaves.
- The process according to any one of claims 2 to 4, wherein the maceration includes mincing and does not involve the addition of water.
- 6. The process according to any one of the preceding claims, wherein the cladodes are peeled and sliced prior to cooking.
- The process according to any one of the preceding claims, further comprising the step of refrigerating the cooked cladodes or leaves before carrying out step c).
- 8. The process according to any one of the preceding claims, further comprising the step of allowing the extracted mucilage to age for about 5 days, before use.

- 9. The process according to any one of claims 1 to 7, further comprising the step of allowing the cooked cladodes or leaves to age for about 5 days, before carrying out step c).
- 10. The process according to claim 8 or 9, wherein the step of ageing involves refrigerating the cladodes, leaves or mucilage.
- 11. The process according to any one of the preceding claims, wherein the cooking is carried out in a microwave oven having a power in the range of between 300W to 3000W.
- 12. The process according to claim 11, wherein the cooking is carried out in a microwave oven having a power in the range of between 800W to 1700W.
- 13. The process according to claim 12, wherein the cooking is done in a 900W microwave oven.
- 14. The process according to claim 13 wherein the cooking is done in a 900W microwave oven at 100% power for about 4 -6 minutes.
- 15. The process according to any one of the preceding claims, wherein the cladodes or leaves are fresh or dried.
- 16. The process according to any one of claims 2 to 15, wherein the maceration step includes the addition of water.
- 17. A mucilage extract obtained from the process according to any one of the preceding claims.
- 18. The extract according to claim 17, for use as a functional ingredient in food processing.

- 19. The extract according to claim 17 or 18, for use as an emulsifier or vegetable oil replacement in mayonnaise.
- 20. The extract according to claim 17 or 18, for use as a fat replacement in meat emulsions or baked products.
- 21. The extract according to claim 17 or 18, for use as a gelling agent in candy, including Turkish Delight.
- 22. The extract according to claim 17 or 18, for use as a foaming agent in marshmallow.
- 23. A food product including a foodstuff, dietetic foodstuff or food supplement, the food product comprising mucilage obtained by the process according to any one of claims 1 to 16.
- 24. The food product according to claim 23, wherein the food product is mayonnaise, a meat emulsion, a baked product, candy including Turkish Delight or marshmallow.
- 25. The food product according to claim 23 or 24, wherein the food product is mayonnaise and wherein 25%, 33.3%, 50%, 75% or 100% of oil or egg yolk intended to be present in the mayonnaise is replaced with the mucilage.
- 26. The food product of claim 25, wherein the mayonnaise further comprises 1% xanthum gum.
- 27. The food product according to claim 23 or 24 wherein the food product is a meat emulsion and wherein 25%, 50%, 75% or 100% of fat intended to be present in the meat emulsion is replaced with the mucilage.
- 28. The food product according to claim 23 or 24, wherein the food product is a baked product and wherein 25%, 50%, 75% or 100% of

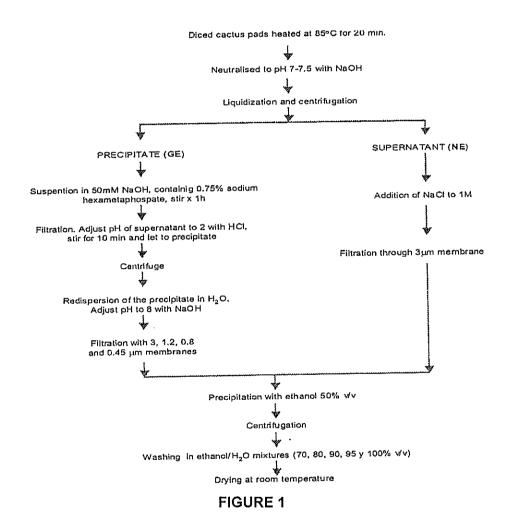
oil intended to be present in the baked product is replaced with mucilage.

- 29. The food product according to claim 23 or 24, wherein the food product is a candy, including Turkish Delight, and wherein 25%, 50%, 75% or 100% of gelatin intended to be present in the candy is replaced with mucilage.
- 30. The food product according to claim 23 or 24, wherein the food product is marshmallow and wherein 25%, 50%, 75% or 100% of gelatin intended to be present in the marshmallow is replaced with mucilage.

DATED THIS 27<sup>TH</sup> DAY OF JULY 2012 H. VAN SCHALKWYK

SPOOR & FISHER

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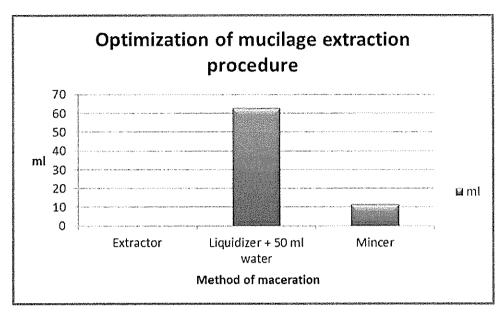
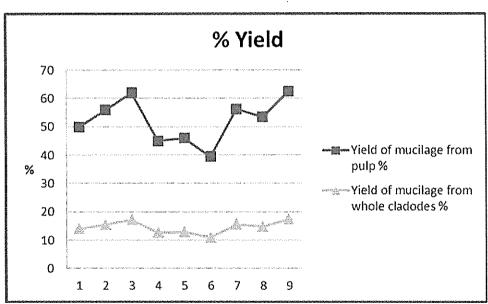


FIGURE 2



Sample 1. Cladode was peeled, minced, and centrifuged. (Raw)

Sample 2. Cladode was peeled, cooked by steaming, minced, and centrifuged. (Steamed)

Sample 3. Cladode was peeled, minced, cooked in the microwave oven, and centrifuged. (Microwaved)

Sample 4. Cladode was peeled, minced, refrigerated for 5 days, and centrifuged. (Raw)

Sample 5. Cladode was peeled, minced, centrifuged, and refrigerated for 5 days. (Raw)

Sample 6. Cladode was peeled, cooked, minced, centrifuged, and refrigerated for 5 days. (Steamed) Sample 7. Cladode was peeled, cooked, minced, refrigerated for 5 days and centrifuged. (Steamed)

Sample 8. Cladode was peeled, minced, cooked in the microwave oven, centrifuged, and refrigerated for 5 days. (Microwaved)

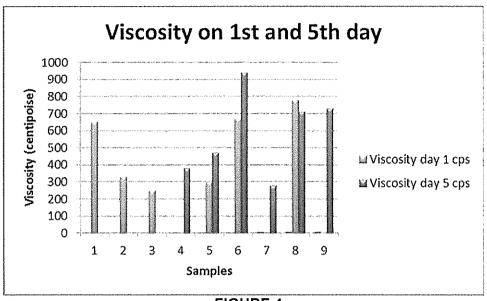
Sample 9. Cladode was peeled, minced, cooked in the microwave oven, refrigerated for 5 days and centrifuged. (Microwaved)

FIGURE 3

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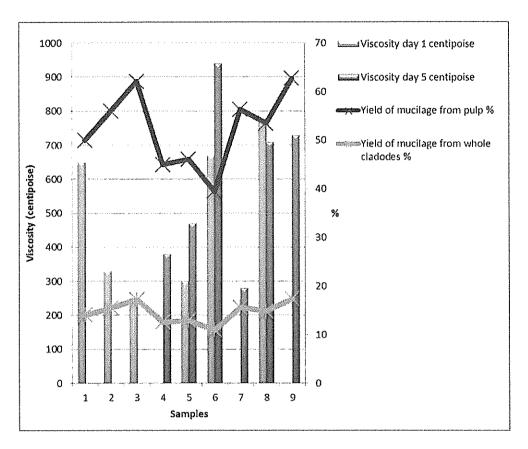
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#### FIGURE 4

- Sample 1. Cladode was peeled, minced, and centrifuged. (Measured on day of extraction)
- Sample 2. Cladode was peeled, cooked by steaming, minced, and centrifuged. (Measured on day of extraction)
- Sample 3. Cladode was peeled, minced, cooked in the microwave oven, and centrifuged. (Measured on day of extraction)
- Sample 4. Cladode was peeled, minced, refrigerated for 5 days, and centrifuged. (Measured after 5 days of refrigeration)
- Sample 5. Cladode was peeled, minced, centrifuged, and refrigerated for 5 days. (Measured on day of extraction as well as after 5 days of refrigeration)
- Sample 6. Cladode was peeled, cooked, minced, centrifuged, and refrigerated for 5 days. (Measured on day of extraction as well as after 5 days of refrigeration)
- Sample 7. Cladode was peeled, cooked, minced, refrigerated for 5 days and centrifuged. (Measured after 5 days of refrigeration)
- Sample 8. Cladode was peeled, minced, cooked in the microwave oven, centrifuged, and refrigerated for 5 days. (Measured on day of extraction as well as after 5 days of refrigeration)
- Sample 9. Cladode was peeled, minced, cooked in the microwave oven, refrigerated for 5 days and centrifuged. (Measured after 5 days of refrigeration)

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#### FIGURE 5

- Sample 1. Cladode was peeled, minced, and centrifuged. (Raw)
- Sample 2. Cladode was peeled, cooked by steaming, minced, and centrifuged. (Cooked)
- Sample 3. Cladode was peeled, minced, cooked in the microwave oven, and centrifuged. (Cooked)
- Sample 4. Cladode was peeled, minced, refrigerated for 5 days, and centrifuged. (Raw)
- Sample 5. Cladode was peeled, minced, centrifuged, and refrigerated for 5 days. (Raw)
- Sample 6. Cladode was peeled, cooked by steaming, minced, centrifuged, and refrigerated for 5 days. (Cooked)
- Sample 7. Cladode was peeled, cooked by steaming, minced, refrigerated for 5 days and centrifuged. (Cooked)
- Sample 8. Cladode was peeled, minced, cooked in the microwave oven, centrifuged, and refrigerated for 5 days. (Cooked)
- Sample 9. Cladode was peeled, minced, cooked in the microwave oven, refrigerated for 5 days and centrifuged. (Cooked)

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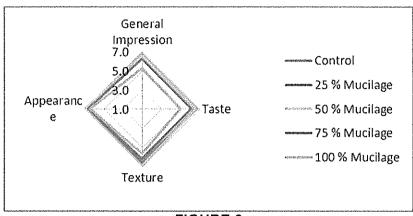


FIGURE 6

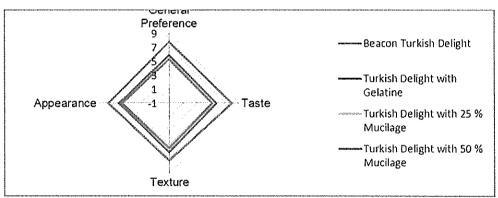


FIGURE 7

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