GELLING PROPERTIES OF CACTUS PEAR MUCILAGE-HYDROCOLLOID COMBINATIONS IN A SUGAR-BASED CONFECTIONERY

by

Liezl du Toit

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Promoter: Dr. C. Bothma
Co-Promotor: Prof. Arno Hugo
Co-Promotor: Dr. M. De Witt

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DECLARATION

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Liezl du Toit

Student number: 2008046902

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GLOSSARY OF ABBREVIATIONS

i.e.	-	id est / that is
LBG	-	locust bean gum
CMC	-	carboxymethyl cellulose
e.g.	-	exempli gratia / for example
KGM	-	konjac gum / glucomannan
pH	–	potential of hydrogen / scale of acidity from 0-14
κ-carrageenan	-	kappa carrageenan
G	-	100% gelatin
MX	-	75% mucilage + 25% xanthan
MA	-	75% mucilage + 25% agar
MG	-	75% mucilage + 25% guar
8M2X	-	80% mucilage + 20% xanthan
8M2A	-	80% mucilage + 20% agar
8M2G	-	80% mucilage + 20% guar
MXA	-	75% mucilage + 12.5% xanthan + 12.5% agar
MXG	-	75% mucilage + 12.5% xanthan + 12.5% guar
MAG	-	75% mucilage + 12.5% agar + 12.5% guar
L*	-	lightness
C*	-	Chroma
H^o	-	Hue Angle
a_w	-	Water activity
B.C.	-	Before Christ
spp.	-	several species
O.	-	*Opuntia*
L-arabinose	-	Levo arabinose
D-galactose	-	Dextro galactose
L-rhamnose	-	Levo rhamnose
D-xylose	-	Dextro xylose
et al.	-	et alia / and others
W	-	Watt
min	-	Minute / s
Inc.	-	Incorporated
CA	-	California

USA	-	United States of America
rpm	-	Revolutions per minute
°C	-	Degrees Celsius
°F	-	Degrees Fahrenheit
mm	-	Millimeters
SA	-	South Africa
LSV	-	line spread value
cm	-	Centimeters
ASTM D217	-	standard for cone penetration of lubricating grease
%	-	Percentage
UTM	-	Universal Testing Machine
kN	-	kilo Newton
h	-	Hours
WW	-	Woolworths white
WTW	-	Woolworths traditional white
BW	-	Beacon white
MW	-	Manhattan white
ANOVA	-	Analysis of Variance
NCSS	-	Number Cruncher Statistical Systems
kg	-	kilogram
IMF	-	intermediate moisture foods
GMIA	-	Gelatin Manufacturers Institute of America
CO_2	-	Carbon dioxide
CaC_2O	-	Calcium oxalate
Mg	-	Magnesium
P	-	Phosphate
Na	-	Nitrogen
Sn	-	Zink
Mn	-	Manganese
Fe	-	Iron
$CaCl_2$	-	Calcium Chloride
Ca^{2+}	-	Calcium
K^+	-	Potassium
(PTY) LTD	-	Proprietary Limited

LSD-test	-	least significant difference test
RVA	-	Rapid visco analyser
DSC	-	Differential scanning calorimetry
SEM	-	scanning electron microscopy
cP	-	centipoise
g	-	grams
mg	-	milligrams
µl	-	microliter
Al	-	Aluminum
N_2	-	Nitrogen
Hg	-	Mercury
ca	-	Circa / approximately
nm	-	nanometer
FE-SEM	-	Field Emission scanning electron microscopy
mJ / g	-	millijoule per gram
temp.	-	temperature
H_2O	-	water
(w / w)	-	% weight per weight
ppm	-	parts per million
TM	-	measurement temperature
TD	-	dissolution temperature
g / ℓ	-	gram per liter
CABI	-	Centre for Agriculture and Biosciences International
g / ml	-	gram per milliliter
(w / v)	-	weight per volume
WHC	-	water-holding capacity
OHC	-	oil-holding capacity
NCA	-	National Confectioners Association
DE	-	Dextrose Equivalent

CHAPTER 1

1. Introduction

The main aim behind the liberal use of hydrocolloids in the food industry is their capacity to modify the rheology of food systems, including two basic characteristics, i.e., viscosity and texture. These alterations aid the modification of sensory properties, and hence, hydrocolloids are used as important food additives to perform specific purposes, such as thickening and gelling (Saha and Bhattacharya, 2010).

Hydrocolloids, such as starch, agar, carrageenan, alginates, furcellaran, pectin and gelatin, have been used as gelling agents, while xanthan, galactomannans like guar gum and locust bean gum (LBG), gum karaya, gum tragacanth and carboxymethyl cellulose (CMC) have been used as thickening agents in various food systems (Milani and Maleki, 2012). Blending of different hydrocolloids offers an alternative route to development of new textures and a major interest lies in the development of synergistic mixtures, with improved or induced gelation (Saha and Bhattacharya, 2010).

There is a continuous search for new and interesting sources of hydrocolloids. These thickening and gelling agents are the most useful edible products of modern chemistry (Imeson, 2000). It is derived from natural sources and they make it easy to transform liquids or purees in ways that are hard or even impossible to accomplish with traditional ingredients (Armisen and Galatas, 1987).

Some of the hydrocolloids that are used today date back hundreds, even thousands of years, e.g. gum Arabic was used by the ancient Egyptians in mummy binding and for the paint that was applied to murals in the pyramids (Glicksman, 1982). Agar was discovered accidently by a Japanese innkeeper in the 17^{th} century (Matsuhashi, 1990) and carrageenan in 1809 (Mitchell and Guiry, 1983). Even xanthan, which is a relatively 'young' hydrocolloid, has been in use for the past 67 years (García-Ochoa *et al.*, 2000).

The most exciting of the 'new generation' of hydrocolloids is konjac gum / glucomannan (KGM), which is blended with a variety of 'old-school' hydrocolloids to produce desired textures. There is a 1000-year written history of konjac tubers being consumed as a high-grade food, offered as presents to the Samurai and noble classes, and used as a cure for certain

diseases in China and Japan. It only became an industrialized food product in Japan when a way was developed to dry and separate the glucomannan from the starch and cellulose in the tuber. The two largest uses of the 125 000 tons of konjac annually produced in Japan are for Shirataki noodles and as Konnyaku gel, which has similar food uses to tofu (soybean curd) (Thomas, 1997). Xanthan (30%) possesses a synergistic interaction with KGM (70%) during gel formation, producing thermoreversible physical gels at neutral pH (Toba *et al*., 1987). Mixtures of xanthan and KGM are reported to produce 'melt-in-the-mouth' gels, having a texture like that of gelatin. Hence, they provide a useful replacement in applications where 'melt-in-the-mouth' characteristics are important for product quality and where moderate acidity is acceptable or necessary (e.g., fruit jellies) (Agoub *et al*., 2007). Konjac gum also interacts strongly with κ-carrageenan, forming strong elastic gels, having rupture strength four times higher than that of κ-carrageenan gel alone (Imeson, 2000).

A lot of research has been done on lesser-known hydrocolloids of plant origin. Production on fenugreek seed-endosperm galactomannan only started in 1993. Fenugreek has a bitter taste and strong aromatic odour, since the seed contains spicy oil, saponins and edible protein. It has possible applications for medicinal purposes, because its soluble dietary fibre promotes beneficial pre-biotic colon bacteria (Mathur and Mathur 2005). According to BahrabmParvar and Goff (2013), basil seed gum can be used to stabilise ice-cream, by reducing the rate of ice crystal growth and decreasing meltdown rate. Tamarind gum is easily dispersed in cold water and forms a viscous liquid upon heating (Huanbutta *et al*., 2015). It also has a favourable resistance to heat, acids, salts, freezing and thawing, and exhibit stabilising, emulsifying, thickening, coagulating, water retention and film forming properties (Sahoo *et al.*, 2010).

Mucilage is a slimy substance found in the young leaves of the *Opuntia ficus-indica* plant (Sepúlveda *et al.*, 2007) and is classified as a hydrocolloid, as it is a long-chain polymer that dissolves in water to give a thickening or viscosity producing effect (Glicksman, 1983). These interesting flow properties, together with the nutritional and functional characteristics, compelled researchers to investigate the application of mucilage as a functional ingredient (Cárdenas *et al*., 1997; Medina-Torres *et al*., 2003; Saenz *et al*., 2004; Leon-Martinez *et al*., 2011).

The first aim was to replace gelatin in marshmallows with different formulations of liquid *O. fikus-indica* mucilage, in combination with different concentrations of powdered hydrocolloids, to see if gelatin could be substituted with mucilage or mucilage-blend.

The following hypothesis was formulated:

It is proclaimed that mucilage form *O. ficus-indica* has gelling properties and can be used to replace other gelling agents in food products (Medina-Torres *et al*., 2002). A hypothesis for mucilage / mucilage-blend gelling capabilities would, thus, be that by replacing gelatin in a high sugar high albumin aerated food product, such as marshmallows, a gel would be formed, as will be evident in physical parameters, such as consistency, percentage sag, shear, colour and water activity (a_w).

The second aim was to determine the sensory acceptability of the mucilage / mucilage-blend marshmallows and to compare it to the liking of commercially available marshmallows.

The following hypothesis was formulated:

Texture of aerated food products are the most important aspect influencing consumers' acceptability of a product. It is also a well-known fact that colouring and flavouring of a food product, with an altered textural profile, may convince the consumer in still eating the product (Spence, 2015). The hypothesis for 'the liking of' sensory test for mucilage / mucilage-blend containing marshmallows would, thus, be that even if the texture of the marshmallow is not perceived as the same as the gelatin-containing sample, colouring and flavouring of the marshmallows would increase the liking of the samples.

The third aim was to determine the effect of mucilage / mucilage-blend on the formation of the various food systems in the making of a high sugar high albumin aerated food product, such as marshmallows.

The following hypothesis was formulated:

To form a gel, water must be bound in a three-dimensional network (Gulrez *et al.,* 2011). Marshmallows contain saturated sugar syrup, which also binds water. The gel that is formed should be strong enough to stabilise this sugar syrup. A hypothesis for the effect of mucilage / mucilage blend would, thus, be that different hydrocolloids form three-dimensional networks and bound water in different ways and are influenced by different factors, as determined by

cooling and heating cycles, accompanied by shear, unfolding, glass transition, solids-melting and decomposition temperatures, as well as micrographs.

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CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Hydrocolloids are a varied collection of polymers with long chains, categorised by their characteristic of producing thick dispersions and / or gels when distributed in water (Saha and Bhattacharya, 2010). These substances were originally discovered in exudates from trees or brushes (e.g. gum Arabic), isolates from seaweeds (e.g. agar), flours from endosperms (guar), mucilages from aerobic fermentation procedures (xanthan) and various natural products (e.g. gelatin from animal hides and bones) (Nishinari *et al*., 2017). The occurrence of a considerable amount of hydroxyl groups noticeably enhances the ability to bind molecules of water, making them hydrophilic substances (Li and Nie, 2016). Furthermore, they form a dispersion, which is between a true solution (particle size smaller than 1 nanometer [nm] and a suspension (particle size bigger than 0.1micrometer [μm], exhibiting properties of colloid particles between 1 nm and 0.1 μm. Taking these two properties into account, these substances are suitably named 'hydrophilic colloids' or 'hydrocolloids' (Milani and Maleki, 2012.).

Hydrocolloids have a varied range of practical applications in foods, comprising of coagulating, congealing, blending, stabilization, enrobing, replacement of fat, etc. Furthermore, hydrocolloids have a noticeable effect on the properties of food when added at different concentrations, varying from a few parts per million (ppm) of carrageenan in dairy products that are treated with heat (e.g. chocolate milk) to high concentrations of gelatin in jelly confectionary (e.g. 'jelly babies'). The main purpose behind the frequent use of hydrocolloids in food products is their capacity to change food systems' rheology (Chaplin, 2017). Rheology consists of two rudimentary characteristics of food systems, i.e., viscosity (behaviour regarding flow) and texture (properties of mechanical solids). The alteration of these two factors in food systems assist in changing the sensory properties thereof (Williams and Phillips, 2009; Saha and Bhattacharya, 2010; Milani and Maleki, 2012).

While all hydrocolloids thicken and turn aqueous dispersions into a paste, a small number of biopolymers can also form a gel. When forming a gel, polymer chains cross-link and form a network that is three-dimensional in nature, locking in or immobilising the water inside, forming a solid structure that cannot flow, i.e., it turns into a visco-elastic substance, exhibiting properties of both a liquid and a solid. Texture, opacity, mouth feel and taste of various gels

vary tremendously, and are subject to the hydrocolloid used. Xanthan, guar gum, locust bean gum, gum karaya, gum tragacanth, gum Arabic and cellulose derivatives are used as thickening agents. The gelation agents are starch, gelatine, pectin and seaweed exudates (agar, furcellaran, carrageenan, and alginate) (Saha and Bhattacharya, 2010).

2.2 Hydrocolloids of importance for this study

2.2.1 Guar

Cyamopsis tetragonoloba, a member of the family Leguminosae (Prem *et al*., 2005), which has been grown in India and Pakistan since antiquity, contains the seed endosperm which is the source for guar gum (Mudgil *et al*., 2011). The guar gum industry only started in the United States of America (USA) in the 1940s and 1950s (BeMiller, 2009).

Mannans and galactomannans are particularly plentiful in the cell walls of the seeds. Guar gum mainly comprises of glatomannans, namely high molecular weight polysaccharides, with (1→4)-linked β-D-mannopyranosyl units forming a linear chain and (1→6)-linked α-D-galactopyranosyl residues being side chains (Figure 2.1) (Cui *et al*., 2005; Nishinari, *et al*., 2007; Mudgil *et al*., 2011). The proportion of mannose to galactose units is about 1.6:1 to 1.8:1 (Williams and Philips, 2009; Pathak, 2015). More branching in the guar molecule is accountable for very simple hydration properties and consequently, hydrogen bonding activity (Kuravadi *et al.*, 2013). Aggregates that occur in guar systems may be prominent in its visco-elastic behaviour, subject to interlinkages between these aggregates (Khouryieh *et al*., 2007).

Guar gum contains 33-40 % (w / w) galactose and dissolves in water at 25 ºC / 77 ºF (Balhagi, 2015). With a decrease in particle size and an increase in temperature, the rate of dispersion of guar gum increases. In solution, guar gum becomes pseudo-plastic or shear thinning, and when concentration and molecular weight rise, the degree of pseudo plasticity also rises (Mudgil *et al*., 2011; Milani and Maleki, 2012; Wüstenberg, 2015). Guar gum has one of the highest molecular weights of all natural occurring water soluble polymers, namely 10^6 to 2 x 10^6 (Mudgil *et al*., 2011; Kuravadi *et al*., 2013; Wüstenberg, 2015).

Guar gum expands and / or dissolves in polar solvents and forms robust hydrogen bonds, while in non-polar solvents, the bonds are frail. With reductions in particle size and pH, along with an increase in temperature, there is an increase in the dispersion and viscosity development rate

of guar gum. These rates reduce when dissolved salts and other water-binding agents, such as sucrose, are present (Mudgil *et al*., 2011; Tripathy *et al.*, 2013).

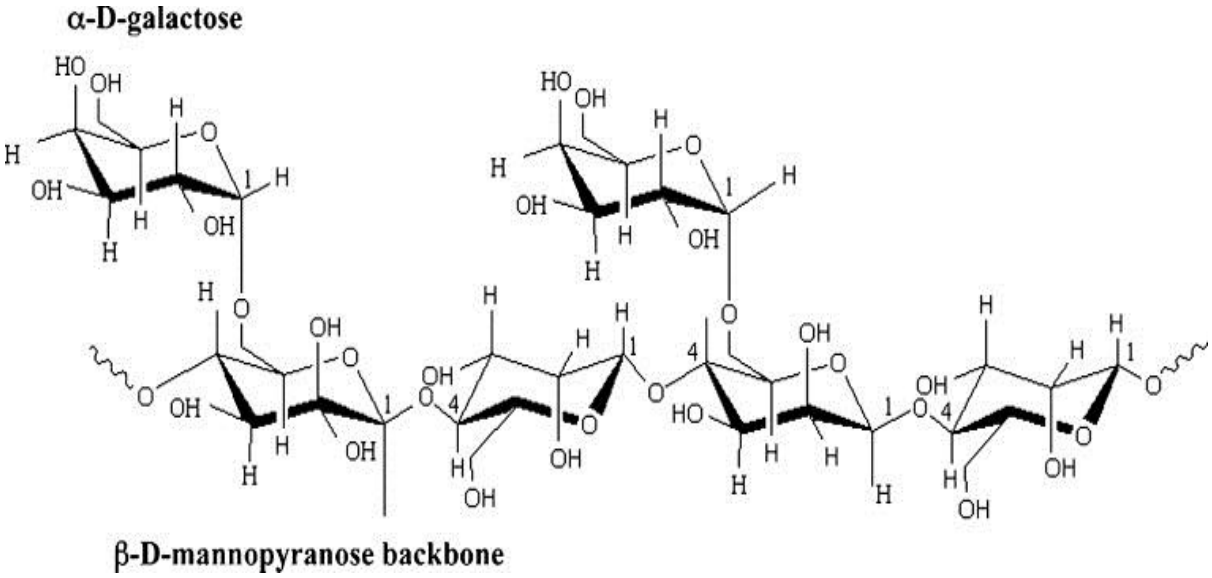

Figure 2.1: Chemical structure of guar gum (Mudgil *et al*., 2011).

The capacity of guar gum to hydrate rapidly in cold water to form very thick mixtures is its most important attribute. When fully hydrated, the formed thick colloidal dispersion can form a gel, return to the sol state and gel again, known as thixotropy (Mudgil *et al*., 2011). Viscosity is also reliant on time, temperature, concentration, pH, ionic strength and type of agitation (Pegg, 2012). A 1 % aqueous dispersion of guar gum has a high viscosity in the range of 10 000 centiPoise (cP) (Parija *et al*., 2001).

Hydration of about two hours (h) is needed to reach optimum viscosity, which is mainly dependent on the size of powder particles (Mahmoud, 2000). Hydrogen bonding activity is caused by the presence of hydroxyl groups in the guar gum molecules, especially with cellulose-type materials and water-bound minerals. Even small additions of guar gum to a system alter the electro-kinetic properties markedly (Panda, 2010). Viscosity and hydration rate of guar gum is influenced by temperature, pH, solute, concentration, etc. (Mudgil *et al*., 2011).

The tempo of hydration and optimum viscosity are mostly affected by temperature. At higher temperatures maximum viscosity is reached quicker; however, sustained heat can cause degradation. When guar gum solutions are heated during preparation, the final viscosity decreases. The opposite is achieved when the solutions are prepared with cold water and allowed to hydrate slowly. Optimum viscosity of dispersions is reached in a temperature range of 25 °C to 40 °C / 77 ºF to 104 ºF (Srichamroen, 2007).

At even very low concentration, guar gum solutions are very viscose. For food applications, concentrations of below 1 % are recommended. Viscosity increases proportionally with increases in hydrocolloid concentration (Moser *et al*., 2013), because of more interactions between galactose side chains with the water molecules (Zhang *et al*., 2005). Doubling the concentration of guar gum shows a tenfold increase in viscosity (Mudgil *et al*., 2011). Guar gum solutions, with concentrations of up to 0.5 %, behave like Newtonian systems; above this concentration, it behaves like non-Newtonian and thixotropic systems (Srichamroen, 2007).

Being non-ionic and uncharged render guar gum solutions stable over a pH range of 1.0–10.5. The pH does not affect final viscosity, but any changes in pH do have an influence on the hydration rate. At pH 8-9, fastest hydration is attained and above pH 10 and below pH 4, hydration rate is the slowest (Pilgaard, 2016).

In the presence of sugar, hydration of guar gum molecules is delayed, because sugar competes with the guar molecules for the available water. This results in a decrease in the viscosity of guar-sugar solutions and is inversely proportional to the sugar concentration. However, non-nutritive sweeteners, like aspartame, acesulfame-k, cyclamate and neotame, have no significant effect on the intrinsic viscosity of guar gum solutions (Samavati *et al*., 2008).

Salt does not influence the hydration rate of guar gum solutions; however, the presence of sodium chloride (NaCl) causes a slight increase in the final viscosity of a fully hydrated 0.5 % guar gum solution (Srichamroen 2007). Hydration is restricted by NaCl (Doyle *et al*., 2006). When salts are present, intermolecular interactions can be assisted, due to change in charge density and the conformation of the hydrocolloid (Tripathy *et al*., 2013).

2.2.2 Gelatin

Hydrolysis in an acidic (Type A) or an alkaline (Type B) solution, followed by hot water extraction, is used to derive gelatin from animal connective tissue (collagen). Commercial gelatin production employs skins or bones of different animal species, such as beef, pork, fish and poultry (Stevens, 2010). Gelatin is nearly tasteless and odourless, and is a clear, brittle solid, with a pale yellow colour.

Collagen may be deemed an anhydride of gelatin (Pamfil *et al*., 2014). Molecules of varying mass are formed by the hydrolytic change of collagen to gelatin: each molecule is a fragment of the collagen chain, from which it was split. Gelatin is, thus, a combination of fractions, comprised entirely of amino acids, which are linked by peptide bonds to form polymers, varying in molecular mass from 15 000 to 400 000 (Figure 2. 3) (Stevens, 2010; Elvers, 2017). The molecular weight profile depends on the extraction process (Djagny *et al*., 2001; GMIA, 2012; Milani and Maleki, 2012).

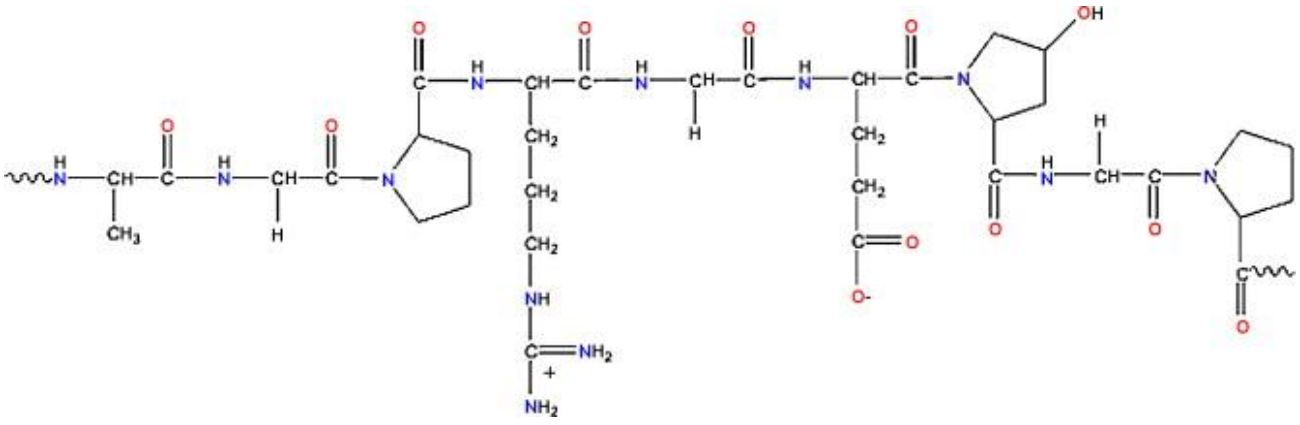

Figure 2.2: Chemical structure of gelatin (Chaplin, 2017).

Gelatin, in terms of basic elements, is composed of 50.5 % carbon (C), 6.8 % hydrogen (H), 17 % nitrogen (N) and 25.2 % oxygen (O) (Sam *et al.*, 2016). Gelatin contains 8-13 % moisture and has a relative density of 1.3g / cm^3 to 1.4g / cm^3 (Chanchal *et al*., 2014). The amino acids that are predominantly present in gelatin are glutamic acid, glycine, hydroxyproline and proline (Chaplin, 2017). Hydrogen bond formation and reactions via side chains, such as amine, imidazole, alcohol, amide and carboxylic acid, are determined by the amino acid profile (Stevens, 2010; GMIA, 2012; Milani and Maleki, 2012).

On soaking in cold water, gelatin granules hydrate into discrete swollen particles. When heated, these granules then dissolve to form solutions that have good whipping and foaming properties (GMIA, 2012; Milani and Maleki, 2012). Temperature, pH, ash content, method of manufacturing, thermal history and concentration all have an influence on gelatin solutions (Cortis *et al*., 2008).

A gelatine solution is amphoteric, meaning that it acts either as an acid or as a base (Zandi, 2008). Gelatine is positively charged in acidic solutions and migrates as a cation in an electric field, while it is negatively charged in alkaline solutions and migrates as an anion. The net charge is zero and no movement occurs at the iso-electric point pH, which is between 7 and 9 for Type A gelatine and 4.7 and 5.4 for Type B gelatine (GMIA, 2012; McClements, 2015).

The most significant property of gelatine is the formation of gels in water that reverse upon change in temperature. When cooling a solution, containing around 0.5% gelatine, to 35 °C to 40 °C / 95 ºF to 104 ºF, viscosity increases and a gel forms later. The rigidity or strength of this gel depends on gelatine concentration, the intrinsic strength of the gelatine, pH, temperature and the presence of any additives (Banerjee and Bhattacharya, 2011; Elvers, 2017).

Locally ordered regions are formed, as first step in the process of gelation, due to partial random re-denaturation of gelatine to collagen-like helices (Guo, 2003). Next, a continuous fibrillary three-dimensional network of fringed micelles is formed throughout the system, due to non-specific bonds that are formed between the more orderly segments of the chains. Bonds that are involved in the cross-bonding included hydrophobic, hydrogen and electrostatic bonds (Guo, 2003; GMIA, 2012). These bonds are disrupted with heat application, making these gels thermo-reversible. The slowest part of the process is the formation of cross-bonds, so that the strength of the gel increases with time as more cross bonds are formed (Bhowmick *et al*., 2014).

2.2.3 Agar

Agar consists of a group of polysaccharides from the red-purple algae of the class *Rhodophyceae*. These algae, from the *Gracilaria* and *Gelidium* genuses, are found in the waters along the coast of Japan, New Zealand, South Africa (SA), Southern California, Mexico, Chile, Morocco and Portugal (Nishinari, *et al*., 2017).

Agar is made up of two major fractions, namely agarose and agaro pectin, which does not gel (Chaplin, 2014). Agarose is a neutral linear molecule, with almost no sulphates, and it exist as chains of repeating alternate units of β-1,3-linked-D-galactose and α-1,4-linked 3,6-anhydro-L-galactose (Figure 2.4). Agaro pectin is a polysaccharide (with 3 % to 10 % sulphate), composed of agarose and varying percentages of ester sulphate, D-glucuronic acid and small amounts of pyruvic acid. The species of seaweed determines the proportion of these two polymers. Agarose normally represents at least two-thirds of the natural agar (Nordqvist and Vilgis, 2011; Milani and Maleki, 2012; Chaplin, 2014; Shankar and Rhim, 2015).

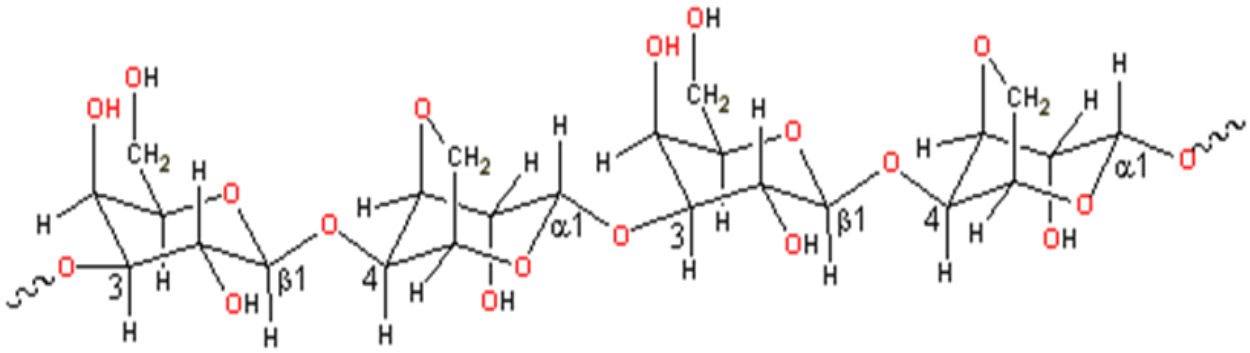

Figure 2.3: Chemical structure of agarose (Shankar and Rhim, 2015; Chaplin, 2017).

Agar does not dissolve in cold water, but swells so much, that it absorbs as much as twenty times its own weight of water. It dissolves very easily in boiling water, forming firm gels at concentrations as low as 0.50 %. Powdered dry agar is soluble in water and other solvents at temperatures from 95º to 100º C / 203 °F to 212 °F (Shankar and Rhim, 2015).

Inside the double helical cavities of agar, as well as inside exterior hydroxyl groups, three-fold left-handed helices are stabilized by the presence of water molecules (Labropoulos *et al*., 2002; Milani and Maleki, 2012). Aggregates of up to 10 000 of these helices form, resulting in micro domains of spherical micro gels (Boral *et al*., 2008). Agar helices are more compact, because it contains smaller amounts of sulphate groups. Agar is well-known for being a thermo-reversible hydrocolloid, setting at 30 ºC to 40 ºC / 86 ºF to 104 ºF. Strong gels are formed, which are subjected to pronounced syneresis, a property that is attributed to the strong aggregation of the double helices (Williams and Phillips, 2009). The ability to form reversible gels, just by cooling hot aqueous solutions, is the most important property of agar. Gel

formation depends exclusively on the formation of hydrogen bonds, where the random coils associate to form single helices (Imeson, 2010) and double helices (Guo, 2003) (Figure 2.5).

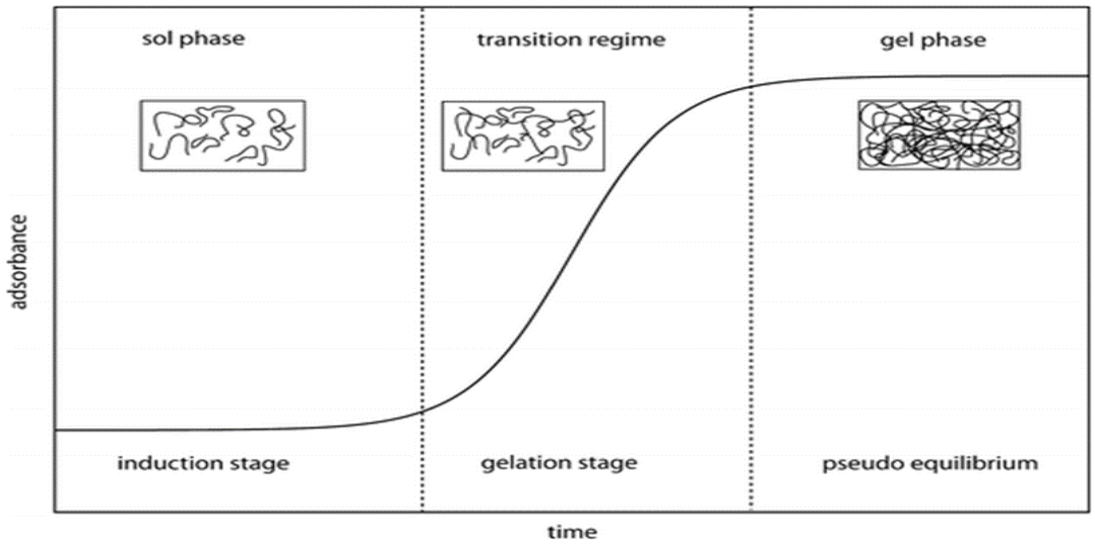

Figure 2.4: Gelation process in agarose solutions (Nordqvist and Vilgis, 2011).

Agar is outstanding amongst hydrocolloids regarding its gelling power. Agar gels form even at concentrations as diluted as 0.5 to 1.0 % (Porto, 2003). The gels are rigid, brittle, have clean cutting edges, as well as clear melting and gelling points. Furthermore, these gels are also subjected to the release of water through the surface of the gel (syneresis) and an extreme hysteresis lag, i.e., substantial temperature ranges between melting and gelling temperatures. A gel is formed, with 1.5 % agar, on cooling to about 32 ºC to 45 º C / 89.6 ºF to 113 ºF, which will only melt at 85º C / 185 ºF or higher (Armisen and Galatas, 1987). This hysteresis interval is a unique property of agar that finds many uses in food products. Gel strength of agar is influenced by concentration, time, pH and sugar content. The pH has a definite effect on gel strength: as the pH decreases, the gel strength weakens (Youssef, 2011). Increasing sugar content results in harder gels, with a less cohesive texture (Porto, 2003; Imeson, 2010).

Agar solutions are slightly negatively charged (Bohidar and Rawat, 2014). Stability is dependent on two factors, namely hydration and electric charge, and flocculation will follow upon removal of both factors. Prolonged exposure to high temperatures degrades agar solutions and lowers gel strength; these effects are accelerated by decreasing pH (Armisen and Galatas, 1987; Porto, 2003; Imeson, 2010).

Agar has the following general properties: maximum (max.) of 18 % moisture content; max. water absorption of 75 c.c.; max. of 0.5 % acidic insoluble ash; max. of 6.5 % total ash; pH 6.8 to 7; gel strength (1.5 % sol at 20 ºC / 68 ºF) of 700 to 1 000 g / cm^3; viscosity (1.5 % sol at 60 ºC / 140 ºF) of 10 to 100 cP; melting point of 85 to 95 ºC / 185 to 203 ºF; setting point of 32 to 45 ºC / 89.6 ºF to 113 ºF; solubility in boiling water; arsenic content of max. of 3 ppm; heavy metals of max. of 10 ppm; and lead content of max. of 10 ppm (Armisen and Galatas, 1987; Porto, 2003).

2.2.4 Xanthan

The bacterium, *Xanthomonas campestris*, produces an extracellular polysaccharide, called xanthan (Vu *et al*., 2009). Its primary structure consists of a cellulosic backbone of β-(1→4) linked D-glucose units, substituted on alternate glucose residues with a trisaccharide side chain (Sworn, 2009). Two mannose units, separated by a glucoronic acid, form this side chain (Figure 2.5) (Sworn and Kerdavid, 2012). About half of the terminal mannose units are linked to a pyruvate group, while the non–terminal residues usually carry an acetyl group. The carboxyl groups on the side chains add to the anionic nature of the hydrocolloid (Milani and Maleki, 2012). It is suggested that xanthan gum is in the shape of a helical structure, with the side chains positioned almost parallel to the helix axis, thus stabilising the structure. Xanthan gum forms very thick solutions, and, at high enough polymer concentrations, it acts like a weak gel (Cui *et al.*, 2005).

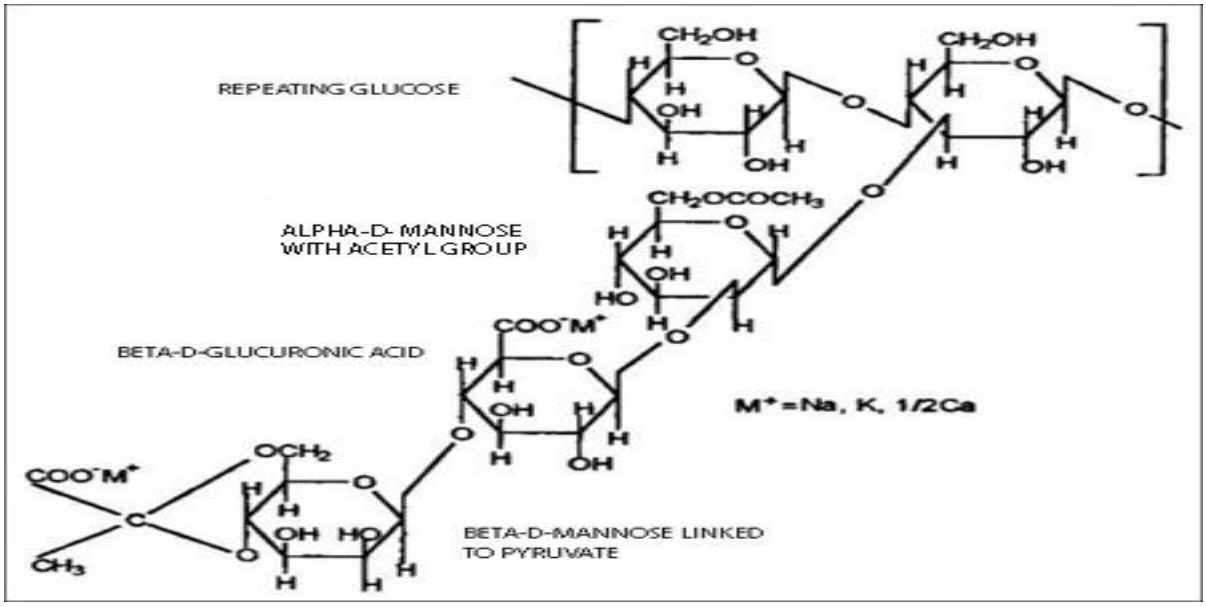

Figure 2.5: Chemical structure of xanthan (Chaplin, 2014).

Solubility of xanthan gum is good in either cold or hot water, due to the polyelectrolyte nature of the molecule. The formed solutions are very thick, even at low concentrations, resulting in food applications, such as thickening and stabilising of suspensions and emulsions (García-Ochoa *et al*., 2000).

The ability to thicken is related to the viscosity of the xanthan solutions. A high viscosity resists flow. Xanthan solutions appear to be solid at rest, hence the term pseudo-plastic. The apparent solid turns into a liquid as shear rate increases, which is also referred to as shear thinning. Viscosity also depends on temperature, biopolymer concentration, concentration of salts and pH (García-Ochoa *et al*., 2000).

Measurement and dissolution temperatures will determine viscosity of xanthan solutions. Measurement temperature is the temperature at which the viscosity is measured and dissolution temperature is the temperature at which the xanthan dissolves. As temperature increases, viscosity decreases and this behaviour is fully reversible between 10 °C and 80 °C / 50 °F and 176 °F (García-Ochoa *et al*., 2000; BeMiller, 2014; Kelco, 2018). With temperature increases, the optical rotation angle and circular dichroism of xanthan change. Conformational transition observed corresponds to a helix - coil transition of the backbone, with simultaneous release of the lateral chains, followed by progressive decrease of the rigidity of the (1±4) – β –D - glucan chain, as the temperature rises between 40 °C and 60 °C / 104 °F and 140 °F (Cui and Wang, 2005).

The typical properties of xanthan gum are as follows: 8 - 15 % moisture; 7 - 12 % ash; 0.3 - 1 % N; 1.9 - 6.0 % acetate content; 1.0 - 5.7 % pyruvate content; 3.6 - 14.3 g / ℓ monovalent salts; 0.085 – 0.17 g / ℓ divalent salts; and 13 – 35 cP viscosity (García-Ochoa *et al*., 2000).

The viscosity of solutions increases dramatically with increasing concentration of the hydrocolloid, due to intermolecular interaction or entanglement. This, in turn, increases the effective macromolecule dimensions and molecular weight. At low concentrations, the viscosity declines slightly when a small amount of salt is added, because diminished intermolecular electrostatic forces occur (Vega *et al.*, 2015). Viscosity increases at higher concentrations or when a large amount of salt is added, due to increased interaction between the xanthan molecules (Kelco, 2008; Dário *et al*., 2011).

Between pH 1 and 13 viscosity of xanthan solutions is not affected. At pH 9 or higher, xanthan is gradually deacetylated (Mahanta and Mahanta, 2016), while at pH lower than 3, xanthan loses the pyruvic acid acetyl groups (Dessipri, 2016). However, both these reactions have no effect on the viscosity of xanthan solutions (García-Ochoa *et al*., 2000).

Xanthan solutions show non-Newtonian rheology and apparent viscosity decreases as shear rate increases. No hysteresis is noted, and shear-thinning and recovery are instantaneous (Song *et al.*, 2006). However, xanthan solutions exhibit an initial yield stress that needs to be overcome before flow is possible (Amundarain *et al*., 2009).

There is a synergistic interaction between xanthan and guar gum, resulting in increased viscosity for the mixture (Saha and Bhattacharya, 2010). As noted above, xanthan changes its conformation when in solution, depending on the dissolution temperature. When xanthan is dissolved at a temperature below 40 °C / 104 °F, it has an ordered conformation that allows better interaction between xanthan and guar molecules (Figure. 2.6) (Casas and García-Ochoa, 1999). Dissolution temperature also influences the nature of the dissolved guar. As described

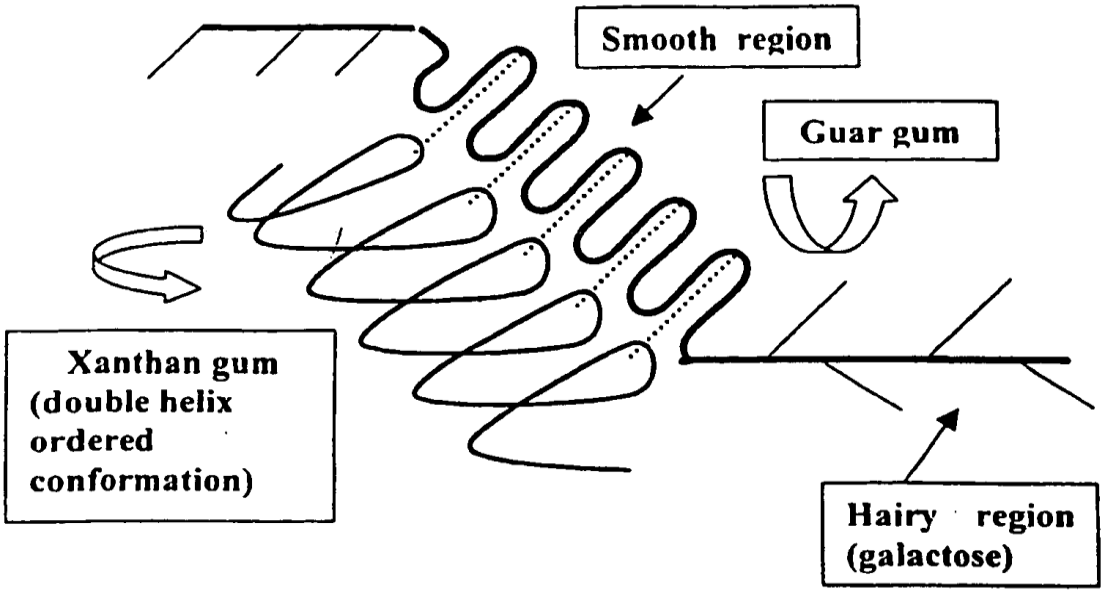

Figure 2.6: Interaction between xanthan and guar (García -Ochoa *et al*., 2000)

under heading 2.2.1, guar consists of a backbone chain of mannose units, which is linked to a monomolecular unit of galactose (Mudgil *et al*., 2011). These galactose residues are not uniformly distributed: there are regions without galactose (smooth regions) and others with many galactose residues (hairy regions). The smooth regions favour interaction with the

xanthan molecule, but this region is soluble only at ~ 80 °C / 176 °F (García-Ochoa and Casas, 1999). Thus, interaction between xanthan and guar is favoured when xanthan is dissolved at 40 °C / 104 °F and guar is at 80 °C / 176 °F (García-Ochoa *et al*., 2000).

2.3 *Opuntia ficus-indica*

Opuntia is a genus of fewer than 200 species of readily recognizable cacti from the Cactaceae family (Wilson, 2007). In nature, they grow in tropical and semitropical regions, but are very well adaptable to arid and semi-arid regions (Cárdenas, 1997; Sáenz *et al.*, 2004; Gebresamual and Gebre-Mariam, 2012; Isaac, 2016). This crop is well known, especially in Mexico, Argentina, Peru, Bolivia, Brazil, Chile, the USA (especially in the state Texas), Spain, Italy, North and Eastern Africa (Algeria, Morocco, Tunisia, Eritrea and Ethiopia), SA and Israel, as well as Central America and the Caribbean (Costa Rica, Cuba, Honduras, Nicaragua, Puerto Rico) (Figure 2.7) (Sepúlveda *et al.*, 2007; Isaac, 2016; CABI, 2018). This crop originated in Mexico, where it extended to Central America and the southern parts of the USA. Later, it spread to Africa (Cape Verde, Egypt, Eritrea, Ethiopia, Madagascar, Reunion, Seychelles, Somalia), Asia (Fujian, Guangdong, Guangxi, Guizhou, Sichuan, Yunnan, Zhejiangm Taiwan), Southern Europe and also to the islands Galapagos and Hawaii (Isaac, 2016; CABI, 2018). In SA and Australia, this crop is seen as a toxic weed. According to Isaac (2018), large areas in Chile, Algeria, Mexico and Brazil are used for cactus cultivation. Cactus pear is only native to Mexico and was introduced to the rest of the world (Isaac, 2018).

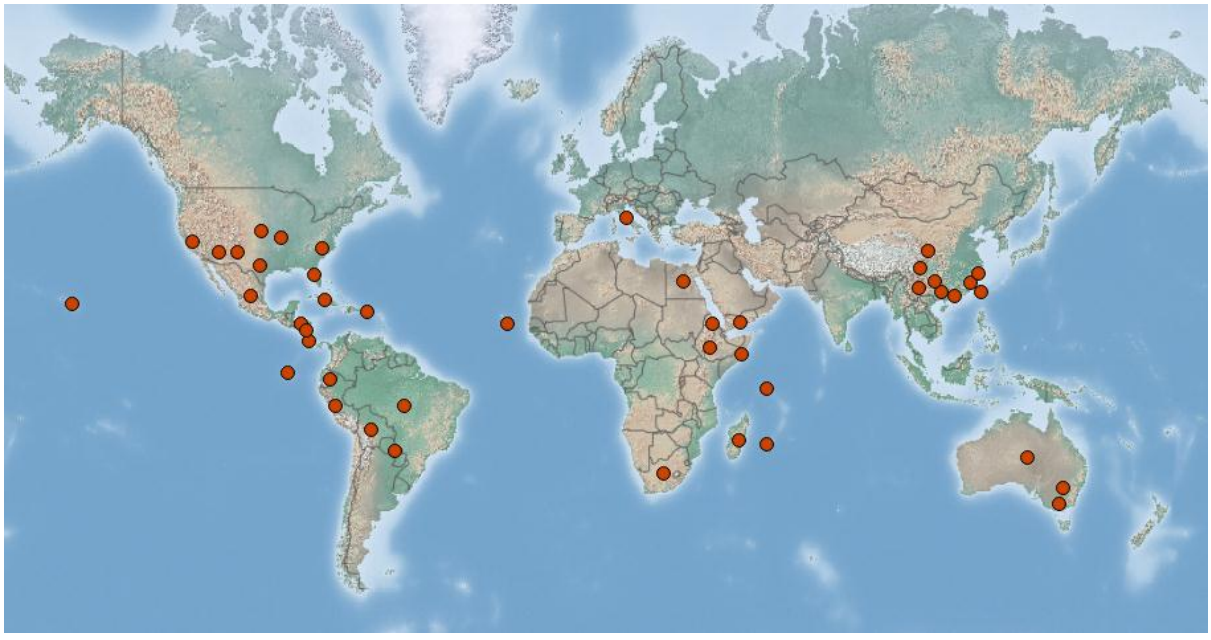

Figure 2.7: Most important areas in the world where *O. ficus-indica* are cultivated (CABI, 2018)

Customarily, *Opuntia* has been applied for both healing and edible purposes. Studies have indicated that extracts from the cladodes can lower cholesterol levels (Cárdenas *et al*., 1997), regulate low blood sugar (Andrade-Cetto and Heinrich, 2005), diminish ulcers (Galati *et al*., 2001), play a role in protecting neuronal structures (Dok-Go *et al*., 2003) and show anti-inflammatory and pain-relieving characteristics (Loro *et al*., 1999; Galati *et al*., 2001).

Opuntia starts as small ground-hugging plants and grow into massive trees. Many of these perennials are branched with distinctive jointed fleshy, flattened, rounded stem-segments, which are known as cladodes. The flowers that grow on the cladodes are often spectacular and symmetric, and vary in colour from yellow, orange, pink, red, magenta and sometimes white or bi-coloured (Figure 2.8). The fruits are club-shaped or cylindrical to ovoid, or nearly spherical, spineless to spiny, fleshy or dry, and range in colour from green to yellow, red orange or purple in the fleshy types or tan to grey in the dry species. The many seeds in each fruit have hard, bony aril or funicular envelopes surrounding them, which are characteristic to the subfamily *Opuntioideae*, to which *Opuntia* belongs (Sepúlveda *et al*., 2007; Wilson, 2007; Chauhan *et al*., 2010; Isaac, 2016).

Figure 2.8: *Opuntia ficus-indica* (Eaton, 2018).

Cactaceae are well adapted to arid and hot dry lands, where the plants have a marked capacity to withstand prolonged drought. The ability of the *Opuntia* cladodes to retain water under unfavourable climatic conditions is due, at least in part, to the water-binding capacity of a water-soluble pectin-like polysaccharide, called mucilage (Sepúlveda *et al*., 2007;

Gebresamual and Gebre-Mariam, 2012). There are a few potential uses for this plant material, but it is not yet an industrial hydrocolloid. The mucilage biosynthesis takes place in specialized cells that excrete it into the apoplasts, where it helps regulate the cellular water content and has a role in Ca^{2+}-saving in the plant (Cardenas, 1997). This bio-mineral in mucilage has a strong effect in molecular conformation, which leads to a positive effect on the WHC of the mucilage. The difference in calcium oxalate (CaC_2O_4) content could be responsible for differences in WHC, which causes a difference in the swelling power of the mucilage and moisture content (Gebresamuel and Gebre-Mariam, 2011).

2.3.1 Cladodes

The weight and length of the harvested cladodes fluctuate, depending on the specie. However, they generally weigh from 40 to 100 g and are 11 to 20 cm long (Cantwell *et al*., 1992; Mizrahi *et al*., 1997; Berger *et al*., 2013), and the plants are generally one to three years old (Le Houérou, 1996). Due to the daytime acidity changes in the cladodes, harvesting a few h after sundown delivers the best quality cladodes to be used for human consumption, which are plump, full of sugars and are pro-vitamin A and C (Le Houérou, 1996).

Generally, cladodes are rich in pectin, mucilage and minerals (Habibi *et al*., 2004). The fresh young stems are a source of proteins, including amino acids and vitamins. The major amino acid detected is glutamine, followed by leucine, lysine, valine, arginine, phenylalanine and isoleucine (Feugang *et al*., 2006). The free amino acid contents in cladodes, in g / 100 g, are: alanine (0.60-1.25); arginine (2.40-5.01); asparagine (1.50-3.13); asparaginic acid (2.10-4.38); glutamic acid (2.60-5.43); glutamine (17.30-36.12); cysteine (1.04); glycine (0.50); histidine (2.00-4.18); isoleucine (1.90-3.97); leucine (1.30-2.71); lysine (2.50-5.22); methionine (1.40-2.92); phenylalanine (1.70-3.55); serine (3.20-6.68); threonine (2.00-4.18); tyrosine (0.70-1.46); tryptophane (0.50-1.04); and valine (3.70-7.20) (Feugang *et al.*, 2006). α-Aminobutyric acid, carnosine, citrulline, ornithine, proline, taurine and glycine are only available in trace amounts (Feugang *et al*., 2006). Vitamin and antioxidant contents, in mg per 100g, in the cladodes, are: ascorbic acid (7.00-22.00); vitamin B_1 (0.14); vitamin B_2 (0.60); vitamin B_3 (0.46) niacin (0.46); riboflavin (0.60); thiamine (0.14); total carotenoid (0.14), with beta-carotene consisting of 11.30-53.50 µg (Stintzing *et al*., 2001; Kuti, 2004; Stintzing and Carle, 2005).

The cladodes are characterised by high malic acid contents due to CAM-based daytime rhythm (Ben Salem *et al*., 2005; Stintzing and Carle, 2005). Crassulacean Acid Metabolism (CAM) is a diurnal cycle of (i) night-time uptake of CO_2 and fixation via phosphor-enol-pyruvate carboxylase and storage in vacuoles as malic acid, and (ii) day-time remobilization and absorption of CO_2 in the Calvin cycle (Winter, 1996). The mineral contents in mg per 100 g dry weight for the spineless cladodes, are as follows: calcium (Ca) (5.64-17.95); CaC_2O_4 (4.30-11.5); magnesium (Mg) (0.19-8.80); potassium (K) (2.35-55.20); phosphorus (P as PO_4) (0.15-2.59); sodium (Na) (0.30-0.40); zinc (Sn) (0.08); manganese (Mn) (0.19-0.29); and iron (Fe) (0.14 μg-0.09 mg) (Ben Salem *et al*., 2005; Feugang *et al*., 2006; Ayadi *et al*., 2009; Contreras-Padilla *et al*., 2011).

Phenolic compounds comprise of a wide variety of compounds, divided into several classes, such as hydroxybenzoic acids, hydroxycinnamic acids, anthocyanins, proanthocyanidins, flavonols, flavones, flavanols, flavanones, isoflavones, stilbenes and lignans (Cieslik *et al*., 2006). These compounds are usually by-products of plant metabolism and they possess antioxidant potential, which is involved in health benefits, such as the prevention of inflammation (Laughton *et al*., 1991). El-Mostafa *et al*. (2014) reviewed the phenols and flavonoids in *O. ficus-indica* cladodes, as follows, in mg per 100 g: gallic acid (0.64-2.37); coumaric (14.08-16.18); 3,4-dihydroxybenzoic (0.06-5.02); 4-hydroxybenzoic (0.50-4.72); feruli acid (0.56-34.77); salicylic acid (0.58-3.54); isoquercetin (2.29-39.67); isorhamnetin-3-*O*-glucoside (4.59-32.21); nicotiflorin (2.89-146.50); rutin (2.36-26.17); and narcissin (14.69-137.10) (Gallegos-Infante *et al*., 2009; Ginestra *et al*., 2009; Guevara-Figueroa *et al*., 2010; Valente *et al*., 2010). Cladode age, environment, soil type and climate could explain the variations in these polyphenol contents (El-Mostafa *et al*., 2014).

Chromatographic analyses of total lipids, extracted from *Opuntia* cladodes, show that palmitic acid (C16:0), oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3) contribute 13.9, 11.2, 34.9 and 32.8 %, respectively, of the total fatty acid content. These four fatty acids, thus, represent over 90 % of the total fatty acids, with linoleic and linolenic acids, the major polyunsaturated fatty acids, accounting for 67.7 % (Abidi *et al*., 2009). The linoleic acid content in cactus cladode (34.9 %) is, thus, close to the percentage found in argan oil (31.3 %) (Charouf and Guillaume, 2007). It is, however, lower than extracts from barely (51.3 %) and soybean oil (50.1 %) (Abidi *et al*., 2009).

When cladodes are three to four weeks old, they are called *nopales* (Figure 2.9), with a nutritional content in-between that of lettuce and spinach (Betancourt-Dominguez *et al*., 2006).

Figure 2.9: *Nopales* are sold at markets in Mexico (Bowman, 2018)

The nutritional value of *nopales* is similar to that of many vegetables: mostly water (88-95 %); some carbohydrates (3-7 %), and minerals (about 1.3 %, mainly Ca). Like most vegetables, *nopales* are low in proteins (about 1%) and fibre (about 1%, which is still more than twice that of lettuce). *Nopales* are less nutritious than spinach, but more nutritious than lettuce (Rodriquez-Felix and Cantwell, 1988) and are cut into slices, known as *nopalitos*, and sold fresh, bottled, or canned and less often even dried. They have a light, slightly tart flavour, and a crisp, mucilaginous texture. *Nopalitos* are often eaten with eggs as a breakfast and in salads and soups as lunch and dinner meals (Figure 2.10 a-d) (Jinich, 2014; Bowman, 2018). Furthermore, *nopalitos* contain galactogamin gums, which retard the absorption of their sugars by the digestive system and are, thus, considered to have a low glycaemic index (Gutierrez, 1998).

Figure 2.10: a. *Nopales* being sliced into *nopalitos*; b. *nopalitos*; c. canned *nopalitos*; and d. *nopalito* salad ('cactus paddle salad') (Jinich 2014; Bowman, 2018).

2.3.2 Mucilage

The Cactaceae family, to which *O. ficus-indica* belongs, is characterized by the ability to produce slime when cutting through the cladodes, where it is stored in cells. This slimy material, also referred to as *nopal* dribbles, mainly consists of mucilage, a soluble fibre. This fibre is a complex polysaccharide carbohydrate, with a highly branched structure, and is referred to as a gum or hydrocolloid. The carbohydrates included in the structure are L-arabinose, D-galactose, L-rhamnose, D-xylose and galacturonic acid in various proportions (Figure 2.11 a-e) (Sepúlveda *et al*., 2007). It also contains glycoproteins (Pichler *et al*., 2012) and other substances such as tannins, alkaloids and steroids (Gebresamual and Gebre-Mariam, 2012). The mucilage composition differs among the various *Opuntia* spp. and the regions in which they grow (Sáenz *et al*., 2004; Gebresamual and Gebre-Mariam, 2012).

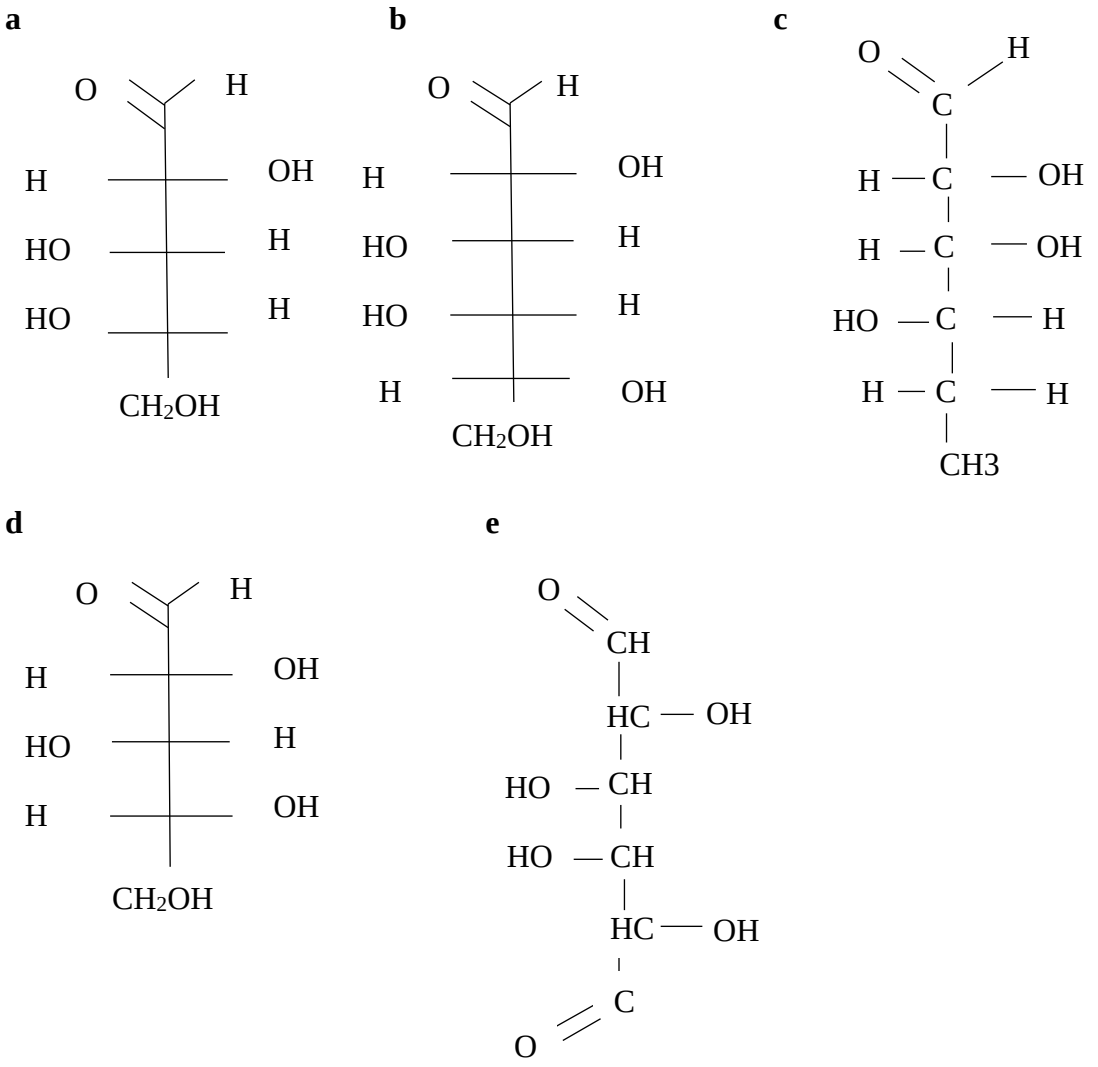

Figure 2.11: Chemical structures of carbohydrates included in structure of mucilage: a. L-arabinose (BeMiller, 2014); b. D-galactose (BeMiller, 2014); c. L-rhamnose (Mortada, 2009); d. D-xylose (BeMiller, 2014) and e. galacturonic acid (BeMiller, 2014).

It is suggested that the mucilage structure consists of two distinct fractions that are soluble in water. One fraction is pectin, which, in the presence of Ca^{2+} has the ability to form gels, while the other fraction is mucilage, with no gelling properties (Goycoolea and Cárdenas, 2003). The water-soluble polysaccharide fraction, with thickening properties, represents less than 10 % of the water-soluble material (Majdoub *et al*., 2001) in the case of *O. ficus-indica*.

Two layers in the epidermis of the cladodes, namely the chlorenchyma and the parenchyma store water and also contain mucilaginous cells, which store mucilage (Granados and Castañeda, 1996; Terrazas and Mauseth, 2002). Research on the tissues in the cladodes showed

that the mucilage only exists in the Golgi body (Figure 2.12), where synthesis of the mucilage possibly occurs (Trachtenberg and Mayer, 1981).

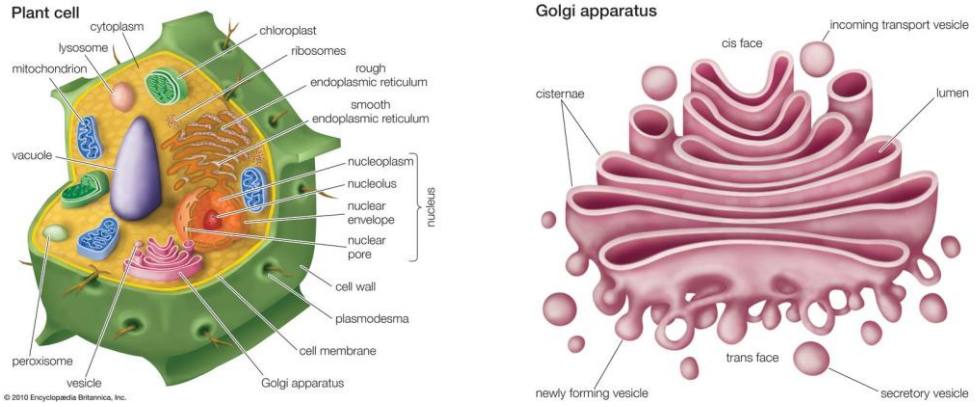

Figure 2.12: Golgi body in plant cell (Rogers, 2017).

In order for mucilage from *O. ficus-indica* to be used commercially, an extraction procedure need to be established that would provide an expectable yield. The liquid mucilage extracted from cladodes may be useable as is, or may be processed to produce a powder-like material for application in food processing. Furthermore, cactus mucilage has the advantage of being natural, healthy and cheap (El-Mostafa *et al.*, 2014); however, only around 20 % of fresh weight of cladodes are by-products (Bensadón *et al.*, 2010*)*, including fibre, minerals and proteins.

2.3.2.1 Extraction and average yield of mucilage

The mucilage content in a cladode is directly related to the moisture content, because of its components having the ability to absorb water. Furthermore, moisture content is higher in young cladodes than in older cladodes (Monrroy *et al*., 2017). Over the past 40 years various extraction procedures have been studied (Sepúlveda *et al*., 2007; Rodríguez-González *et al*., 2014; Felkai-Haddache *et al*., 2016; Monrroy *et al*., 2017).

Thermal processes can be done on fresh and dried cladodes. Fresh cladodes are peeled and cut into pieces (2x2x2 cm), crushed and homogenised in a ratio of 7.5 g in 15 ml distilled water. This ratio is equivalent to suspending 100 mg of dry basis in 5 ml of water. Samples are placed in a water bath at different temperatures for various reaction times and then filtered. The mucilage are then precipitated by adding 45 ml ethanol and dried in an oven at 60 ˚C / 140 ºF (Monrroy *et al*., 2017). Fresh cladodes can also be cut into pieces (2x2x2 cm) and dried at

60˚C / 140 ºF for 48 h, and then milled. The mucilage are extracted by mixing 100 mg of the milled sample with 5 ml water at different temperatures (44 ˚C to 86 ˚C / 111.2 ºF to 186.8 ºF) and for different periods of time (54–96 min).The mucilage are then precipitated by adding 15 ml ethanol and dried in an oven at 60 ˚C / 140 ºF for 54–96 min (Monrroy *et al*., 2017).

Non thermal processes are based on hydration, agitation, and a combination of these two processes. For extraction by hydration, a solution is prepared by mixing the milled sample (100 mg) with water (5 ml), and allowing it to stand for 24 h. The solution is filtered and the mucilage precipitated by adding 15 ml ethanol and drying it in an oven at 60 ˚C / 140 ºF (Monrroy *et al*., 2017). Extraction by agitation follows the same procedure as described above, but the mixture is stirred for 30 min with a magnetic stirrer and then filtered. The mucilage is precipitated by adding 15 ml of ethanol for 30 min and then dried in an oven at 60˚C / ºF 140 (Monrroy *et al*., 2017). Extraction by agitation and hydration follows the same combined procedures as described above (Monrroy *et al*., 2017).

Microwave-assisted extraction (MAE) from cladodes are performed in a domestic microwave oven system. The apparatus is equipped with a digital control system for irradiation time and microwave power (the latter is linearly adjustable from 100 to 1000 W). The oven are modified in order to condense the vapours generated during extraction into the sample. The MAE procedure is performed in a 500-ml volumetric flask with three volumes of distilled water at different powers (500, 700 and 900 W) during 7 min, with 1 min steps. The extracted mucilage is immediately cooled in an ice bath (4 °C / 39.2 ºF) and filtered through a double layer cheesecloth, to remove the pulp. It is centrifuged, filtrated in 95 % ethanol at 4 °C / 39.2 ºF, where after the precipitate is washed with 75 % ethanol. Finally, lyophilisation is carried out at -55 °C / - 67 ºF for 12 h (Matsuhiro *et al*., 2006; Felkai-Haddache *et al*., 2016).

Powdered cladodes are hydrated with water for 90 min and rigorously stirred to remove the mucilage (Habibi *et al*., 2004). Samples are separated from the mucilage extract by centrifugation at 4 000 rpm for 15 min. The supernatants are removed and stored at 4 °C / 39.2 ºF, until needed for extraction. Residues are dried overnight at 60 °C / 140 ºF. A dried pre-treated 5.0 g is mixed with water and placed in an ultrasound water bath, using a frequency of 40 kHz and input power of 330 W. The liquid phase is separated from the insoluble residue by centrifugation at 4 000 rpm for 15 min and precipitated with two volumes of isopropanol overnight at 4 °C / 39.2 ºF. The precipitate are recuperated and dried at 50 °C / 122 ºF until a

constant weight is reached (Bayar *et al*., 2017).

2.3.2.2 Nutritional contents of mucilage

The total carbohydrate content ranges from 13.0 to 64.0 % (Cota-Sánchez, 2016; Nharingo and Moyo, 2016). In addition, mucilage is an excellent source of essential minerals, such as Ca, Cu, Fe, Mg, Mn, P, Se and Zn (Isaac, 2016). *Opuntia ficus-indica* is a better source of Ca than spinach, soy and grains, suggesting that *Opuntia* spp. may have potential in the prevention and treatment of diseases such as osteoporosis (Hernández-Urbiola *et al*., 2010). Crude protein content is 3.66 to 8.08 %, which is higher than those for xanthan (1-2 %) and agar (1%), but in the range for guar gum (5.0-6.0 %) (Hernández-Urbiola *et al*., 2010; Mudgil *et al*., 2011; Gebresamuel and Gebre-Mariam, 2012; Murwan *et al*., 2012; Isaac, 2016; Du Toit, 2017). The interactions that occur between the hydrophilic groups of the polysaccharides and proteins are important, because they form a three-dimensional network that can stabilise the consistency of the system matrix. Possible applications for the food industry can arise from this (Rincón *et al*., 2008), as proteins and polysaccharides are safe food additives that can form physically stable emulsions (Chityala *et al*., 2016). Saponins, flavonoids and alkaloids are also present in the mucilage and vary, depending on chemical characteristics of the soil, geographical location, environmental conditions and age (Sepúlveda *et al*., 2007; Hernández-Urbiola *et al*., 2010).

2.3.2.3 Functional properties of mucilage

The functional properties of mucilage can be affected by the chemical composition. The mucilage density, at a concentration of 8.0 %, is 0.85 g / ml. This density is comparable to those reported for xanthan (1.50 g / ml), guar (0.80-1.00 g / ml), agar (0.55 g / ml) and gelatine (0.98 g / ml). Xanthan is mostly used as a substitute in baking and thickening, while guar is added as a stabilising agent, fibre source and as thickener for hot or gold beverages (Mudgil *et al*., 201l). Gelatine and agar are mostly applied in the food industry as gelling agents; gelatine perhaps more so, because of its thermally reversible gelling properties with water, e.g. to produce table jellies (Cole, 2000).

According to Gebresamuel and Gebre-Mariam (2012), the conductivity values for mucilage for 0.20 g / 1 % (w / v); 0.80 g / 4 % (w / v); 1.60 g / 8 % (w/v) and 2.40 g / 12 % (w / v), are 2.73 g / ml^{-1}; 9.69 g / ml^{-1}; 12.04 g / ml^{-1} and 13.12 g / ml^{-1}, respectively. Differences in conductivity concentrations may be ascribed to the incidence of a greater number of divalent and monovalent ions, which increases the conductivity. According to Gebre-Samuel and Gebre-

Mariam (2012), the presence of these electrolytes in the mucilage can be valuable in the flocculation of suspension formulations.

Contreras-Padilla *et al*. (2016) reported the pH value to be 5.5 to 6.0, which is comparable to those reported for gelatine (8.0-9.0), xanthan (6.0-8.0), agar (6.5-7.5) and guar (5-7). The pH is also a critical factor in coagulation / flocculation processes, wherein the optimal pH should be between 5 and 7.5 (Contreras-Padilla *et al*., 2016). The viscosity for mucilage solutions of 1 and 4% are 1.6cP and 4.6 cP, respectively. These values are comparable to those for solutions of the same concentrations of pure gelatine (2.0-7.0 cP). Agar (35 cP for 1%), xanthan (1 550cP for 1%) and guar (5 000 cP for 1%) has much higher viscosities for solutions of the same concentrations (Prasad *et al*., 2005; Sharma *et al*., 2006; Mudgil *et al*., 2011; Ninan *et al*., 2014). The ability of mucilage to form low viscosity solutions at low concentrations, in the same manner as gelatine, should enable it to form solutions in a wide range of concentrations, with excellent properties as an emulsifier and stabiliser (Monrroy *et al*., 2017)

Opuntia ficus-indica mucilage presents a better WHC than oil-holding capacity (OHC) (Du Toit, 2017). The WHC influences the formation of viscous solutions that can assist in industrial practises. Mucilage forms a three-dimensional network when coming in contact with water, trapping it and resulting in very viscous solutions. Mucilage mainly consists of galactose, mannose, xylose and other sugars, and, thus, has a good ability to bind or hold water, comparable to pectins, gums and some seaweed polysaccharides. Because of this large water absorption capacity, mucilage may find uses in foods, cosmetics and pharmaceuticals, in which it can dissolve, be dispersed and form colloids (Del-Valle *et al*., 2005). In addition, the good OHC value suggests that mucilage could improve the texture of food products (Monrroy *et al*., 2017).

Additionally, as a negatively charged polyelectrolyte molecule, due mainly to its sugar composition, mucilage also has the ability to attract or retain certain types of positively charged substances (Pichler *et al*., 2012), such as sugars and fats, which are caught within the matrix of mucilage and water (Sáenz *et al.*, 2004).

Thus, it has the potential to be used in food applications as a thickener, stabilizer and emulsifier due to its functional properties, including high viscosity, high WBC and gelatinisation ability (Bensadón *et al.*, 2010). Mucilage has emulsifying capabilities in that it reduces surface and

interfacial tensions, stabilise oil-in-water emulsions, and forms small droplets and absorbs onto oil-water under-surfaces. It is suggested that the stabilizing properties are similar to those of xanthan gum and guar gum (Bensadón *et al*., 2010). Xanthan and guar gum traps water by forming hydrogen bonds with the water molecule, thus, acting as a stabilizer (Mudgil *et al*., 2011). Other mucilage applications include its use in foods as a flavouring agent and fat substitute (Sáenz *et al.*, 2004). An exciting application is as edible coating, where thin layers are formed on the surface of food, e.g. it was found useful to extend the shelf life of strawberries, by acting as an edible coating (Del-Valle *et al*., 2005). Mucilage can also be used in egg foams to increase stability, as well as in gelled dessert and powdered drinks available in Mexico, which are prepared with water or milk (Sáenz *et al.*, 2004).

2.4 Marshmallows

Marshmallows are one of the most popular candies on the planet and, in the USA only, 90 million pounds are eaten every year, i.e., 40 823 313.3 kg. Half of these are roasted over a flame or coals (Figure 2.13a) (Petkewich, 2006). In SA, 100 million chocolate marshmallow eggs (Figure 2.13b) are consumed during the Easter season, resulting in one chocolate manufacturer, Beacon Sweets and Chocolates, already starting in October each year manufacturing one million of these eggs per day (Naidu, 2006). Marshmallows even have a chemical formula, namely C6H12O6 + H2O + C2HC5H9NOC5H10NO2 (Gomezalcorta, 2015).

Marshmallows qualify as aerated confectionery and consist of sugar, carbohydrates and various types of glucose syrups, stabilizing agents, whipping agents, colours and flavours. Aeration increases volume, reduces density, stabilises, decreases stickiness and cold flow, and reduces sweetness. It also decreases product texture and makes the product less satiating, while improving flavour and overall appeal, thus modifying the texture and organoleptic characteristics of the confectionery products. Aeration levels and textures in aerated confectionery products vary from product to product and are achieved either by chemical or mechanical aeration, e.g. whipping and agitation (Edwards, 2000; Zanizdra, 2015; Hartel *et al*., 2018 a).

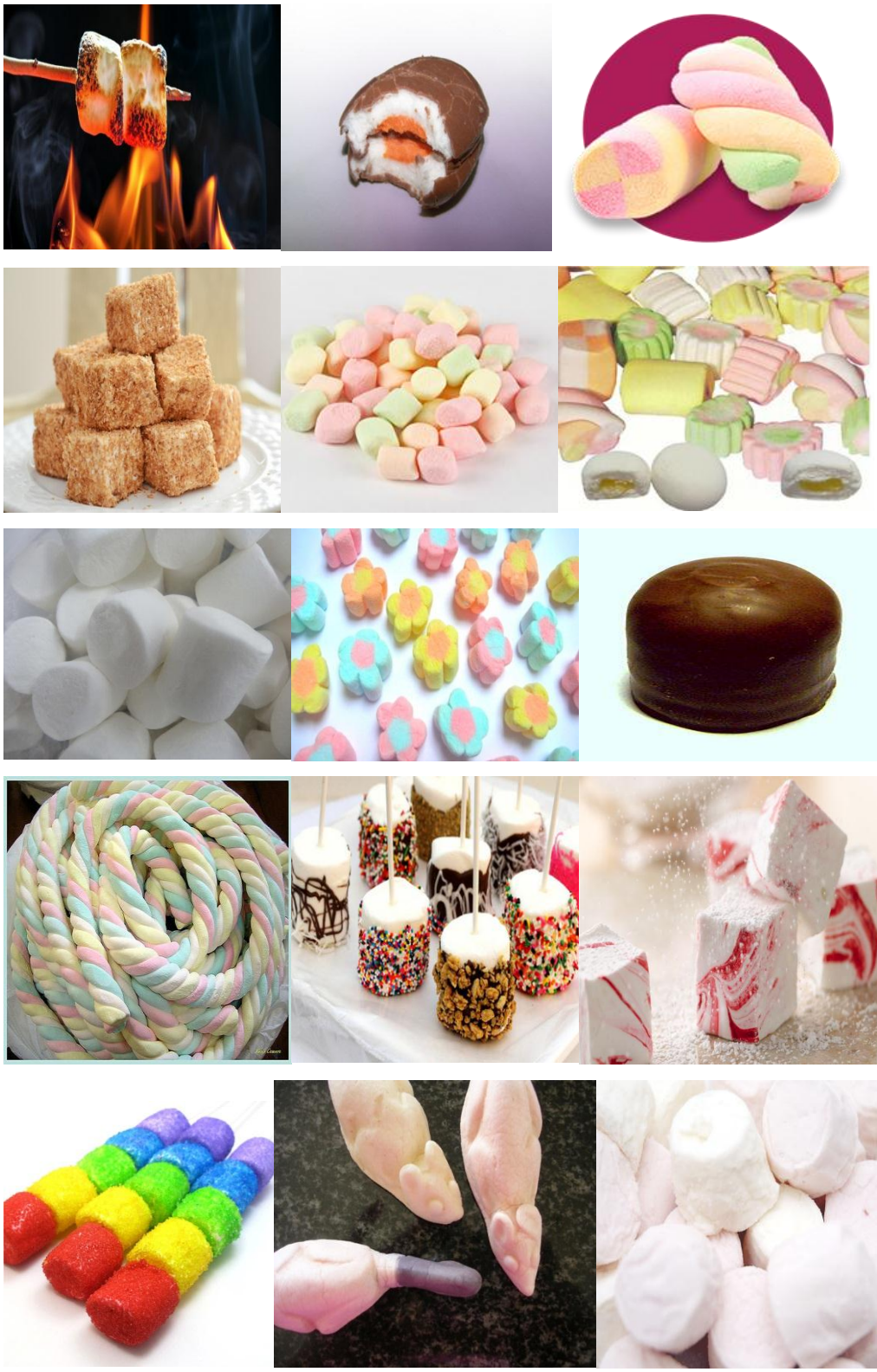

Figure 2.13: a. marshmallows roasted over flame; b. chocolate marshmallow Easter egg; c. extruded multi-colour strips; d. roasted coconut covered marshmallows; e. mini marshmallows; f. extruded and centre-filled marshmallows; g. extruded marshmallows; h. extruded marshmallows with coloured centres; i. cutie pie; j. extruded multi-colour twisted marshmallow ropes; k. dipped marshmallows; l. marbled marshmallows; m. sparky colourful marshmallow kebab; n. marshmallow mice; and o. traditionally-shaped marshmallows (Husband, 2014; Gomezalcorta, 2015; Getty Images, 2016; Bowman, 2017; NCA, 2018).

The aeration process is accomplished by using aerating agents, such as hydrolysed proteins, intact proteins and saponins, which are dissolved to be activated. Stabilizers used in the aeration process include gelatine and whipping agents. Gelatine plays the role of foaming agent, stabilizer and binder in aerated confectioneries, which provide creamy and chewy textures. Similarly, the whipping agent increases aeration and improves the texture (Zanizdra, 2015; Hartel *et al.*, 2018 a).

Marshmallows are one of the earliest confections known to mankind and are available today in many shapes and sizes (Figure 2.13c-o). They can be solid (soft pillows dropped in cocoa or roasted on a stick) to semi-liquid (covered in chocolate or formed into eggs for Easter) to the crème-like (used as a base in other candies or as an ice cream topping) (Olver, 2000; Tan and Lin, 2008).

Originally, marshmallows were made from the root sap of the marshmallow (*Althaea officinalis*) plant, which is a herb native to parts of Europe, North Africa and Asia, where it grows in marshes and other damp areas (Sadighara *et al*., 2012). The plant has a fleshy stem, leaves and pale flowers with five petals (Figure 2.14 a and b). The first marshmallows were made by boiling pieces of the marshmallow root pulp with sugar, until thick, where after the mixture was strained and cooled (NCA, 2018). As far back as 2000 B.C., the Egyptians mixed marshmallow root with honey, making a candy that was only reserved for the gods and royalty. Until the mid-nineteenth century, access to marshmallow confections was limited to the wealthy. Common people only tasted marshmallows when they took pills, as doctors sometimes hid medicine inside the candy to cover its undesirable taste (Olver, 2000; Petkewich, 2006; NCA, 2018).

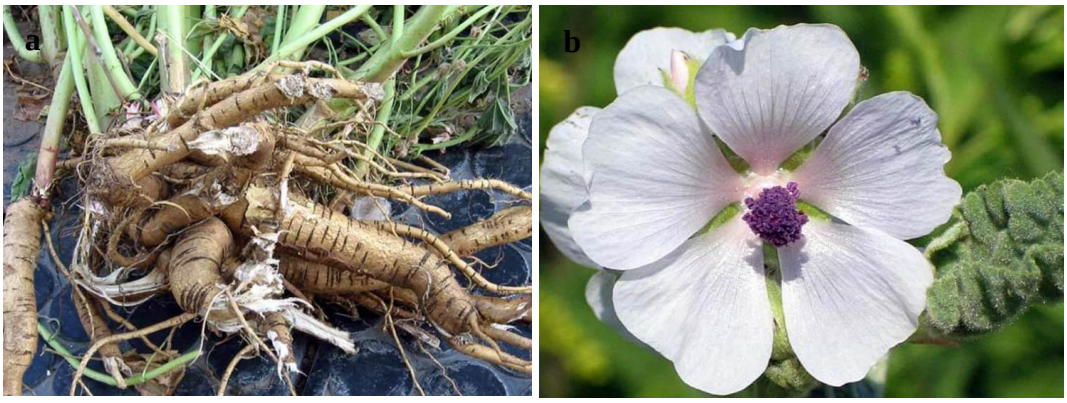

Figure 2.14: a. Root and b. flower of *Althaea officinalis* (Alicandro-Mace, 2017).

Contemporary marshmallow confections were first prepared in France around 1850. This was an expensive and slow manufacturing method, as each marshmallow had to be individually casted and moulded. French confectionary makers used the mallow root sap as binding agent for the egg whites, corn syrup and water. The fluffy mixture was heated and poured onto the corn starch in small moulds, forming the marshmallows. At this time, marshmallows were still not mass manufactured; instead, they were made by confectioners in small stores or candy companies (Groves, 1991; Bowman 2017).

Today, marshmallows are made using the extruding process, consisting of five steps, which is more time efficient than the traditional process. Sugar and corn syrup is dissolved in water and cooked to a syrup in a cook kettle at 115 °C / 240 °F. The mixture is poured into a pump and beaten to a foam, increasing two to three times in volume. Flavouring is added and the heated mixture is transferred to a heat exchanger, where air is pumped into the mixture. The mixture is left to cool in a tempering kettle, where after marshmallows are extruded through a machine or deposited onto bands.

Prior to extrusion, the aerated mixture can be diverted into a number of streams and individually coloured, thus, creating multi-coloured ropes (Yixun machines, 2017). The extrusion process involves the foam being squeezed through a die to produce marshmallow's familiar pillow shape. Usually, they get a coating of corn starch to counter stickiness and help maintain their form after they have been extruded. Sometimes the pillows are formed into a rope of pillows. If so, they are cut and dried on a rubber conveyor belt (Zanzidra, 2016). The extruded marshmallow line can also be adapted to produce traditionally starched products, as well as products enrobed in chocolate or a compound coating (Yixun machines, 2017). After the products have been formed, they are sent through a cooling drum, where excess starch is removed. After cooling, the marshmallows are weighed and packaged (Groves, 1991; Zanzidra, 2016). Though the basic ingredients are mixed in a different ratio, the basic process for deposited marshmallows is similar to the extruded marshmallows; the difference lies in the manifold and finishing line (Yixun machines, 2017).

2.4.1 Major ingredients used in the making of marshmallows

Marshmallows are made by soaking gelatin in cold water. Sugar, salt, syrup and water are heated and stirred until all the sugar has dissolved. After the mixture reaches a temperature of 118 °C / 244.4 °F, gelatin is mixed in. Stiffly beaten egg whites are added to the hot syrup

mixture, continuously mixing it until the mixture becomes thick and creamy. The mixture is then poured into a greased pan, cooled overnight and cut into 25 mm squares. The squares are rolled in a mixture of corn flour and icing sugar, and stored in an airtight container (Foods and Cookery, 1991).

2.4.1.1 Egg whites

Egg whites, also known as egg albumin, are used as a protein-whipping agent in marshmallows. Its function is to provide lightness of texture and low density in all-sugar formulations that, without such modification, would be essentially hard candies (Vaclavik and Christian, 2014). Egg albumin foams consist of a discontinuous air phase that is dispersed in a continuum of liquid and are produced by whipping (Tyapkova *et al*., 2016). Because of its ability to increase the volume up to six times (Lomakina and Míková, 2006), these foams hold large volumes of air, resulting in a much lighter marshmallow (Kamosawa and Talbot, 2006).

2.4.1.2 Sugar

Sucrose (Figure 2.15a), commonly known as table sugar, is derived from either sugar cane or sugar beets and is a disaccharide that consists of one glucose (Figure 2.15b) and fructose (Figure 2.15c) molecule (BeMiller, 1996). For marshmallows, a saturated sugar syrup solution is made by dissolving sugar in water, until no more sugar can be added. Sucrose is the most commonly sugar used and other sugars, such as glucose, invert sugar or corn syrup is added to make sucrose more soluble and less likely to form large crystals (Brown, 2008). The main functions of sugar in marshmallows are for sweetness, while aiding in foam formation, enhancing flavour and acting as preservative (Parker and Pace, 2016). The sugar syrup also stabilizes and sweetens the foam (BeMiller, 1996).

Marshmallows are an amorphous solid, because of the way in which the sugars crystallize. The crystals are not grained, but very fine in size, in contrast to a crystalline solid, where the crystals are grainy and larger in size. Temperature is responsible for an amorphous solid, like marshmallow. Sucrose, corn syrup and fructose, dissolved in water, are heated to a high temperature and then cooled down so rapidly, that no crystallisation can occur. This rapid cooling of a liquid in open air does not allow the sucrose molecules to form crystals, so amorphous crystals are created instead (Husband, 2014). When a combination of different sugars is used, each will influence the solubility concentration of the others. Presence of invert

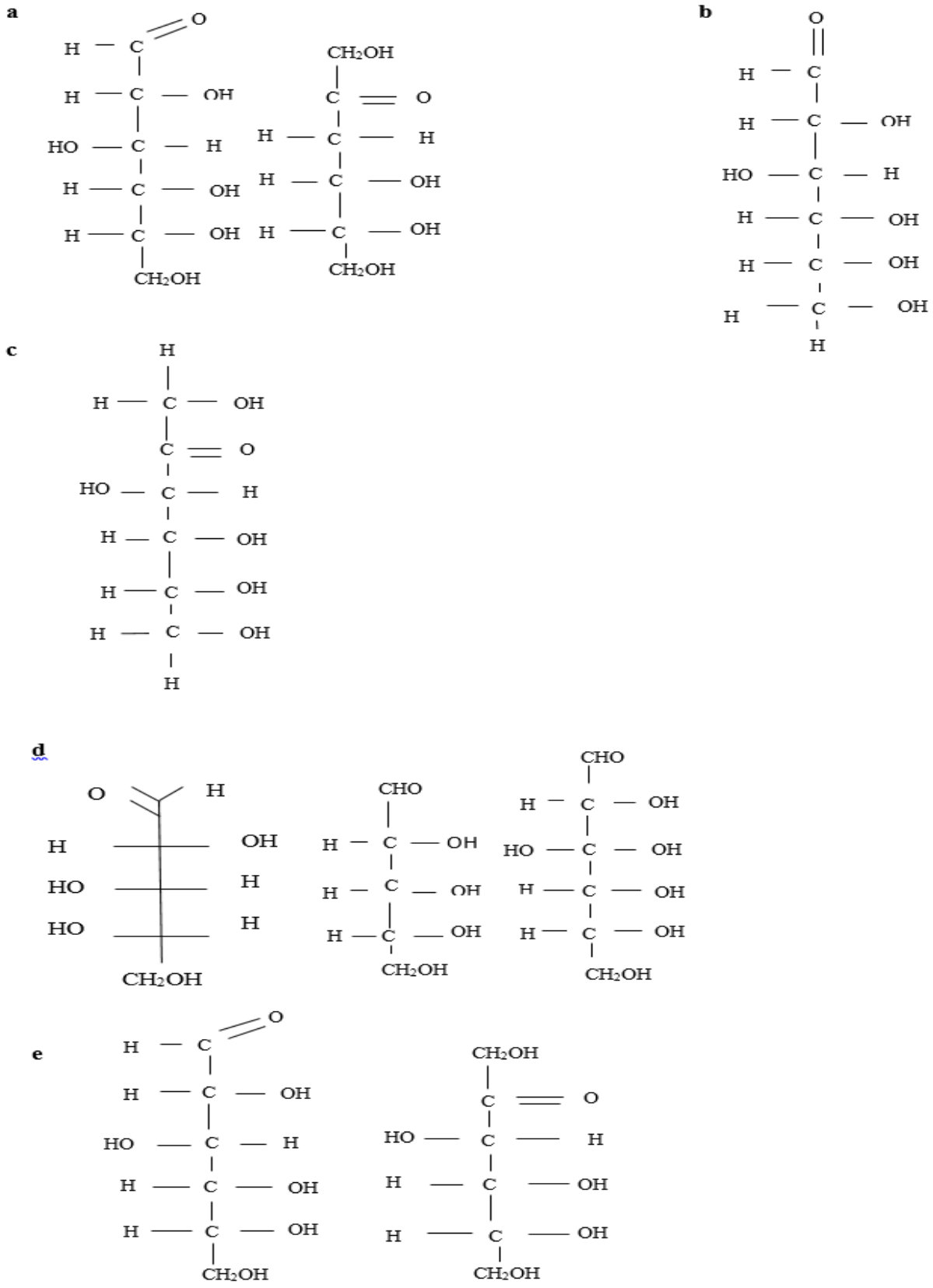

Figure 2.15: Chemical sturctures of a. sucrose, b. glucose, c. fructose (Mcgee, 2004), d. corn syrup and e. invert sugar (Skura *et al.*, 2010)

sugar and / or corn syrup causes a substantial decrease in sucrose solubility, due to the competition among the sugar molecules for water (Hartel *et al*., 2018 b).

In general, sugars weaken the potential of foaming, but increase stability once a foam is formed. Therefore, sucrose is used in combination with a protein, like gelatine. The protein can adsorb, unfold and form a secure network, while the sugar can increase the viscosity (BeMiller, 1996).

Liquid drainage of the dispersion medium should also be diminished. According to Takagi and Huppert (2011), high viscosity liquids drain slower than low viscosity liquids; thus, by increasing the viscosity of the dispersion medium drainage will be reduced. It is essential to achieve high viscosity, if a stable foam is the objective (Tan *et al*., 2015). For this reason, sucrose is used in combination with other sugars (Raikos *et al*., 2007; Yang, 2008).

When the sugar solution cools down, crystals start to form. The principle of Le Châtelier explains that, when crystallisation occurs, a system is shifted away from its equilibrium. Reactions will start to oppose the shift, thus, restoring stability. An increase in temperature, therefore, decreases the system's energy, in an attempt to lower the temperature. The energy is then absorbed, as the chemical bonds in the sucrose molecules break. This cools down the system and more sucrose molecules break apart, dissolving into the solution (Husband, 2014). The opposite is also true, according to Le Châtelier's principle. A decrease in temperature causes a system to generate energy to increase the temperature. The formation of chemical bonds emits energy, so more sucrose molecules will join crystals which have already been formed, to increase the temperature. This explains why crystallization occurs when temperature decreases (Husband, 2014).

Corn syrup (Figure 2.15d), also known as glucose syrup, consists of dextrin, maltose and dextrose, and is formed due to partial hydrolysis of corn starch (BeMiller, 2014). It prevents crystallization of other sugars, such as sucrose, contributes to body, reduces sweetness, and alters flavour release and hygroscopicity, depending on its Dextrose Equivalent (DE). Dextrose Equivalent is the measurement of the amount of reducing sugars present in a sugar product, in relation to glucose. Lower-DE glucose syrups will provide a chewier texture, while higher-DE syrups will make the product more tender (BeMiller, 1996).

A customary marshmallow formulation may consist of about 60 % corn syrup, 30 % sugar and 1 to 2 % gelatine. The corn syrup / sugar proportion will supply only about 35 to 40 % solids,

to avert crystallization. It can further be avoided by choosing a suitable type of corn syrup. A higher conversion corn syrup (glucose rich) will supply more invert sugar to the formulation, which will inhibit formation of crystals (Hegenbert, 1995).

Invert sugar (Figure 2.15e) is formed when sucrose breaks down due to hydrolysis or the addition of water. This molecule displays all the attributes of honey, except the flavour, as it is the main sugar found in honey. Invert sugar has the capacity to stop crystallization, thus, producing a tender marshmallow. On the other hand, it is also an effective humectant, meaning that it can bind water, preventing the marshmallow from drying out. For marshmallows, it is an advantage, since it has an elevated moisture content (Hartel & Hartel. 2014).

2.4.1.3 Gelatin

Gelatin is a very effective foam stabilizer, as it prevents air bubbles in the foam system from collapsing (Tan and Lim, 2008). Gelatin granules are hydrated in cold water to prevent clump formation, by sprinkling it over water to separate the granules. When this sponge-like structure is added to the hot syrup mixture, protein granules are dispersed in the mixture. The increase in the temperature of gelatin causes protein molecules in the gelatin molecules to denature and unfold. When the marshmallow mixture is left to cool, the protein molecules of the gelatin aggregate again and consequently form a gel (Saha and Bhattacharya, 2010). Gelatin plays the role of foaming agent, stabilizer and binder in marshmallows, which give the confectionary its creamy and chewy textures (Fellows and Hampton, 1992).

2.4.1.4 Acids

Acids, such as cream of tartar, or even lemon juice and vinegar, will assist in increasing the stability of foams, as it decreases the pH of the system. This will reduce the charges of the protein molecules, bringing them closer to their iso-electrical point, resulting in a stronger, more stable inter-facial film. However, when mixed with egg whites, acid inhibits too much excessive accumulation at the interface. For this reason, acid delays foam formation and should be added at the end of the whipping process, after a stable foam has already been formed (Bovšková and Míková, 2011).

2.4.2 Food systems involved in the making of marshmallows

Marshmallows are foams, comprising of an aqueous dispersion medium and a gaseous dispersed phase (i.e., a liquid with gas bubbles dispersed throughout) (Tyapkova *et al*., 2016), resulting in a candy which consists of up to 50 % air. The thin layers of the dispersion medium are referred to as the lamellar phase (Zuniga and Aguilera, 2008). The aim of such an aerated product, such as marshmallows, is to include gas into a sugar mixture and then stabilize the tiny air bubbles before the gas escapes (Vaclavik and Christian, 2014).

2.4.2.1 The formation of a saturated sugar solution

A saturated solution can be defined as a solution holding the maximum amount of dissolved solute at room temperate (Brown, 2008). For marshmallows, 250 ml water and 500 ml sugar is added to a pot and boiled to the soft-ball stage, 112 °C-115 °C / 233.6 °F-239 °F, to form a saturated sugar solution (Foods and Cookery, 1991). A highly concentrated sugar solution is achieved by using high temperatures for short periods of time, where after it is rapidly cooled down. This produces a highly viscose and amorphous mixture, decreasing the mobility of the sugar molecules, thus preventing crystallization (Quintas *et al.*, 2006).

2.4.2.2 The formation of a gel

Proteins usually form gels when heat is applied. Partial denaturation causes exposure of hydrophobic parts of the molecule to the aqueous environment, leading to aggregation (Banerjee and Bhattacharya, 2011). Once gelatine is dissolved in warm water (the so-called "blooming stage"), it forms a dispersion, which results in a cross-linking of its helix-shaped chains. The linkages in the gelatine protein network trap air in the marshmallow mixture and immobilize the water molecules in the network, resulting in the well-known spongy structure of marshmallows. This is why the omission of gelatine from a marshmallow recipe will result in marshmallow crème, since there is no gelatine network to trap the water and air bubbles (Schrieber and Gareis, 2007; Karim and Bhat, 2008;).

2.4.2.3 The formation of two foams

Proteins are the major surface-active agents responsible for the creation and maintenance of the dispersed air. Due to their structure, surface-active molecules collect at the surface area of a portion of water-based liquid. Each protein molecule consists of two parts: one part is hydrophilic or polar; and the other part is hydrophobic or non-polar. The non-polar part has little or no attraction, while the polar section is attracted to water and has little or no affinity

for air. Therefore, the molecule orients with the polar part in the water and the non-polar part in the air. Albumen, or egg whites, and gelatine are commonly used as aerators in marshmallows (Schrieber and Gareis, 2007; Karim and Bhat, 2008).

2.4.2.3.1 Albumen (egg whites)

Egg whites or albumen are used to create foams in marshmallows. For commercial application, dried albumen is used, as fresh egg whites are too expensive, causes problems in regard to food safety (high risk of *Salmonella*) and increases water activity of the final product (90.0 % water in fresh eggs). Albumen is seldom used alone in the making of marshmallows and is combined with gelatine (Greweling, 2013; McKee, 2013).

2.4.2.3.2 Gelatine

Gelatine, prepared from collagen, a structural protein derived from animal skin, connective tissue and bones, is the foaming agent most often used in the production of marshmallows. Like albumen, it stabilizes foams and contributes to the uniform distribution of fine air cells in the structure of foods. Small bubbles are desired as it modifies texture, extends the range of physical properties, increase the interfacial area, decrease syneresis and disperse air and aromas gradually, thus reducing the caloric density (Zuniga and Aguilera, 2008). Furthermore, when combined with water it forms a thermally-reversible gel, meaning that it can melt and return to the sol state, and reset again to form a gel. This is due to its sensitivity to temperature, since it has a melting point of 35 - 37.7 ˚C / 95 – 98.6 ˚F (body temperature). The melt-in-the-mouth sensation is caused by the melting of the marshmallow when it is put into the mouth, where it immediately starts to melt, due to body temperature; thus, it turns from a solid into a liquid (Schreiber and Gareis, 2007; Karim and Bhat, 2008).

When making marshmallows, the temperature needs to be just above the melting point of gelatine, so that as soon as it is formed it cools quickly, and the gelatine will set, retaining the desired shape. If a marshmallow rope mixture, exiting the extruder during processing, is too hot, the marshmallow will flow before the gelatine sets, resulting in an oval shape, instead of a round marshmallow. Excessive heat can also degrade the gelatine itself. Therefore, for home-made marshmallows gelatine is added after the hot sugar syrup has cooled down slightly (Hartel and Hartel. 2014).

When marshmallows are commercially made, the gelatine is added to the boiling syrup. Kinetics plays a significant role here, along with time and temperature. If the gelatine is added to the batch at the beginning of a cooking period of 20 - 30 min at 112.7 - 115.6 ˚C / 235 –40 ˚F, a significant amount of gelatine would break down, resulting in decreased springiness. However, little to no degradation of gelatine occurs, because the time the syrup spends at elevated temperatures in modern cookers is short (Hartel & Hartel, 2014).

2.5 Conclusions

From the Literature Review it is clear that there are a myriad of hydrocolloids available, either naturally occurring or semi – or fully produced by some sort of chemical reaction. These compounds have very unique characteristics, which are the result of specific composition and chemical structure. Various factors are responcible for the hydrocolloid's behaviour, such as temperature and the addition of other ingredients. Some hydrocolloids show synergistic effects when combined with one or more hydrocolloids, resulting in varying textural and rheological characteritics.

Confectionary products are amongst the top foodstuffs consumed on earth. Texture is one of the most important attributes in these products and hydrocolloids are responsible for the extreme variety of textures found in candies. In the making of any confectionary, certain food systems must be formed, resulting in the desired final product. Successful food systems, such as foams and gels, are crucial for the textural properties of such product. Unsuccessful food systems will not result in the perfect product and no consumer interest in such product.

Cactus mucilage is currently under investigation for further exploitation of its properties. Just because it is classified as a hydrocolloid because of its water holding capacities, does not automatically qualify it as as a functional ingredient for a specific section of the food industry, e.g. confectionary. Its behaviour in the presence of huge amounts of protein and sugar, as well as under high temperature conditions, need to be closely investigated.

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CHAPTER 3

INFLUENCE OF THE REPLACEMENT OF GELATIN WITH LIQUID *OPUNTIA FICUS-INDICA* MUCILAGE ON THE PHYSICAL PARAMETERS OF MARSHMALLOWS

[This chapter was published in *Journal of the Professional Association for Cactus Development* volume 18: pp. 25-39, under the heading. *'Replacement of gelatin with liquid Opuntia ficus-indica mucilage in marshmallows. Part 1: Physical parameters.* (See Annexure 1)]

ABSTRACT

The objective of this study was to replace gelatin in marshmallows with different concentrations of fluid mucilage, combined with different concentrations of powdered hydrocolloids. Nine different formulations were prepared: control (100% gelatin); 75% mucilage + 25% xanthan (MX); 75% mucilage + 25% agar (MA); 75% mucilage + 25% guar (MG); 80% mucilage + 20% xanthan (8M2X); 80% mucilage + 20% agar (8M2A); 80% mucilage + 20% guar (8M2G); 75% mucilage + 12.5% xanthan + 12.5% agar (MXA); 75% mucilage + 12.5% xanthan + 12.5% guar (MXG); and 75% mucilage + 12.5% agar + 12.5% guar (MAG). Consistency, texture, tenderness of gel and shear measurements were determined, along with colour (L* values, as well as C* and H^o values) and water activity ($a_{w)}$. There were significant ($p<0.05$) differences between the different samples for all measurements. The best formulation for gelatin replacement was found to be the 75% mucilage + 12.5% xanthan + 12.5% agar combination (MXA), as it only differed significantly ($p<0.05$) from the control (100% gelatin) sample in regard to shear, as measured by the Warner Bratzler Shear. It was significantly ($p<0.05$) less tender and resembled the shear of commercially available marshmallows in South Africa. All samples had a light, greyish yellow colour.

3.1 Introduction

Marshmallows are one of the earliest confections known to man (Olver, 2000) and were originally made from the root sap of the marshmallow (*Althaea officinalis)* plant. The first marshmallows were made by boiling pieces of the marshmallow root pulp with sugar until it thickened, whereafter the mixture was strained and left to cool. In 2000 B.C., Egyptians combined the marshmallow root with honey, and made a candy which was reserved for gods and royalty (Groves, 1995; Olver, 2000).

Modern marshmallow confections were first made in France around 1850 when marshmallows were cast and molded individually. When mass production became possible, by 1900, marshmallows were made by implementing the starch mogul system. By 1955, Alex Doumak of Doumak, Inc. patented a new manufacturing method, called the extrusion process, which changed the history of marshmallow production and is still used today (Groves, 1995).

Marshmallows are made by using sweeteners and emulsifying agents. Proportionally, there is more corn syrup than sugar, because it increases the ability of the sugar to dissolve and retards crystallisation. Corn starch, modified food starch, water, hydrocolloids, gelatin, and/or whipped egg whites are used in various formulations, resulting in a specific texture. Hydrocolloids act as emulsifiers in marshmallows and provide the aeration that makes marshmallows puffy; however, they also act as gelling agents. Most marshmallows also contain natural and/or artificial flavouring (Groves, 1991; Olver, 2000).

Opuntia spp. is a genus of < 200 species of readily recognizable cacti, growing from small ground-hugging plants to massive shrubs. The fiber content has the capacity to absorb large amounts of water, forming viscous or gelatinous colloids and absorbing organic molecules (Sáenz *et al.*, 2004; Anderson, 2008).

The Cactaceae family, of which *O. ficus-indica* is a part, is characterized by the production of slime, which mainly consists of mucilage. When cutting through a cladode, mucilage is the soluble fiber found in the thick slimy fluid, where they are stored in cells, commonly referred to as "nopal dribbles". Thus, these complex hydrophilic polysaccharide carbohydrates molecules, with a highly branched structure and containing varying proportions of L-arabinose, D- galactose, L-rhamnose, and D-xylose, as well as galacturonic acid, may not only bind water as they increase in volume, but also sugars and fats, which are caught within the matrix of mucilage and water (Goycoolea and Cárdenas., 2003; Sáenz *et al.*, 2004; Ramírez-Tobías *et al*., 2012).

Thus, mucilage is, therefore, commonly referred to as a gum or hydrocolloid. Hydrocolloids are water-soluble dietary fiber that can be used as healthy additives for profitable food products. None of the mucilage found in cladodes is hydrolyzed or absorbed by the human digestive system, but they can make up alimentary fiber (Sepúlveda *et al.*, 2007; Osuna-Martínez *et al*., 2014).

Mucilage has a strong emulsifying capability in that it reduces surface interfacial tensions, stabilises oil-in-water emulsions and does not contribute to the viscosity of the system. Its stabilising properties are similar to those of xanthan and guar gums. It can also form part of gelled dessert and powdered drinks, to be reconstituted with water or milk (Bensadón *et al*., 2010).

Another possible function for mucilage could be as a fat mimic, as it has the required tendency to increase the viscosity of the water phase. With increased concentration, it will exhibit gelling capabilities. Mucilage can, therefore, be used to emulsify systems, but as it is not a true emulsifier, processing costs would be reduced, while providing the final product with a natural and healthy ingredient as a replacement for fat (Bensadón *et al.*, 2010; Milani and Maleki, 2012).

Today, new facts at the molecular level of chemistry, as well as on functional properties, allow improved knowledge on the selection of a suitable hydrocolloid for a specific product. Cactus mucilage definitely has the advantages of being natural, healthy and cheap. However, despite having potential uses, it is not yet an industrial hydrocolloid (Sáenz *et al.*, 2004).

The aim of this study was to replace gelatin in marshmallows with different concentrations of liquid mucilage, in combination with different concentrations of powdered hydrocolloids. Various physical parameters, such as consistency, percentage sag, shear, colour and water activity (a_w), were determined.

3.2 Materials and methods

3.2.1 *Extraction of liquid mucilage*

Mucilage was extracted from the *Opuntia* cultivar Algerian, according to the patented method of Du Toit and De Wit (2011). The cladodes (one-year-old with an average mass of ± 700 g, delivering a wet mucilage yield of ± 34% per cladode) were scrubbed and disinfected with chlorinated water to lengthen the shelf life period of the mucilage, and to eliminate impurities and thorns present. The green outside peel was removed with a vegetable peeler. The cladodes were sliced into squares and cooked in a microwave oven (900 W) for 3 min at 100% power, until soft. The cooked, soft cladode pieces were pulverised in a food processor (300W) to break the skin, which aids in the extraction of the mucilage. The pulp was centrifuged in a

Beckman centrifuge; model J2-21, (Beckman Coulter Inc. 2505 Kraemer Boulevard. Brea, CA 92817 USA) for 15 min at 8000 rpm, to separate the liquid mucilage from the solids.

3.2.2 Preparation of marshmallows

The formulation for the marshmallows that was used as the control sample is given in Table 3.1. The gelatin was soaked in cold water. Sugar, salt, syrup and the remaining water were heated and stirred to dissolve the sugar. After the mixture reached a temperature of 118 °C / 244.4 °F, the gelatin was added. The stiffly beaten egg whites were added to the hot mixture, whilst continuously mixing it until thick and creamy. The mixture was then poured into a greased pan, cooled overnight and cut into 25-milimetre (mm) squares. The squares were rolled in a mixture of corn flour and icing sugar, and stored in an airtight container (Foods and Cookery, 1991).

Table 3.1: Formulation of control marshmallow (Foods and Cookery, 1991).

Ingredient	**Percentage (%)**
Gelatin*	2.30
Water cold, to hydrate gelatin	11.52
Sugar	46.09
Water	28.81
Egg whites	9.22
Syrup	1.93
Salt	0.13

***** Libstar, Johannesburg 1st Floor 62 Hume Road, Dunkeld, 2196, SA.

3.2.3 Replacement of gelatin with liquid mucilage and powdered hydrocolloids

Nine different formulations were prepared according to Table 3.2. The three hydrocolloids that were chosen to be used in combination with the fluid mucilage, to replace gelatin in the formulation, were: gum guar (Health Connection Wholefoods™, Unit 17 – River Park, 77 de Waal Road, Diep River, 7800, SA); agar (Health Connection Wholefoods™, Unit 17 – River Park, 77 de Waal Road, Diep River, 7800, SA); and xanthan (Nature's Choice, 73 Cyprus Avenue, Meyerton, SA, 1960). Guar was chosen because of the synergistic effect that it displays when used in combination with xanthan (Pangborn, *et al*., 1978). According to Bensadón *et al*. (2010), the stabilising properties of mucilage are similar to those of xanthan and guar gums. Agar was also chosen, because it is commercially used in SA in chocolate candies to stabilise the marshmallow layer, such as *Cadburys Chocolate log* (Made in SA by

Mondelez South Africa (PTY) LTD. 18 Harrowdene Office Park, Kelvin Drive, Woodmead, Sandton, 2129, SA.).

The control sample contained 100% gelatin and one of the experimental samples 100% liquid mucilage. The lowest concentration at which the mucilage was used in the formulations was 75%. The lowest concentration, at which the other hydrocolloids were added, was 12.5 %. The 100% liquid mucilage sample did not gel and could not be analysed for any of the physical texture properties.

3.3 Physical texture analysis of marshmallow samples

3.3.1 Consistency

A glass plate, placed on paper with concentric circles drawn on it, at intervals of 0.5 cm was used. An open-ended tube, 2 cm x 2 cm in diameter, filled with 5 mL of the liquid sample, was placed in the center of the circles. After the tube was lifted, the liquid sample was allowed to flow for 2 min. The distance, traveled by the liquid, was then measured at each 90º section of the circle and the average calculated; the line spread value (LSV) was the mean of the four values obtained (Kim, 2007) and expressed in cm.

3.3.2 Texture

The penetrometer was used to determine the comparative tenderness and penetration properties of semi-solid substances (Mohos, 2010). The cone penetration test (ASTM D217) was used and the depth was measured in mm. The larger the reading, the more tender is the product. The more tender the sample, the deeper the penetrometer will sink into the sample and, thus, the higher the penetration number will be. The flat attachment tests resistance against pressure; thus, the higher the value the more compression is achieved, indicating a more tender softer product. The results were expressed in mm.

3.3.3 Tenderness of gel

The percentage (%) sag test was used to determine the gel tenderness and was expressed as % of the height, before unmolding. For this test, the depth of the marshmallow sample was measured in its container, by using a probe. After the sample was unmolded onto a flat surface (McWilliams, 1989), the depth was measured again. The % sag was then calculated as the change in height of the sample measured in the container or mold, compared to that of the

freestanding gel placed on a flat surface. The greater the % sag value, the more tender the gel (McWilliams, 1989).

Table 3.2: Substitution hydrocolloids, combinations of hydrocolloids, substitution percentages and weights used in the preparation of marshmallows samples.

% substitution	Actual weight replacement (g)	Gelatin substitution	Formulation Code
Control	143.27	gelatin	G
100	143.27	mucilage*	M
75+25	107.45+35.82	mucilage* and xanthan	MX
75+25	107.45+35.82	mucilage* and agar	MA
75+25	107.45+35.82	mucilage* and gum guar	MG
80+20	114.62+28.65	mucilage* and xanthan	8M2X
80+20	114.62+28.65	mucilage* and agar	8M2A
80+20	114.62+28.65	mucilage* and gum guar	8M2G
75+12.5+12.5	107.45+17.90+17.90	mucilage* and xanthan and agar	MXA
75+12.5+12.5	107.45+17.90+17.90	mucilage* and xanthan and gum guar	MXG
75+12.5+12.5	107.45+17.90+17.90	mucilage* and agar and gum guar	MAG

* Liquid mucilage, while other hydrocolloids were powdered.

3.3.4 Shear

Shear analysis was performed with the Instron Universal Testing Machine (UTM Model 3344). The samples from each of the treatments were cooled down to room temperature (19 to 25°C / 66.2 to 77°F) for at least 24 h, before shear force, measurements were taken and for each sample, six repetitions were done. Samples were prepared, with a core diameter of more or less 12.7 mm and a length of 10 mm, as advised by the prescribed method. The load cell size was 1 kilo Newton (kN). A Warner Bratzler shear device, mounted on the Instron Universal Testing Machine, was used and the reported value in kilogram (kg) represented the average of the peak force measurements of each sample. The Instron Bluehill software (Instron, 2004) generated sample compression data.

3.3.5 Colour analysis

Colour measurements were performed on the marshmallow samples, 24 h after preparation, using a Minolta CR 400 Chroma Meter, with an 8 mm measuring area at a 0º viewing angle. Calibration was done by using the white calibration plate and measurements were done in sextuplicate. The CIELAB colour space (CIE, 1986) was used for measurements, with L* representing lightness. Chroma (C*), as well as Hue Angle (Hº), was calculated by using a* and b* values in the respective formulas: $C^* = \sqrt{(a*)^2 + (b*)^2}$ and $H^o = \tan^{-1}\left[\frac{b*}{a*}\right]$ (Ripoll

et al., 2011; Tapp *et al*., 2011), where C* represents saturation and H^o the tone, which indicates colour variation in the plane formed by the coordinates a* and b* (Chervin *et al*., 1996).

3.3.6 Water activity (a_w)

Water activity of the marshmallow samples, in a_w containers (height of 5 mm and diameter of 39 mm), was measured with a Novasina Thermoconstanter TH 200 water activity meter, at room temperature (20 ºC / 68 ºF). The means of the replications for the marshmallow samples were recorded (Mathenjwa *et al.*, 2012).

3.3.7 Comparison between best experimental formulation and commercial marshmallows

The best experimental formulation sample was then tested against four commercial marshmallow brands in SA: Woolworths white (WW); Woolworths traditional white (WTW); Beacon white (BW); and Manhattan white (MW). Only WW is made according to the traditional process, while the other three are extruded. The texture of the extruded marshmallows is tougher than marshmallows made the traditional way. For this comparison, the line spread test was omitted, because the commercial samples could not flow upon melting. All the other textural tests were performed, as described above.

3.3.8 Statistical Analysis

All results were captured in multiple spreadsheets in Microsoft Excel 2007. A one-way analysis of variance (ANOVA) procedure (NCSS, 2007) was used to determine the effect of mucilage/ hydrocolloid formulations on the physical parameters of the marshmallow samples. Differences were considered statistically significant at $p<0.05$ or lower. Treatment means were then compared by means of the Tukey-Kramer multiple comparison test at $\alpha = 0.05$.

3.4 Results and discussion

The replacement of gelatin with mucilage, in combination with guar, agar agar and xanthan, had a significant ($p<0.05$) effect on all the evaluated physical parameters of the marshmallows (Table 3.3).

3.4.1 Consistency

For the line spread test, the MX sample had the lowest value (1.42 cm), signifying that the consistency of this formulation was the highest of the all the samples. This sample differed significantly ($p<0.05$) from all the other samples (Table 3.3). The consistency of the control

Table 3.3: Physical and colour properties of different formulations of hydrocolloids, in the making of marshmallows.

Property	G	MX	MA	MG	8M2X	8M2A	8M2G	MXA	MXG	MAG
Consistency (cm)	1.97±0.02^{d}	1.42±0.06^{a}	2.14±0.10^{e}	2.32±0.07^{f}	2.27±0.12^{f}	2.05±0.01^{de}	2.33±0.07^{f}	1.83±0.11^{c}	1.66±0.15^{b}	1.98±0.05^{d}
Texture (flat)* (mm)	2.26±0.33^{f}	0.77±0.22^{ab}	0.53±0.20^{a}	0.90±0.19^{bc}	0.53±0.03^{a}	1.33±0.15^{d}	0.90±0.09^{bc}	1.90±0.05^{e}	1.10±0.21^{cd}	0.53±0.07^{a}
Texture (cone) * (mm)	19.70±0.83^{f}	14.87±0.80^{b}	0.15±0.08^{a}	16.03±1.00^{c}	14.13±0.17^{b}	0.14±0.02^{a}	0.19±0.06^{a}	18.47±0.56^{e}	17.27±0.28^{d}	0.17±0.00^{a}
Tenderness of gel (%)	0.96±1.11^{a}	9.18±1.10^{bc}	19.84±1.76^{d}	16.38±3.18^{d}	8.85±1.65^{bc}	9.74±1.50^{c}	29.78±3.63^{e}	5.41±0.86^{b}	9.70±3.18^{c}	27.90±6.51^{e}
Shear(kg)	0.07±0.03^{a}	0.45±0.20^{bc}	0.06±0.02^{a}	0.05±0.03^{a}	0.33±0.15^{b}	0.02±0.01^{a}	0.02±0.01^{a}	0.69±0.10^{d}	0.56±0.22^{cd}	0.02±0.01^{a}
a*	-0.12±0.09^{f}	-0.57±0.25^{de}	-1.08±0.18^{c}	-2.39±0.07^{a}	-0.64±0.13^{de}	-1.09±0.24^{c}	-1.86±0.08^{b}	-0.71±0.05^{d}	-0.44±0.11^{e}	-1.13±0.13^{c}
b*	9.29±0.87^{b}	8.16±2.53^{ab}	16.67±0.20^{c}	20.61±0.72^{e}	7.89±2.11^{ab}	16.26±0.31^{c}	18.13±0.42^{cd}	9.64±0.35^{b}	6.34±2.15^{a}	19.20±0.63^{de}
L*	90.03±1.88^{d}	90.63±1.96^{d}	85.67±1.80^{c}	82.52±0.85^{b}	89.25±1.77^{d}	86.40±0.50^{c}	81.27±1.11^{b}	91.33±0.36^{d}	89.47±1.79^{d}	77.59±2.86^{a}
C*	9.29±0.07^{b}	8.18±2.54^{ab}	16.70±0.18^{c}	20.74±0.71^{e}	7.91±2.12^{ab}	16.30±0.30^{c}	18.22±0.41^{cd}	9.67±0.35^{b}	6.35±2.15^{a}	19.23±0.63^{de}
H^{o}	90.27±0.55^{a}	93.82±0.62^{bc}	93.71±0.66^{bc}	96.62±0.42^{e}	94.69±0.34^{d}	93.84±0.89^{bc}	95.87±0.32^{e}	94.24±0.44^{cd}	94.09±0.42^{bcd}	93.37±0.34^{b}
a_w	0.78±0.01^{de}	0.78±0.01^{ef}	0.76±0.01^{bc}	0.77±0.01^{cd}	0.76±0.01^{ab}	0.80±0.01^{g}	0.78±0.01^{de}	0.75±0.01^{a}	0.79±0.01^{fg}	0.78±0.01^{de}

Means with different superscripts in the same row differed significantly ($p<0.05$).

Texture (flat) =penetrometer flat; texture (cone) =penetrometer cone; a=redness; b*= yellowness; L*=lightness; C*= chroma (saturation index); H^{o}=hue angle; a_w=water activity.

Samples:

G	Gelatine
MX	75% Mucilage + 25 % Xanthan
MA	75% Mucilage + 25% Agar
MG	75% Mucilage + 25%Guar Gum
8M2X	80% Mucilage + 20% Xanthan
8M2G	80% Mucilage + 20% Guar Gum
8M2A	80% Mucilage + 20% Agar
MXA	75% Mucilage + 12.5% Xanthan + 12.5% Agar
MXG	75% Mucilage + 12.5% Xanthan + 12.5% Guar Gum
MAG	75% Mucilage + 12.5% Agar + 12.5% Guar Gum

sample (G = gelatin) (1.97 cm) did not differ from that of 8M2A (2.05 cm) and MAG (1.98 cm); however, the consistency was significantly ($p<0.05$) higher than MA (2.14 cm), MG (2.32 cm), 8M2X (2.27 cm) and 8M2G (2.33 cm), and significantly ($p<0.05$) more fluid than MX (1.42 cm), MXA (1.83 cm), and MXG (1.66 cm). Sample 8M2G (2.33 cm) had the highest value for the line spread test, indicating that it was the most fluid and was significantly ($p<0.05$) more fluid than all the other samples (Table 3.3).

Since the control sample contained 100% gelatin and resulted in a successful product, a line spread test value of around 1.97 cm could result in a successful product. The combinations 8M2A (2.05 cm) and MAG (1.98 cm), did not differ from the consistency of the control sample (1.97 cm) (Table 3.3). In both samples, agar was used in combination with mucilage, which could be responsible for the satisfying results. Agar is used in confectionaries, because of its solubility and the strength it confers upon the product (Nussinovitch, 1997). It was also found that, as soon as the percentage mucilage increased, so did consistency (Table 3.3).

3.4.2 Texture

The sample with the highest measurement for the flat attachment was the control sample (G) (2.26 mm). According to McWilliams (1989), the larger the reading, the tenderer is the product, as the attachment will sink deeper into a tender sample, resulting in a higher penetration value. The samples with the lowest reading were MA (0.53 mm), 8M2X (0.53 mm), and MAG (0.53 mm), indicating that these samples were less tender (Table 3.3). Sample MX (0.77 mm) did not differ from MG (0.90 mm) and 8M2G (0.90 mm), but was significantly ($p<0.05$) harder than G (2.26 mm), 8M2A (1.33 mm), MXA (1.90 mm), and MXG (1.10 mm). Although MXA (1.90 mm) was also significantly ($p<0.05$) harder than G (2.26 mm), it was the mucilage combination closest to the tenderness achieved by the control sample, containing 100% gelatin. Agar is known as a ‘gelling type’ hydrocolloid, like gelatin (Saha and Bhattacharya, 2010), but it has stronger setting properties (five times greater), so agar form gels at lower concentrations (Condrasky, 2014).

For the cone attachment, the lowest value was measured for the 8M2A (0.14 mm) sample, which did not differ from MA (0.15 mm), MAG (0.17 mm), and 8M2G (0.19 mm); these samples were the tenderest and differed significantly ($p<0.05$) from all the other samples (Table 3.3). The MG (16.03 mm) sample was significantly ($p<0.05$) less tender than MXG (17.27 mm), MXA (18.47 mm) and G (19.70 mm), and significantly ($p<0.05$) tenderer than all the

other samples (Table 3.3). Finally, MXA (18.47 mm) was significantly ($p<0.05$) less tender than the control sample G (19.70 mm), which contained 100% gelatin.

3.4.3 Tenderness of gel

The sample that scored the lowest % sag was the control sample (G) (0.96%), indicating a strong gel texture, as the greater the % sag value, the more tender the gel is (McWilliams, 1989); this sample differed significantly ($p<0.05$) from all the other samples (Table 3.3). The 8M2G (29.78%) and MAG (27.90%) samples had the highest % sag, meaning that they were the tenderest of all the samples. The sample closest to the control sample (G) (0.96%), again, was MXA (5.41%), which was also the best sample in the penetrometer tests. Although it did not differ from MX (9.18%) and 8M2X (8.85%), it had the lowest numerical % sag.

3.4.4 Shear

For the physical attribute shear, the lowest value was scored by three samples, 8M2A (0.02 kg), 8M2G (0.02 kg), and MAG (0.02 kg). These did not differ from the values recorded by the control (0.07 kg), MA (0.06 kg) and MG (0.05 kg). Sample MXA (0.69 kg), the toughest of all the samples, differed significantly ($p<0.05$) from all the other samples. This sample, which is a combination of 75 % mucilage + 12.5 % xanthan + 12.5 % agar, was the most promising hydrocolloid formulation thus far. For this attribute, it measured at the opposite end of the scale, compared to the control sample G (0.07 kg). As mentioned earlier, xanthan does not form a gel when used alone. In combination with certain hydrocolloids, such as guar and locust bean gum (LBG), gels are formed, because of synergistic interactions. Xanthan shows quite spectacular synergistic interactions with other non-gelling polysaccharides of the galactomannan family (Saha and Bhattacharya, 2010), giving an increase in consistency (Casas and García-Ochoa, 1999) and gel formation (Rodriguez - Hernández and Tecante, 1999). It might be possible that there may also be an interaction between xanthan and mucilage, which requires further investigation.

The MA (0.06 kg) combination had a low score which did not differ from that of the control sample. This indicated that the shear increase was introduced by the 12.5% xanthan in the MXA combination. This agrees with the findings of Al-Assaf *et al*. (2006), who stated that xanthan shows a very high consistency at low shear rate and is, thus, preferred as a thickener or stabiliser for dispersed solids or emulsions.

3.4.5 Colour analysis

Values for a* and b* measurements will not be discussed and is only included in Table 3.3 for clarification of the C* and H^{o} calculations. L* values distinguish between bright and dull colours (Konica Minolta, 1998). All the samples tested showed high values for L*, with MXA (91.33) having the highest value; this sample did not differ from the control sample (90.03) and appeared to be the best formulation of hydrocolloids to replace gelatin (Table 3.3). The MAG (77.59) sample had the lowest score for lightness, indicating that it was significantly ($p<0.05$) darker when compared with the other samples. All the samples containing xanthan had the highest L* values, suggesting that xanthan had a lightening effect on the final product. Except for sample MXG (89.47), which contained xanthan, guar had a darkening effect on all the marshmallow samples (Table 3.3). In a study by Bortnowska and Makiewicz (2006), on the influence of xanthan and guar on the colour of mayonnaise, sensory profiling indicated that both hydrocolloids intensified the creamy colour of low-fat mayonnaise. With an increase in the hydrocolloid, the characteristic yellow colour disappeared and became a light creamy colour, which increased with increasing concentration of the hydrocolloid. Considering that the colour of a food is the first contact point of the consumer (Alvarenga *et al*., 2012) and that the consumer may want a bright white marshmallow, the L* value may play an important role.

Chroma is an indication of the saturation of colours, indicating whether they are vivid/strong to dull, to weak/greyish (McGuire, 1992; Konica Minolta, 1998). All the samples had values ranging from 6.35 to 20.74 (Table 3.3), putting them horizontally closer to the center or greyish/ weak area in the plane formed by the coordinates a* and b* (Chervin *et al*., 1996; Konica Minolta, 1998). The sample with the lowest C* value was MXG (6.35), which was the dullest or most greyish sample; it differed significantly ($p<0.05$) from G (9.29), MA (16.70), MG (20.74), 8M2A (16.30), 8M2G (18.22), MXA (9.67) and MAG (19.23), but not from MX (8.18) and 8M2X (7.91). This could be attributed to xanthan that was used in the formulations. With the L* values, the xanthan-containing samples were also the lightest. Sample 8M2X (7.91) differed significantly ($p<0.05$) from MA (16.70), MG (20.74), 8M2A (16.30), 8M2G (18.22) and MAG (19.23), but not from G (9.29), MX (8.18), MXA (9.67), and MXG (6.35). Sample MA (16.70) differed significantly ($p<0.05$) from G (9.29), MX (8.18), MG (20.74), 8M2X (7.91), MXA (9.67), MXG (6.35) and MAG (19.23), but not from 8M2A (16.30) and 8M2G (18.22). Sample 8M2G (18.22) differed significantly ($p<0.05$) from all the samples, but not from 8M2A (16.30) and MAG (19.23), while MG (20.74) had the highest value for C* and

differed significantly from all the samples, except from MAG (19.23). Samples containing guar had thus a stronger saturation of colour than the other formulations.

Hue is the term used for the classifications of colours, for example red, yellow, blue, etc. For this attribute, all the samples had values ranging between 90º and < 96º (Table 3.3), and when the hue angle is in this region, the colour is yellow (Konica Minolta, 1998; XRite, 2007; Zhang *et al*., 2007). The highest values were found for both samples containing guar, namely MG (96.62) and 8M2G (95.87), while the lowest Hº value was for the sample containing gelatin (90.27) (Table 3.3). For the samples where xanthan was added to the formulations, the hue angle increased significantly ($p<0.05$) for samples 8M2X (94.69), MXA (94.24) and MXG (94.09).

Although the various marshmallow formulations differed significantly ($p<0.05$) amongst each other, it could be concluded that all samples had a light greyish yellow colour.

3.4.6 Water activity

All the samples fell within the range of intermediate moisture foods (IMF). These are foods with a_w of 0.65 - 0.85, which is largely responsible for its protection from microbial spoilage (Garbutt, 1997). The sample with the lowest a_w value was MXA (0.75); this sample differed significantly ($p<0.05$) from all the other samples (Table 3.3). The sample with the highest a_w of 0.80, namely 8M2A, differed significantly ($p<0.05$) from all the samples, but still fell within the range of IMF. The MXA (0.75) combination had a significantly ($p<0.05$) lower a_w than the control (G) (0.78) sample, rendering it even more acceptable as a substitute for gelatin in the marshmallow formulation. This water dispersing property is common to all hydrocolloids; however, the extent of thickening varies with the type and nature of the hydrocolloid (Saha and Bhattacharya, 2010). Nammakuna *et al*. (2009) found that addition of hydrocolloids to rice crackers increased its moisture content, which correlated to significantly higher a_w in treated samples, as compared to the controls.

It can be concluded that the best textural attributes were associated with sample MXA, consisting of 75% mucilage, 12.5% xanthan, and 12.5% agar. This sample showed good results in all the above mentioned physical evaluations and it was the closest sample to the control. It is then clear that in combination with xanthan and agar, mucilage can be used as a substitute for gelatin in the production of marshmallows.

3.4.7 Comparison between best experimental formulation and commercial marshmallows

3.4.7.1 Texture

The sample with the highest measurement for the penetrometer with the flat attachment was the MXA (1.90 mm). As noted earlier, the larger the reading, the tenderer is the product, as the attachment will sink deeper into a soft sample, resulting in a higher penetration value. The sample with the lowest reading was BW (0.52 mm), indicating that this sample was not tender (Table 3.4). The two Woolworths samples did not differ, although the one was made in the traditional way and the other one was extruded. All the other samples differed significantly ($p<0.05$). The gelling agent for all four commercial samples was gelatin. For this test, the MXA (1.90 mm) sample resembled the tenderness of the MW (1.43 mm) sample the most, although they differed significantly ($p<0.05$).

When the cone attachment was used on the penetrometer, the samples were divided into three groups: WW (17.38 mm) and BW (17.13 mm), with the lowest scores, which differed significantly ($p<0.05$) from the next group, MXA (18.47 mm) and MW (18.92 mm), which in turn differed significantly ($p<0.05$) from WTW (20.10 mm), which had the highest score (Table 3.4), indicating that this sample was the tenderest. For these two measurements, the BW (17.13 mm) samples scored the lowest values in each instance, confirming that it was the least tender sample. It should be noted that again the measurements for MXA (18.47 mm) and MW (18.92 mm) did not differ for this attachment.

Table 3.4: Physical and colour properties for MXA and four commercial marshmallows brands.

Property	MXA	WW	WTW	BW	MW
Texture (flat)* (mm)	1.90 ±0.06^{d}	0.78 ± 0.15^{b}	1.00 ± 0.14^{b}	0.52 ± 0.13^{a}	1.43 ± 0.16^{c}
Texture (cone)* (mm)	18.47 ± 0.76^{b}	17.38 ± 0.73^{a}	20.10 ± 0.26^{c}	17.13 ± 0.51^{a}	18.92 ± 0.75^{b}
Tenderness of gel (%)	5.41 ± 1.15^{a}	9.18 ± 1.48^{ab}	16.38 ± 4.27^{c}	9.74 ± 2.01^{b}	5.41 ± 1.15^{a}
Shear (kg)	0.69 ± 0.14^{a}	25.36 ± 6.55^{c}	16.47 ± 4.01^{b}	41.00 ± 5.54^{d}	67.42 ± 6.11^{e}
a*	-0.71 ± 0.07^{b}	-0.98 ± 0.10^{a}	-1.13 ± 0.12^{a}	-0.07 ± 0.12^{c}	-0.77 ± 0.09^{b}
b*	9.64 ± 0.47^{d}	5.81 ± 0.58^{a}	6.97 ± 0.85^{b}	8.31 ± 0.94^{c}	8.25 ± 0.15^{c}
L *	91.33 ± 0.48^{a}	96.39 ± 0.21^{d}	94.40 ± 1.02^{bc}	93.61 ± 0.36^{b}	94.90 ± 0.54^{c}
C*	9.67 ± 0.47^{d}	5.89 ± 0.59^{a}	7.06 ± 0.85^{b}	8.31 ± 0.94^{c}	8.29 ± 0.15^{c}
H(°)	94.25 ± 0.59^{b}	99.61 ± 0.72^{c}	99.22 ± 0.65^{c}	90.71 ± 0.89^{a}	95.30 ± 0.61^{b}
a_w	0.75 ± 0.01^{e}	0.52 ± 0.01^{a}	0.65 ± 0.01^{d}	0.63 ± 0.00^{c}	0.60 ± 0.01^{b}

Means with different superscripts in the same row differed significantly ($p<0.05$). *Texture (flat) =penetrometer flat; *texture (cone) =penetrometer cone; a*=redness; b*= yellowness; L*=lightness; C*= chroma (saturation index); H^{o}=hue angle; a_w=water activity.
Samples:
MXA=75% Mucilage + 12.5% Xanthan + 12.5% Agar
WW=Woolworths white
WTW=Woolworths traditional white
BW=Beacon white
MW=Manhattan white

3.4.7.2 Tenderness of the gel

Both MXA (5.41%) and MW (5.41%) had the lowest values for % sag and both samples differed significantly ($p<0.05$) from BW (9.74%) and WTW (16.38%), but not from WW (9.18%); this sample differed significantly ($p<0.05$) from WTW (16.38%) (Table 3.4). Samples MXA (5.41%) and MW (5.41%), thus, had tough gel textures. The greater the % sag value, the more tender is the gel (McWilliams, 1989); therefore, the WTW (16.38%) sample was the tenderest gel.

3.4.7.3 Shear

For the physical attribute shear, the lowest value was scored by MXA (0.69 kg), indicating least shear. Interestingly, all the commercial samples differed significantly ($p <0.05$) from this sample, as well as from one another. From these samples, the sample with the least shear was WTW (16.47 kg). Sample MW (67.42 kg) was the toughest sample, with BW (41.00 kg) second toughest, followed by WW (25.36 kg). This confirms the belief that traditionally made marshmallows are not as tough as the extruded ones. For extrusion to be successful, the mixture contains modified cornstarch, in addition to gelatin. The temperature of extrusion has to be just right so that the mixture will flow under pressure, but hold its shape after leaving the pipe (Mulvaney, 2015).

3.4.7.4 Colour analysis

Values for a* and b* measurements will not be discussed and is only included in Table 3.4 for clarification of the C* and H^o calculations. All the samples, again, showed high values for L* (lightness). The sample with the lowest L* value was MXA (91.33) and it differed significantly ($p<0.05$) from all the other samples, the reason for this being the greenish colour of the fluid mucilage, which lowered the L* value. The BW (93.61) sample differed significantly ($p<0.05$) from MXA (91.33), WW (96.39), and MW (94.90), but not from WTW (94.40); this sample differed significantly ($p<0.05$) from MXA (91.33) and WW (96.39). The commercial sample with the highest value for lightness was WW (96.39) (Table 3.4). In a study by Periche *et al.* (2015), it was found that the factor that had the greatest influence on lightness, was the percentage of gelatin; the higher the level of gelatin, the higher the lightness. Although there were significant differences ($p<0.05$) between the samples for this colour attribute, all samples were light, which is of importance for the consumer looking for a white marshmallow.

The C* values were significantly ($p<0.05$) different amongst the samples, except for samples BW (8.31) and MW (8.29), which did not differ from each other. The coordinates, again, placed the samples in the greyish / weak yellow spectrum (McGuire, 1992; Konica Minolta, 1998; Sepúlveda, *et al*., 2007). Sample MXA had significantly ($p<0.05$) the highest value for C* (9.67), while sample WW (5.89) had significantly ($p<0.05$) the lowest value. Despite these significant ($p<0.05$) differences, the experimental sample and the commercial samples had values in the same range, and the use of mucilage, along with agar and xanthan, would have a favourable impact on the acceptability of the consumer.

For Hº, all the values were between 90º and 100º (Table 3.4), placing the colour again in the yellow region (McGuire, 1992; Konica Minolta, 1998; Zhang *et al*., 2007). The highest value was found for the WW (99.61º) and WTW (99.22º) samples and the lowest Hº value was calculated for the BW (90.71º) sample. The mucilage sample MXA (94.25º) differed significantly ($p<0.05$) from the three previously mentioned samples, but not from the commercial brand MW (95.30º).

Although the samples differed significantly ($p<0.05$) amongst each other for the colour analysis, they all had a light greyish yellow colour.

3.4.7.5 Water activity

Again, all the samples fell within the range of intermediate moisture foods (IMF) and even lower. The sample with the highest a_w value was MXA (0.75), which differed significantly ($p<0.05$) from all the other samples (Table 3.4). The liquid mucilage has a high a_w of 0.96 (De Wit, 2015), which contributed to the high a_w of the MXA (0.75) sample. It is interesting to note that all the commercial samples had values that differed significantly ($p<0.05$). Furthermore, the traditional commercial sample, WTW, was the only commercial sample with a_w of 0.65, while the other commercial samples had lower values. Sample BW, an extruded commercial brand, had a_w of 0.63, which is lower than the recommended range of 0.65 and 0.85. This low a_w ensures the microbial stability of the product and lengthens the shelf life. Sample MW had an even lower a_w of 0.60, qualifying it to be a low moisture food. The lowest a_w was achieved by WW, with a value of 0.52, also qualifying as a low moisture food.

3.5 Conclusions

Marshmallows were selected as the potential vehicle for the incorporation of cactus pear mucilage in different formulations, using a variety of hydrocolloids. The MXA sample, containing 75% mucilage, 12.5% xanthan, and 12.5% agar, obtained the best results for all the physical parameters tested and could be a potential formula requiring further research. Marshmallows containing mucilage in combination with agar and xanthan might also be a good alternative for similar products, where only one hydrocolloid, e.g. agar is used as a gelling agent, resulting in a tough texture. Furthermore, gelatin-less marshmallows might also open up a market which was restricted by the use of gelatin, namely for Halaal and Kosher consumers.

Since the consumer has the final decision in whether a newly developed product is acceptable, it is necessary to submit it to a sensory panel of regular marshmallow consumers to determine its overall acceptability, taste, aftertaste, as well as the two most important attributes in the case of this aerated gelled product, namely appearance and texture.

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CHAPTER 4

INFLUENCE OF THE REPLACEMENT OF GELATIN WITH LIQUID *OPUNTIA FICUS-INDICA* MUCILAGE ON THE CONSUMER LIKING OF MARSHMALLOWS

[This chapter has been published in *Journal of the Professional Association of Cactus Development* volume 18: pp. 40-51, under the heading. *'Replacement of gelatin with Opuntia ficus-indica mucilage in flavoured pink and unflavoured white marshmallows. Part 2: Consumer liking of mucilage-containing marshmallow versus control and a commercial brand* (See Annexure 2)

ABSTRACT

Mucilage from the cladodes of *O. ficus-indica* is under investigation for application in several foodstuffs. Since it can't form gels on its own, it is advised to be used in combination with other hydrocolloids. The aim of this study was to compare consumer liking of flavoured and unflavoured marshmallows made with liquid mucilage, to that of a flavoured and unflavoured control sample (with 100% gelatin), as well as a flavoured and unflavoured commercial brand. Ninety-two consumers tasted the following six samples: white commercial (*Manhattan*); white control (gelatin); white mucilage (75% mucilage + 12.5% agar + 12.5% xanthan); pink commercial (*Manhattan*); pink control (gelatin); and pink mucilage (75% mucilage + 12.5% agar + 12.5% xanthan). Consumer liking was tested for taste, aftertaste, texture, as well as an overall acceptability of liking. The white mucilage marshmallows had the lowest ranking for taste, aftertaste, texture and overall acceptability, and differed significantly ($p<0.05$) from all the other samples. However, the pink mucilage marshmallow did not differ from the pink commercial one (which had the highest rankings for taste, aftertaste, texture, and overall acceptability) and pink control marshmallow. The differences between the white and pink mucilage marshmallows ranged between 2.75 and 2.89 on the hedonic scale. It could be concluded that flavouring successfully masked the distinctive aroma of the mucilage in the marshmallows, thereby also increasing scores for texture and overall acceptability.

Keywords: Consumer liking; Marshmallows; Mucilage; Gelling agent; *Opuntia ficus-indica*

4.1 Introduction

Gelatin is used in whipped confections, such as marshmallows, where it serves to lower the surface tension of the syrup, stabilise the foam through increased viscosity, set the foam via gelation and prevent sugar crystallisation (GMIA, 2012). Furthermore, it is also responsible for the chewy, bouncy texture of marshmallows (Karim and Bhat, 2008). Gelatin is unique, because when mixed with water, it forms a thermally reversible, elastic and clear gel (De Vries *et al*., 2004). These gelatin gels have a melting temperature just below body temperature (lower than 35ºC / 95 ˚F), so the gel product literally melts in the mouth and releases intense flavour immediately as it dissolves, which is a difficult quality to replicate with other hydrocolloids (Karim and Bhat, 2008).

Gelatin makes marshmallows chewy by forming a tangled three-dimensional network of polymer chains (Glicksman, 1982). Once gelatin is dissolved in warm water (dubbed the 'blooming stage'), it forms a dispersion, which results in a cross-linking of its helix-shaped chains. The linkage in the gelatin protein network, called 'junction zones', trap air in the marshmallow mixture and immobilize the water molecules in the network (Burey *et al*., 2008). The junction zones are only bound by weak hydrogen bonds (De Vries *et al*., 2004).

Gum-on-gum interaction tends to produce synergistic textures and there are many combinations to choose from. One of the most used is when guar gum is often added to xanthan gum to reduce cost, increase viscosity and improve texture, while xanthan gum prevents syneresis (Sakharam and Meschi, 2008).

Two distinctive, water-soluble, high molecular weight, pectic polysaccharide materials occur in *Opuntia* cladodes and fruits, namely mucilage and a Ca-sensitive gelling fraction. Mucilage does not gel in the presence of Ca. Pectin, with a low degree of methoxylation, occurs in the cell wall and can be extracted, using a mild alkali process, aided by a chelating agent; this pectin shows remarkably good gelling properties in the presence of $CaCl_2$, by a cooperative Ca^{2+} "egg-box" binding mechanism (Goycoolea and Cárdenas, 2003).

Although mucilage and pectin share similarities in the composition profile of their neutral constituent sugar residues, pectin has a significantly greater amount of linear poly-galacturonic acids. This difference causes very different physicochemical and functional properties,

underlying the potential applications of these polysaccharides in a wide variety of fields (e.g. foods, biotechnology and medicine).

Many popular uses in different countries shed light on the numerous mucilage properties, making this an interesting ingredient for the food industry, because of its viscosity properties. In SA, mucilage has been included into various products in various research studies: Turkish delight (confectionary); carrot cake; seed bread; mayonnaise; ice cream; and yogurt, replacing various ingredients (De Wit and Fouche, 2015). Nowadays consumers are becoming more aware of their own health and health foods. Thus, the demand for health foods is increasing, because of their functions of lowering the risks of chronic health diseases. In addition, some religions exclude the use of certain animal by-products, such as gelatin. By replacing such a product with an ingredient of plant origin, a huge range of products will be made available to a broader consumer market.

The aim of this study was, therefore, to evaluate consumer liking of the 75% mucilage + 12.5% xanthan + 12.5% agar formulation (Du Toit *et al*., 2016), with that of the control marshmallows (containing gelatin) and one commercial brand. Three marshmallow samples were left uncoloured and unflavoured, and were compared to the liking of three pink coloured, strawberry flavoured samples.

4.2 Materials and methods

4.2.1 Marshmallows

Six marshmallow samples were prepared: 75% mucilage + 12.5% xanthan + 12.5% agar; 100% gelatin (control); commercial brand white (Manhattan); strawberry flavoured and pink coloured 75% mucilage + 12.5% xanthan + 12.5% agar; strawberry flavoured and pink coloured 100% gelatin; and commercial brand pink (Manhattan).

The formulation of the recipe for the control marshmallows (white and pink) is the same as Table 3.1. The same method of preparation was used as described in section 3.2.2, under the heading ‘Preparation of marshmallows’. Two to three droplets commercial pink food colouring was added, while 0.57% commercial strawberry flavouring was used.

The MXA combination was chosen, as it showed the most desired textural attributes of all the combinations evaluated in the previous study (Du Toit *et al*., 2016). Thus, 75% mucilage was

combined with 12.5% agar and 12.5% xanthan, and the same method was used as with the control sample. As mentioned earlier (Bensadón *et al.*, 2010), the stabilising properties of mucilage are similar to those of xanthan and guar gums. Furthermore, agar was chosen, because it is commercially used in SA in chocolate candies to stabilise marshmallow layers. Half of the samples from each treatment were left unflavoured and uncoloured, (representing the white formulation), while the other half were coloured pink and flavoured with strawberry flavouring (representing the pink formulation).

The commercial brand Manhattan (Manufactured by Premier Foods SA, 1 Joist Street, Islando, 1609, SA) also showed textural properties similar to the control and 75% mucilage + 12.5% xanthan + 12.5% agar combination (Du Toit *et al.*, 2016). The marshmallows were melted in a double boiler and poured into containers, to resemble the shape of the other samples.

Samples were cut into cubes of 1x1x1cm and rolled in a mixture of icing sugar and corn flour (Foods and Cookery, 1991). Samples were evaluated simultaneously, under white lights and served on white polystyrene trays. Bottled water was used as a palate cleanser and between samples. The liking of taste, aftertaste, texture and overall acceptability were evaluated on a structured line scale, ranging from 1 (dislike extremely) to 9 (like extremely) (Figure 4.1). The nine-point hedonic scale has been used routinely in Food Science research for the past 60 years and is a scale of liking. It should be emphasized that the numbers on the scale are alternative names for the categories. For example, if the category 'like extremely' is assigned the number '9', the category has not become a number; it has merely been given a new name: 'nine' and is no more numerical than ranks (Wichchukit and O'Mahony, 2014).

1	2	3	4	5	6	7	8	9
Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Figure 4.1. Nine-point hedonic scale of liking (Stone and Sidel, 2004).

4.2.2 Consumer panel

Ninety-two consumers, 21 male, and 71 female panelists, aged 18 to older than 60 years, were sourced from staff and students from the Bloemfontein campus of the University of the Free State, Bloemfontein, SA (Table 4.1). All had to be regular consumers of marshmallows,

indicating that they consume it at least once every two weeks. The percentage split for the age profile was as follows: younger than 18 years (4%); 20-29 years (74%); 30-39 years (3%); 40 – 49 years (6%); 50-59 years (6%); and older than 60 years (7%). The majority of the panel members were, thus, mainly students, being in the younger age group from 18 to 39 years old (81%), while only 19% were in the age group of 40 years to older than 60 years, representing students and staff members.

Table 4.1: Age and gender profile of consumer panel.

Gender	Number of panelists	Age (Years)	Percentage split of total number of panelists
Female	71	18-19	4
Male	21	20-29	74
		30-39	3
		40-49	6
		50-59	6
		Older than 60	7

4.2.3 Statistical analysis

The significance for the liking of taste, aftertaste, and texture, as well as overall acceptability, measured for each treatment, was tested by means of a one-way analysis of variance (ANOVA). When applicable, Fisher's LSD-test ($p<0.05$) was applied to determine the direction of the differences between treatment mean values (NCSS, 2007). The two sample *t*-test was performed to determine the effect of age (NCSS, 2007).

4.3 Results and discussion

The ANOVA on the effect of gender, age and marshmallow type on the liking of sensory properties of the samples is summarized in Table 4.2. It was decided that p-values smaller than 0.05 would be considered as a significant difference. For age, there was a significant ($p<0.05$) effect for the liking of aftertaste, while all the marshmallow types showed significant ($p<0.05$) effects for liking of taste, aftertaste, texture and overall acceptability.

Table 4.2: ANOVA on the effect of gender, age and marshmallow type on the liking of the sensory properties of marshmallows.

Sensory property	Age	Gender	Marshmallow type	Age X Marshmallow type	Gender X Marshmallow type	Age X Gender X Marshmallow type
Taste	p = 0.0889	p = 0.4163	**p = 0.0123**	p = 0.3542	p = 0.6397	p = 0.5706
Aftertaste	**p = 0.0124**	p = 0.0901	**p = 0.0234**	p = 0.4028	p = 0.7754	p = 0.3435
Texture	p = 0.0576	p = 0.9846	**p = 0.0145**	p = 0.6699	p = 0.8227	p = 0.1346
Overall acceptability	p = 0.0752	p = 0.6602	**p = 0.0165**	p = 0.2987	p = 0.7375	p = 0.1943

For age group, there was a significant (p=0.0124) difference between the liking for aftertaste, meaning that the older group (40 to older than 60 years old) of consumers ranked the liking of the aftertaste higher than the younger group (18 to 39 years old) (Table 4.3). Both ranking were situated in the 'like slightly' region of the hedonic scale, with the rank of the older group situated more to the 'like moderately' side of the scale. Liking of aftertaste was included to determine whether the consumers would detect the grassy aroma of the mucilage (Rothman *et al*., 2012). In especially the pink samples, the aftertaste was predominantly sweet due to the addition of strawberry flavouring, which is one of the top six preferred food-related flavours (Hoffman, *et al*., 2016). Although only 19 % of the panelists in the present study fell within the age group 40 years to older than 60 years, it is still a valid result. This result confirms Hoffman and co-workers' (2016) findings that older adults (older than 55 years) find sugar more pleasant at higher concentrations, such as in confectionaries, compared with young adults (18 to 35 years) and middle-aged adults (36 to 55 years) (Petry, 2002).

Table 4.3 Effect of age group on the liking of sensory properties of marshmallows types.

Sensory property	Younger than 18 to 39 years	40 to older than 60 years	Significance level ($p<0.05$)
Taste	6.43 ± 2.15	6.80 ± 1.64	p = 0.0889
Sweet aftertaste	6.26^{a} ± 2.08	6.78^{b} ± 1.63	p = 0.0124
Texture	6.11 ± 2.32	6.55 ± 1.78	p = 0.0576
Overall acceptability	6.39 ± 2.09	6.76 ± 1.59	p = 0.0752

Means with different superscripts in the same row differed significantly, according to two sample *t*-test.

In Table 4.4 the effect of the mucilage inclusion on the liking of taste, aftertaste, texture and overall acceptability is shown. The lowest ranking for the liking of taste was given to the mucilage white sample, representing 'dislike slightly' on the hedonic scale. This sample differed significantly ($p<0.05$) from the control white and the *Manhattan* white samples, as

well as all the pink samples. The control white sample differed significantly ($p<0.05$) from all the samples, except the *Manhattan* white, while the *Manhattan* white sample differed significantly ($p<0.05$) from the mucilage white, control pink and *Manhattan* pink. The mucilage pink differed significantly ($p<0.05$) from the mucilage white and control white samples. For the liking of taste, the *Manhattan* pink sample had the highest ranking and did not differ from the control pink and mucilage pink samples. It was interesting to note that all the pink samples, including the mucilage pink sample, were ranked higher than 7 on the hedonic scale, indicating that the panel liked these samples 'moderately' to 'very much' (Figure 4.1). The strawberry flavouring was thus successful in masking the grassy flavour of the mucilage (Rothman *et al*., 2012), as there was an increase of almost three categories in ranking, on the hedonic scale, from the white unflavoured to the pink strawberry flavoured mucilage sample.

Table 4.4: Effect of marshmallow type on the liking of the sensory properties of the samples.

Sample	Taste	Aftertaste	Texture	Overall Acceptability
Mucilage White	$4.26^{a} \pm 2.30$	$4.20^{a} \pm 2.23$	$3.93^{a} \pm 2.28$	$4.21^{a} \pm 2.18$
Control White	$6.34^{b} \pm 1.89$	$6.38^{bc} \pm 1.65$	$6.08^{bc} \pm 1.98$	$6.38^{b} \pm 1.76$
Control Pink	$7.32^{d} \pm 1.41$	$7.08^{d} \pm 1.59$	$7.40^{d} \pm 1.65$	$7.38^{c} \pm 1.32$
Manhattan White	$6.47^{bc} \pm 1.84$	$6.10^{b} \pm 1.89$	$5.66^{b} \pm 2.00$	$6.22^{b} \pm 1.81$
Mucilage Pink	$7.11^{cd} \pm 1.58$	$7.01^{cd} \pm 1.51$	$6.68^{cd} \pm 1.72$	$7.10^{c} \pm 1.48$
Manhattan Pink	$7.51^{d} \pm 1.42$	$7.37^{d} \pm 1.30$	$7.42^{d} \pm 1.64$	$7.48^{c} \pm 1.38$

Means with different superscripts in the same column differed significantly (<0.05), according to Fisher's LSD-test.

The white MXA sample scored the lowest ranking for the liking of aftertaste, representing 'dislike slightly' on the hedonic scale. This low rank may be due to the grassy flavour of the mucilage (Rothman *et al*., 2012). This sample was significantly ($p<0.05$) less liked than all the other samples. The white control sample differed significantly ($p<0.05$) from the white MXA, pink control and pink Manhattan samples, the latter having the highest rank of all the samples. The white Manhattan sample differed significantly ($p<0.05$) from the white MXA, pink MXA, pink control and pink Manhattan samples. The pink MXA sample was ranked significantly ($p<0.05$) higher for aftertaste than the white MXA and white Manhattan samples. There was no difference amongst the pink MXA, pink control, and pink Manhattan samples. The pink MXA sample differed significantly ($p<0.05$) from white MXA and white Manhattan samples (Table 4.4).

The white MXA sample had the lowest rank for the liking of texture ('dislike moderately') and differed significantly ($p<0.05$) from all the other samples. The white control sample differed significantly ($p<0.05$) from the white MXA, pink control and pink Manhattan samples. The white Manhattan sample differed significantly ($p<0.05$) from the white MXA, pink MXA, pink control and pink Manhattan samples. Texture liking was the only attribute where the pink MXA sample could not be ranked higher than 7 on the hedonic scale. This sample only differed significantly ($p<0.05$) from white MXA and white Manhattan samples. Although the pink MXA sample did not score higher than 7, the ranking for its liking of texture did not differ from that of the pink control and pink Manhattan samples; the latter scored the highest numerical ranking for the liking of texture (Table 4.4). From these results, it is clear that the addition of strawberry flavouring positively influenced the flavour / taste of the MXA marshmallow and changed the consumer's perception of the texture. There was, again, an increase of almost three categories in the ranking on the hedonic scale, from the white unflavoured MXA marshmallow to the pink, strawberry flavoured MXA marshmallow (Table 4.4).

A positive correlation was found for 'intention of buying and – texture' in a study where isomaltulose replaced sucrose in pink strawberry-flavoured marshmallows. Different combinations of sugars were used, as well as different levels of gelatin. Authors concluded that texture defines acceptability and intention of buying this type of product (Periche *et al*., 2015).

In a study by Gress *et al*. (2009), the use of agar alone to replace gelatin produced marshmallows of inferior quality, as determined by a panel of ten trained judges. It is also known that agar gels can't melt in the mouth, as its melting temperature is 85ºC / 185 °F (Condrasky, 2014) and thus need to be chewed. On the other hand, xanthan gum is a general thickener, which can have a somewhat slimy texture at higher levels, when used on its own. For this reason, it is often used in combination with other hydrocolloids and starches, such as konjac flour, where it produces an extremely elastic gel, suitable for chewy candy (Sakharam and Meschi, 2008). In a previous study (Du Toit *et al*., 2016), it was concluded that the best combination to replace gelatin was 75% mucilage (liquid) + 12.5% xanthan (powder) + 12.5% agar (powder), as it only differed significantly from the control (100% gelatin) sample in regard to shear value, as measured by the Warner Bratzler Shear Force.

In a study by Saint-Eve *et al*. (2004), it was confirmed that a single aroma complex, such as strawberry, led to low-fat yogurts being perceived as thicker and stickier, by using three sensory methodologies, namely sorting, free-choice profiling and descriptive analysis. Kostyra and Barylko-Pikielna (2007) studied the effect of fat levels and guar gum addition in mayonnaise-type emulsions on the sensory perception of smoke-curing and salty taste. They found that it was the addition of guar gum that led to intensity differences in flavour and taste, rather than the different fat levels. Thus, it can be speculated that there could be an interaction between the hydrocolloids and flavourings, resulting in a better sensory perception of the textural properties in the present study. Gelatin, being a protein, already aids in the distribution of flavourings and colourings, despite being responsible for the characteristic foam-like texture of marshmallows (De Vries *et al*., 2004; Karim and Bhat, 2008; GMIA, 2012). Periche *et al*. (2015) used 20 trained panelists and a nine-point hedonic scale to evaluate various attributes, including strawberry flavour of five pink marshmallows formulations, consisting of different sugar mixtures, levels of gelatin and amounts of strawberry flavouring. The marshmallow formulation which had double the amount of flavouring was rated the best, as panelists liked a more intense aroma in this kind of product.

In the present study, three distinct groupings of marshmallows were noted for the liking of overall acceptability, namely, the lowest ranking mucilage white sample, then the other two white samples, and finally the cluster of three pink samples. The mucilage white sample had the lowest ranking for overall acceptability, representing 'dislike slightly' on the hedonic scale; this sample differed significantly ($p<0.05$) from all the other samples. The control white and *Manhattan* white samples both differed significantly ($p<0.05$) from the mucilage white and three pink samples. The three pink samples did not differ, again stressing the importance of flavouring to mask any unwanted flavours, such as the grassy flavour associated with mucilage. The sample that had the highest numerical ranking for overall acceptability was the *Manhattan* pink sample.

It is not possible for the authors to comment on the significant differences between the white and pink *Manhattan* marshmallow samples, as the formulation of these marshmallows is not of public knowledge. It was found in a previous study (Du Toit *et al*., 2016), that the shear values of three brands of pink commercial marshmallows were significantly ($p<0.05$) lower than that of the corresponding white commercial marshmallows. It, thus, appears that the South

African consumer favours a softer, flavoured and coloured marshmallow to a white, unflavoured and uncoloured marshmallow.

It should again be stressed that ranking on the hedonic scale, is a representation of a category on the hedonic scale. The simplest illustration that the assigned number is merely a new label is that if 'eight' is subtracted from 'nine', the answer is 'one'. Yet, if 'like very much' is subtracted from 'like extremely', the answer is hardly likely to be 'dislike extremely' (Wichchukit and O'Mahony, 2014). Furthermore, the fact that commercial marshmallows were ranked as 'liked moderately' can be attributed to the fact that it may not have been the best tasting marshmallows in SA. The main reason for its inclusion was the similarity it showed to the mucilage-containing marshmallow and the control sample (gelatin), in regard to textural properties (Du Toit *et al.*, 2016). From the study done by Du Toit *et al.* (2016), it was concluded that texture was the sensory property mostly affected by the reformulations.

Colour is an important quality attribute in the food industry and it influences consumer's choice and preferences. The correlation between colour and other sensory quality attributes is well established (Pathare *et al*., 2013). In a study by Spence *et al*. (2015), they reviewed the growing amount of scientific research showing that people steadily associate specific colours with particular tastes. The colour white is widely associated with salty, because of the whiteness of sodium chloride, while pink and/or red colours are associated with sweetness, because sweetness is more often present in red foodstuffs, such as berries and cherries, than in yellow, white, brown or orange foodstuffs (Dematté *et al.*, 2006). These cross-modal correspondences are consistent across different cultures and at least over the last three decades, despite the wide cultural differences in the use of colours across cuisines (Zhou *et al.*, 2015). In the present study the same conclusions can be drawn from the consumers, when presented with pink and white samples, awarding higher rankings to sweet taste for the pink samples than to the white samples, when in fact at least four of the samples had exactly the same sugar content (Table 3.1). In a study by Periche *et al*. (2015), a positive correlation was found for 'colour and overall appearance' of pink strawberry flavoured marshmallows, containing different sugars and levels of gelatin.

To illustrate the positive effect of pink colouring and strawberry flavouring on the perception of the consumer, the frequencies of the hedonic scale rankings per marshmallow sample, for the liking of the four sensory properties were determined (Figure 4.2). Each of the graphs can

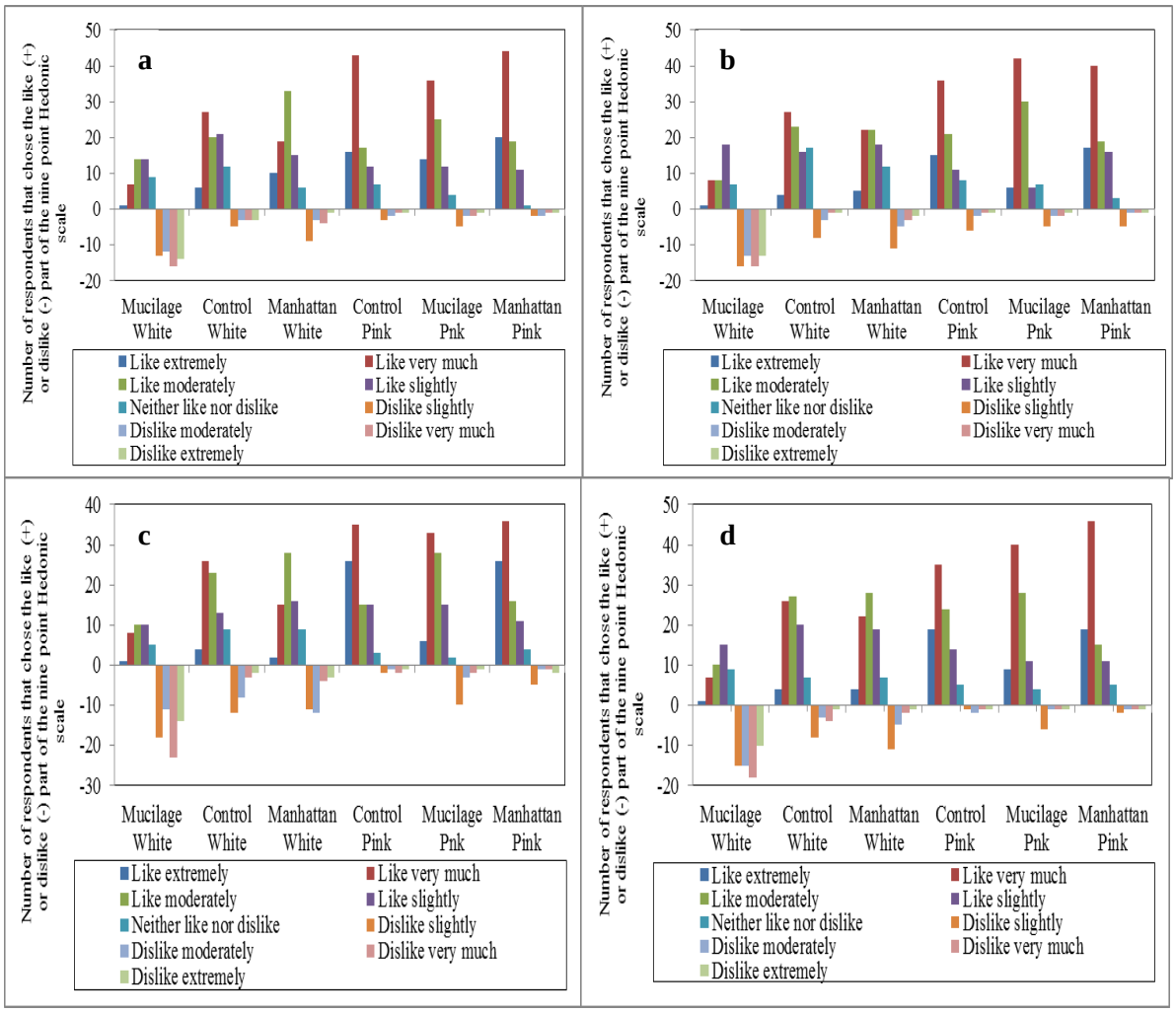

Figure 4.2. Frequency of the hedonic scale rankings per marshmallow sample, for the liking of a) taste, b) aftertaste, c) texture and d) overall acceptability.

be divided into two parts: i) bars pointing upwards, indicating the positive or "like" side of the hedonic scale, ranging from "like extremely" to "neither like nor dislike"; and ii) bars pointing downwards, indicating the negative or "dislike" side of the hedonic scale, categorized as between "dislike slightly" to "dislike extremely". The first three graphs on the left side of each of the figures represent the frequency of use of the hedonic tags for the white samples, while the pink samples are situated on the right side of each figure. In general, the positive hedonic ratings were assigned more regularly than the negative rankings. It is clear that the negative indicators were used more frequently for the white marshmallow samples, with high frequencies being assigned to the mucilage white marshmallow, for all attributes tested. The highest frequency of negative hedonic ranking was recorded for the texture of the mucilage white sample (Figure 4.2c). However, for the liking of this attribute, all three white

marshmallow samples showed an increase in the use of negative hedonic rankings. For the pink samples, the liking of texture had the most frequent usage of negative hedonic ranking by the panel members (Figure 4.2c). All the pink samples also had the highest frequency of use for the positive hedonic rankings.

4.4 Conclusions

Mucilage from the cladodes of *O. ficus-indica* is under investigation for application in several foodstuffs. The results from the present study showed that flavouring successfully masked the distinctive taste and aftertaste of mucilage in marshmallows, thereby also increasing scores for texture and overall acceptability. It was concluded that the inclusion of mucilage in a coloured and flavoured confectionary product is a possibility.

The usage of wet mucilage, however, is questionable, since the quality and quantity of the mucilage are influenced by climate, temperature and rainfall, as noted in literature. Therefore, research should continue on mucilage as a dried powder, as all the other hydrocolloids are dried. At first, the brown colour, produced by heat during the drying process, and grassy flavour of the mucilage powder should be ignored in favour of developing a desirable combination with other hydrocolloids, to find a practical formulation. Furthermore, techniques such as scanning electron microscopy (SEM) might shine light on whether there is a synergistic effect when mucilage is combined with other hydrocolloids in a sugar-based confectionary.

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CHAPTER 5

EFFECT OF *OPUNTIA FICUS-INDICA* MUCILAGE ON TEXTURAL PROPERTIES AND THE MICROSTRUCTURE OF HIGH SUCROSE EGG ALBUMEN FOAM STRUCTURES

ABSTRACT

Rapid visco analyser (RVA), differential scanning calorimetry (DSC) and scanning electron microscopy (SEM) studies were done on 100% gelatin, 100% mucilage (dry), 100% xanthan, 100% agar and 75% mucilage + 12.5% xanthan + 12.5% agar (MXA) marshmallow samples, as well as individual hydrocolloid samples. Rapid visco analyser profiles showed very high viscosities for both 100% xanthan (< 15 000 cP) and 100% mucilage (> 20 000 cP) samples, in contrast to 100% gelatin (just above 5 000 cP) and 100% MXA (< 5 000 cP) samples. The addition of ingredients, such as sugar and egg whites, resulted in lowering of the general RVA viscosity, from a maximum of just below 20 000 cP for mucilage-containing to 730 cP for MXA-containing marshmallows. Xanthan- and MXA-containing marshmallows had final viscosities of 237 cP and 727 cP, respectively, while almost no viscosity was measured for mucilage-containing marshmallows. A 100% mucilage sample showed no thermal events during DSC, indicating that no significant gelation occurred and no gel was formed. All the other samples, namely 100% agar, 100% gelatin, 100% xanthan and MXA, showed very weak exothermal events during heating cycles, indicating that some form of phase change took place; in some cases gels were even formed before samples were placed in the DSC. For the 100% samples, gelatin and agar showed characteristic scaffolding on SEM micrographs, while xanthan had a flaky appearance. Mucilage had irregular round pores, with thick walls and branching. Scanning electron imagery showed sugar crystals suspended in the continuous phase for agar-, xanthan-, mucilage- and MXA-containing marshmallow samples; the crystals were the biggest for the mucilage-containing sample, while the MXA–containing sample also had clear zones like the gelatin-containing sample.

5.1 Introduction

The quest for substitutional innovative and unique sources of hydrocolloids, which are abundantly accessible, but low-key and subsequently underutilized, is critical and urgent. Many of the polysaccharides, currently available as manufacturing hydrocolloids, were first used in an experimental way in domestic food preparation, e.g. starch as a thickening agent in soups and sauces, pectin as the setting-agent in jam and gelatin in jellies (Ndjouenkew *et al*., 1996).

Gelatin has been produced on an industrial scale for almost 120 years and utilized as a structuring agent for providing the required textural properties in toffees, water desserts, meat and dairy products, confectioneries, etc. (Poppe, 1992). The specific kind of confectioneries considered here are marshmallows, which have foam-like structures. Other basic ingredients for their manufacture include sucrose, starch syrup, foaming agents, flavourings and colourings. Other hydrocolloids may be used as a substitute for gelatin, such as agar, pectin and gum Arabic (Karim and Bhat, 2008). However, the early meltdown of gelatin at oral body temperature allows a brilliant flavour release during chewing. It is this distinctive 'melt in the mouth' characteristic of gelatin gels that makes them unacceptable for certain product applications. High sucrose content confectionaries may become gluey when stored in warehouses (temperatures can rise up to 45 ºC / 113 ºF), resulting in partial structural collapse, fusing and crystallisation (encrusting) of the product (Poppe, 1992).

According to Brown (2008), marshmallows are crystalline, syrup phase candies, consisting of a simple syrup mixture (sugar and water), with added flavourings. Crystalline candies are candies formed from sugar solutions which bring about many fine, small sugar crystals, lending to its light and fluffy texture (Brown 2008). The main functions of sugar in marshmallows are for sweetness, while aiding in foam formation, enhancing flavour and acting as preservative (Betts *et al*., 2006).

When the syrup mixture has been heated to 112 to 115 °C / 233.6 to 239 ºF, the so-called soft-ball stage, it is poured slowly over the stiffly whipped egg whites, while continuously beating, until the candy holds its shape (Foods and Cookery, 1991). Egg albumin foams consist of a discontinuous air phase that is dispersed in a continuum of liquid and are produced by whipping (Tyapkova *et al*., 2016). Because of its ability to increase the volume up to six times (Lomakina and Míková, 2006), these foams hold large volumes of air, resulting in a much lighter

marshmallow (Kamosawa and Talbot, 2006). The sugar syrup also stabilises and sweetens the foam.

The addition of gelatin and a cooling process at room temperature (23 °C / 73.4 °F), for several h, produce the soft, juicy and pliable texture of marshmallows (Tyapkova *et a*l., 2016) Gelatin is a very effective foam stabiliser, as it prevents air bubbles in the foam system from collapsing (Tan and Lim, 2008). Gelatin granules are hydrated in cold water to prevent clump formation, by sprinkling it over water to separate the granules. When this sponge-like structure is added to the hot syrup mixture, protein granules are dispersed within the mixture. The increase in the temperature of gelatin causes protein molecules in the gelatin molecules to denature and unfold. When the marshmallow mixture is the left to cool, the protein molecules of the gelatin again aggregate and consequently form a gel (Saha and Bhattacharya, 2010).

Young (2007) states that knowledge of the gelling behaviour of hydrocolloids brings about better development and standardisation of products in the food industry. Some hydrocolloids show synergistic effects if they are used together, e.g. xanthan gum, used with galactomannans, such as locust bean gum, shows a synergistic viscosity increase (Saha and Bhattacharya, 2010). Other hydrocolloids display an enhanced viscosity development in ionic environments and exhibit gelling or setting behaviour at specific temperatures (Young, 2007).

The slimy mucilage that is visible when leaves or cladodes of *O. ficus-indica* (better known as the cactus pear plant) are cut or damaged (Sepulveda *et al*., 2007), is a high molecular weight polysaccharide material, soluble in water (Trachtenberg and Mayer, 1982b). Moreover, it causes a thickening or viscosity-producing effect (Glicksman, 1983). The non-Newtonian viscous colloid that forms when mucilage is dissolved in water, is the result of its capacity to absorb and hold huge amounts of water in its structure (Saenz, 2000; Saenz, 2006). These interesting properties compel researchers to investigate its use as a functional ingredient (Saenz *et al*., 2004).

Up to this point in the present research, mucilage was used in wet form. Due to differences in viscosity of the hydrocolloid due to climate, rainfall and other environmental influences (Rothman *et al*., 2012), it was decided to dry the mucilage to a fine powder, like commercially-available hydrocolloids.

The aim of this study was to use mucilage powder in combination with food hydrocolloids to produce marshmallows. These samples, as well as individual hydrocolloids, were then tested to assess their viscous properties at various heating and shear rates. Furthermore, the unfolding, glass transition, solids-melting and decomposition temperatures were determined for individual hydrocolloids. Scanning electron microscopy studies of the marshmallow and hydrocolloid microstructures were done, as these could be useful to understand their behaviour during cooling and in the presence of high sugar concentrations.

5.2 Materials and methods

5.2.1 Extraction of liquid mucilage

Mucilage, from the *Opuntia* cultivar Algerian, was extracted according to the method described in section 3.2.1, under the heading 'Extraction of liquid mucilage'.

5.2.2 Drying of liquid mucilage

Five sets of 143.27 g of wet mucilage were weighed and put into the E*zidri Snackmaker Dehydrator* (Healthmakers Ltd, Box 333 Utrecht 2980, SA) for 24 h at 63 ˚C / 145.4 ˚F. All the water from the mucilage evaporated and only a film of dry green mucilage was left. These films were removed from the plastic sheets and ground in a Kenwood 400W, BL 370 series (Wittex Agencies CC, Unit 6 Reid Plaza, 21 Reid St., Westdene, Bloemfontein, SA) coffee grinder for 2 min, to a fine powder and weighed again. The food dryer had five drying racks, resulting in a total yield 2.50 g of mucilage powder.

5.2.3 Preparation of marshmallows

The formulation for the marshmallow that was used as the control sample, is given in Table 3.1 and all samples were prepared according to the method described section 3.2.2, under the heading 'Preparation of marshmallows'.

5.2.4 Replacement of gelatin with liquid mucilage and powdered hydrocolloids

According to du Toit *et al*. (2016), food grade agar (Health Connection Wholefoods™. Unit 17 – River Park, 77 de Waal Road, Diep River, 7800, SA), and xanthan (Nature's Choice, 73 Cyprus Avenue, Meyerton, SA, 1960) were used in combination with the powdered mucilage, to replace gelatin, according to the formulation in Table 5.1. Furthermore, the control sample contained 100% gelatin and one of the experimental samples 100% dry mucilage.

5.2.5 Freeze-drying

Samples were divided (±15 ml quantities) into 90 mm Petri-dishes and frozen overnight. The frozen samples were freeze dried in a Labconco Freezone 6 Plus freeze drier (Vacutec, Johannesburg, SA).

5.2.6 Rapid visco analyser (RVA)

The RVA Preten 4500 model was used to measure the water absorption and viscosity properties of the different hydrocolloids (gelatin, xanthan, agar, mucilage and MXA) and marshmallow (gelatin, xanthan, agar, mucilage and MXA) samples, in duplicate. For all the samples, 25 ml of distilled water was added to 5.00 g of sample and thoroughly mixed (Young, 2007). The samples were immediately put in the RVA and the profiles monitored. Each profile was 20 min long, with readings taken every 4 seconds (s). The initial temperature of the system was 15 °C / 59 °F, and it remained constant for 2 min. The sample was then heated for 7.43 min to 95 °C/ 203 °F and held at a constant temperature of 95 °C / 203 °F for 10 min. The samples were cooled down to 25 °C / 77 °F over a period of 15 min. The frequency of the system was 160 rpm (Young, 2007).

Table 5.1: Substitution hydrocolloids, combinations of hydrocolloids, substitution percentages and weights used in the preparation of marshmallows samples.

% substitution	Actual weight replacement (g)	Gelatin substitution	Formulation Code
Control	20.00 g	Gelatin	G
100 %	5.00 g*	Mucilage	M
100 %	20.00 g	Agar	A
100 %	20.00 g	Xanthan	X
75 % +12.5 % +12.5 %	15.00 g+2.50 g+2.50 g	Mucilage + xanthan + agar	MXA

* 5.00 g dry mucilage = 143.27 g liquid mucilage

5.2.7 Differential Scanning Calorimeter (DSC)

Differential scanning calorimeter experiments were performed on a Mettler Toledo DSC822 instrument, equipped with a FRS 5 sensor and controlled by Mettler Toledo STARe software, version 9. Thermograms were prepared for presentation, where after thermal event onset temperatures, peak temperatures and enthalpies were obtained from it, by integration between appropriate temperature boundaries, utilizing the same software.

For this analysis, only hydrocolloid samples were used and no marshmallow samples. Unless otherwise stated, stock solutions consisting of approximately 6.00 mg of sample, accurately weighed to 0.01 mg, was dissolved in 14.00 mg of water at ambient temperatures. When required, larger stock solutions with the same sample / water ratio were prepared. It took several min to obtain a homogeneous composition. An appropriate portion of this stock solution (between 3.00 mg and 22.00 mg) was then placed in a 40 microliter (µl) aluminum (Al) pan and hermitically sealed to prevent moisture evaporating from the system, at temperatures close to the boiling point of water. In cases where the energy associated with phase changes was exceedingly small, it was convenient to prepare 6.00 mg sample / 10.00 mg water stock solutions. If the stock solution already had a gel like texture (indicating gel formation started before homogenizing was complete and the DSC experiment could be commenced), the sample was dissolved in larger volumes of water. Ratios of 6.00 mg sample combined with 28.00 mg or 94.00 mg water were then used. If gel formation still took place too fast, an accurately weighed partially gelled sample was spread evenly over the bottom of the Al pan and the DSC experiment performed, while minimizing all possible delays as much as possible. This procedure undoubtedly led to estimated enthalpies of thermal events at best and any unusually low temperature thermal event may even be unobserved, but onset and peak temperatures still represent useful estimates of where any observed thermal events actually occur.

Once in the DSC instrument, a N_2 gas flow of 4–5 ml min^{-1} was maintained during the entire course of the experiment. Heating and cooling rates were 10 °C min^{-1} / 50 °F min^{-1}. Temperature was controlled to remain between 25 °C and 95 °C / 77 °F and 203 °F; the higher temperature was chosen to prevent water boiling at ambient pressure (ca. 650 mm Hg; water boils at 96 °C / 204.8 °F at this pressure) and the Al pan popping due to a large pressure increase.

5.2.8 Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) was conducted on dried 100% hydrocolloid and marshmallow samples in order to inspect the cell structures of the combined ingredients and to see how the different ingredients fit together in the marshmallow. Material for SEM examination was freeze dried (Freezone 6 Plus, Vacutec, Johannesburg, SA). After drying, material was mounted on Al stubs (Cambridge pin type, 10 mm), using Pratley steel epoxy glue and gold coated (±60 nm), with a Bio-Rad sputter coater (United Kingdom) (Agar, *et al*.,

1974). Specimens were examined and analysed with a JSM-7800F Extreme-resolution Analytical Field Emission SEM (FE-SEM).

5.2.9 Light photography

Photographs of freeze-dried gelatin, agar, xanthan, mucilage and MXA samples were taken with a Nikon D7000 camera body, fitted with a 60 mm F 2.8 micro lens. The samples were displayed on a cold white LED lightbox, to highlight the delicate structure of the fibres.

5.3 Results and discussion

5.3.1 Rapid visco analyser (RVA)

Figure 5.1 shows the RVA graphs for the 100% hydrocolloids, over a period of 20 min and a temperature range from 15 to 95 ºC / 59 to 203 ºF. At first glance it should be noted that the viscosity reached by both xanthan (14 252 cP) and mucilage (23 259cP) were quite high, in contrast to the gelatin (5 616 cP) and MXA (3 502 cP). Both xanthan and mucilage do not form true gels and only act as thickeners (Medina-Torres *et al*., 2002; Saha and Bhattacharya, 2010), while gelatin and agar form true gels (Clark *et al*., 1982).

From the start of the gelatin profile there was an increase in viscosity to 5 616 cP at 2.2 min, at which point the gelatin molecules were hydrated, forming a sponge-like structure (Figure 5.1). This was followed by a gradual decline which was completed at 4.38 min and 40 ºC / 104 ºF. The increase in the temperature caused the protein molecules to denature and unfold (Saha and Bhattacharya, 2010). The gelatin, which is partially degraded collagen, dissolved in the hot water as random coils of polypeptides and became fluid. Until the end of the profile, at 20 min and ±45ºC / 113 ºF, the viscosity remained zero, as it stayed in liquid form. Not shown in the graph is the increase in viscosity, which will happen at 35 ºC to 40 ºC / 95 ºF to 104 ºF, when gelatin cools down and assumes triple helix structures, mediating intermolecular cross-linkages, which will finally result in a firm gel (Bennet, *et al*., 2007).

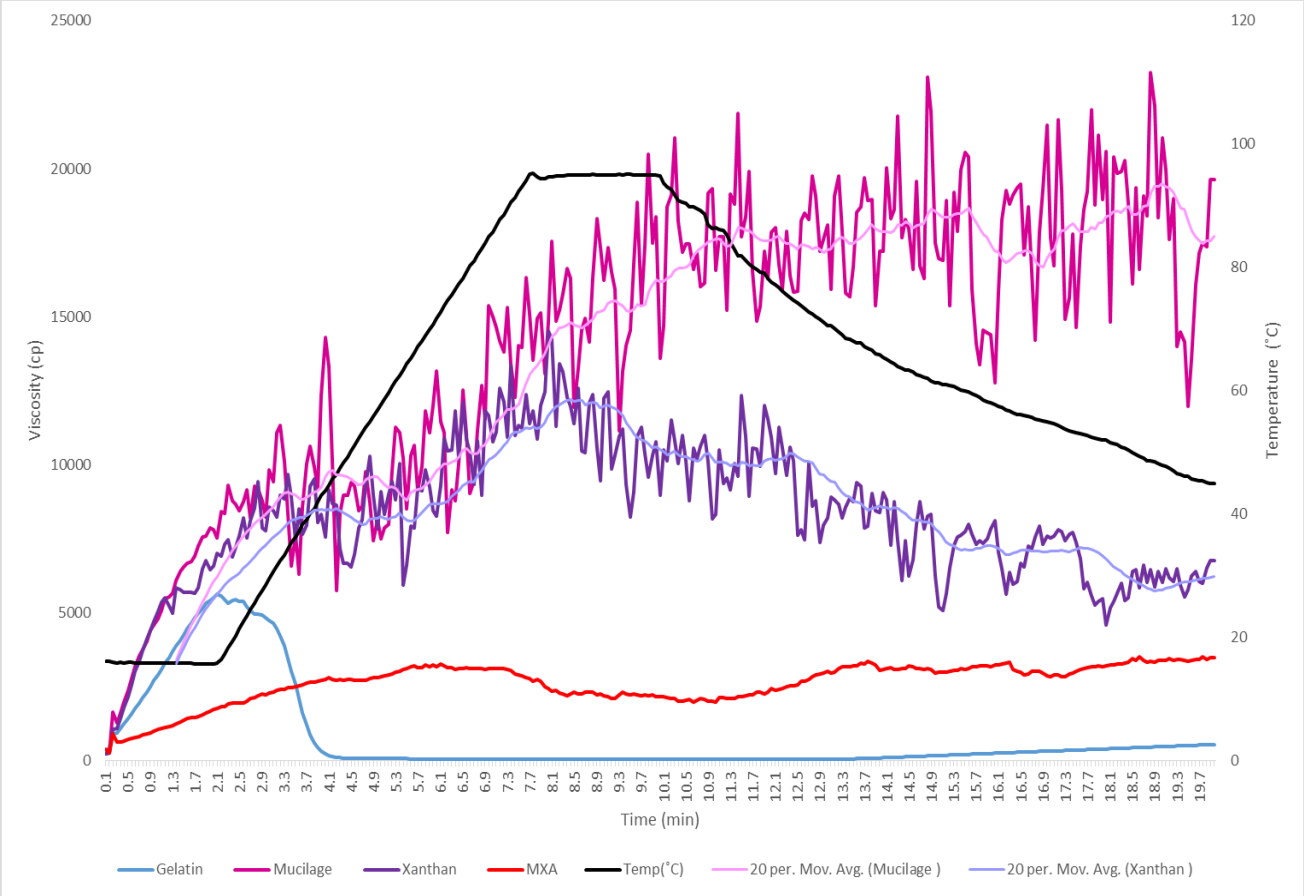

Figure 5.1 Rapid visco analyser heating-cooling cycle displaying the gelling and melting temperatures of gelatin, mucilage, xanthan and MXA

Due to technical error and unit malfunction, an agar profile could not be obtained in the present study. For the purposes of comparison, the agar profile in the Perten Instruments Manual will be discussed (Figure 5.2). Over a period of 180 min the true thermo-reversibility of this hydrocolloid can be analysed, with a viscosity remaining lower than 500 cP, which is in stark contrast to the other hydrocolloids in the present study. Over the first 50 min, there is a gradual decrease in the viscosity, where after it sharply increases to maximum viscosity of 500 cP, at a temperature of around 30 ºC / 86 °F. An agar solution in hot water has a gelling point between 32 ºC to 45 ºC / 89.6 °F to 113 °F. At this temperature double helices aggregate to form three-dimensional structured frameworks, which hold the water molecules within the interstices of the framework (Clark, *et al*., 1982). A gel has formed, due to the three equatorial hydrogen atoms on the 3, 6-anhydro-L-galactose residues, which constrain the molecule to form the above-mentioned helix. The viscosity remains at this peak for about 20 min, where after it declines at a slope of about 45º, until the viscosity is zero at about 100 min and 80 ºC / 176 °F,

thus turning into a liquid phase again. It stays a liquid for the next 40 min, while the temperature heats up to 100 ºC / 212 ºF and cools down to 30 ºC / 86 ºF, where the whole cycle starts again.

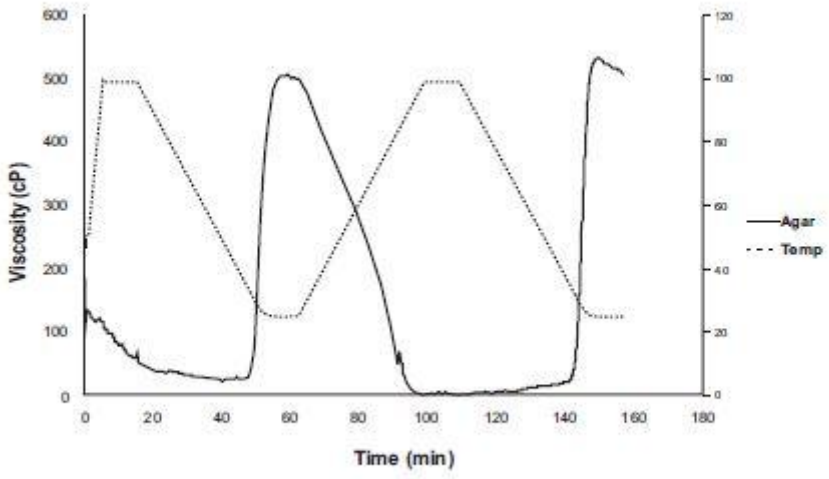

Figure 5.2: Rapid visco analyser heating-cooling cycle displaying the gelling and melting temperatures of agar (Martinez, 2015).

Xanthan's unique solution properties are related to the rigid nature of the molecule, as well as the conformational changes of the side chains, with respect to temperature (Saha and Bhattacharya, 2010). In the present study it was found that an increase in temperature led to an increase in viscosity, and a decrease in temperature to a decrease in viscosity (Figure 5.1). The xanthan profile also displayed profile 'noise', possibly due to the lumpy character of molten xanthan systems. Like most other hydrocolloids, xanthan needs intensive agitation upon introduction into an aqueous medium, to avoid the formation of lumps (Katzbauer, 1998). From the onset of this profile, there was an increase in the viscosity at 3.8 min and a temperature of 40 ºC / 104 ºF, to 9 285 cP. A slight plateau was formed over the next 2.19 min, to a temperature of 50 ºC / 122 ºF. This was followed by an increase to a maximum viscosity of 14 523.5 cP at 8.07 min and 94 ºC / 201 ºF. Under hot conditions, the side chains of the polysaccharide protrude away from the main cellulosic backbone, giving rise to a disordered coil (Katzbauer, 1998). Maximum viscosity was followed by a decrease and another plateau between 9.76 min and 50 ºC / 122 ºF, and 12.99 min and 40 ºC / 104 ºF. Viscosity further declined to 6 007 cP at

30 ºC / 86 ºF and 20 min, because, as the system is cooled, the disordered coils fold over and associate with the back bone, leading to an ordered system (Annable, *et al*., 1994).

With an increase in temperature, there was an increase in the viscosity of the mucilage sample (Figure 5.1). However, contrarily to the profile of xanthan, the viscosity increase continued after the temperature decreased. Also, profile 'noise' was displayed, due to the lumpy character of the mixture not being blended thoroughly. A distinct shoulder was formed at 11 329 cP, after 3.29 min and at a temperature of 32ºC / 89.6 ºF, followed by a small slump at 4.26 min and 46ºC / 114.8 ºF, to a viscosity of 5 739 cP. Hereafter the viscosity increased steadily at 12.99 min and 78ºC / 172.4 ºF to 17 209 cP, where it remained until the end of the cycle, at just below 50ºC / 122 ºF. Mucilage is, like xanthan, also an electrically charged molecule. When it comes into contact with water, the hydrogen atoms split off, leaving negatively charged groups open along the chain. This leads to the long mucilage molecule repelling itself, causing it to uncoil and stretch out. All of these stretched out molecules cause the viscosity of the fluid to increase (Medina-Torres *et al*., 2002).

When looking at the profile for the MXA sample (Figure 5.1), it is noticeable that the general viscosity was distinguishably lower than that of the other samples, at around 3 500 cP, which may be caused by the addition of agar. Figure 5.3 shows the profile of this sample on a reduced scale, displaying the clear formation of three shoulders. There was an increase in viscosity, with an increase in temperature, to a maximum viscosity of 3 202 cP at 5.67 min and 66 ºC / 150.8 ºF. Over the next 3.37 min (to 10.16 min) and at 95 ºC / 203 ºF, the viscosity dropped to a minimum of 2 1976 cP at 10.67 min and 85 ºC / 185 ºF. From here on another shoulder region was formed, with a maximum viscosity of 3 288 cP at 48ºC and 13.87 min, which remained stable as the temperature decreased to 30 ºC / 86 ºF at 20 min. On the combined figure (Figure 5.1), the effect is less dramatic, with a slight gradual increase as the temperature increased, followed by a shallow trough at maximum temperature of 95 ºC / 203 ºF. As the temperature decreased, the viscosity increased slightly. The increase in viscosity over the first 5 min can be attributed to both the xanthan and mucilage, because their viscosities both increased as temperature increased. The slight decrease at maximum temperature may be the influence of agar, which is a liquid at this temperature. As the sample cooled down, the increase in viscosity may be attributed to both agar and mucilage, as both of these hydrocolloids showed an increase at the end of the cycle and agar being able to form a gel at these temperatures (Saha and Bhattacharya, 2010).

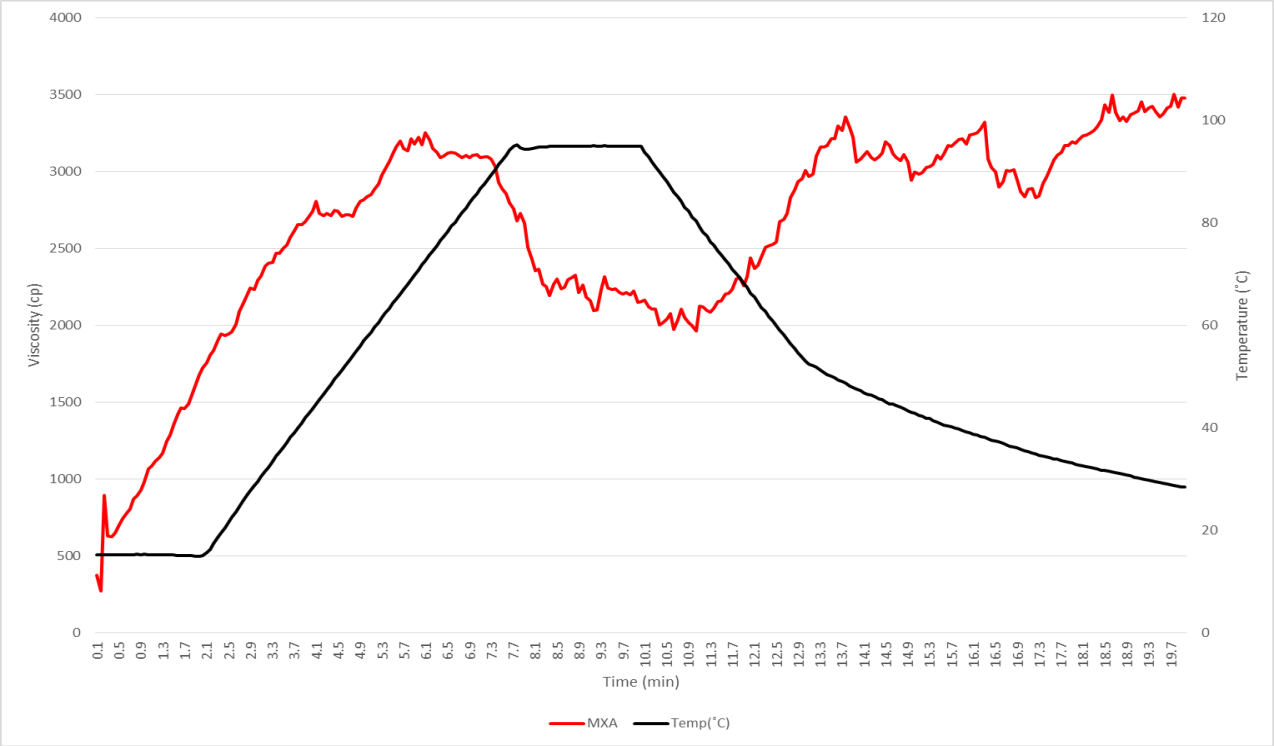

Figure 5.3: Rapid visco analyser heating-cooling cycle displaying the gelling and melting temperatures of the MXA sample

It will be attempted to explain the alteration of the viscoelastic properties and the time dependent behavior of the mucilage by the addition of the two hydrocolloids. First of all, it is not known how successfully the large and stiff xanthan molecules were able to diffuse into the agar network. Both agar and xanthan consist of double helixes, which should aid intermolecular physical interactions. The main difference between xanthan and agar is their flexure; xanthan is very rigid and has a rod-like character and in contrast, agar is dominated by flexible structures. Furthermore, both hydrocolloids are negatively charged, xanthan more so than agar. It is known that agar has less gel strength in the presence of sodium alginate, which is also negatively charged (Armisen and Galatas, 1987). The normally unyielding agar gel will be week in the presence of xanthan. Add to that the long uncoiled repelling molecules of mucilage, and the gel was even more weekend.

Figure 5.5 shows the RVA graphs for the marshmallow samples, over a period of 20 min and a temperature range from 15 to 95 ºC / 59 to 203 °F. At first glance the addition of ingredients, such as sugar and egg whites, resulted in lowering of the general viscosity, from

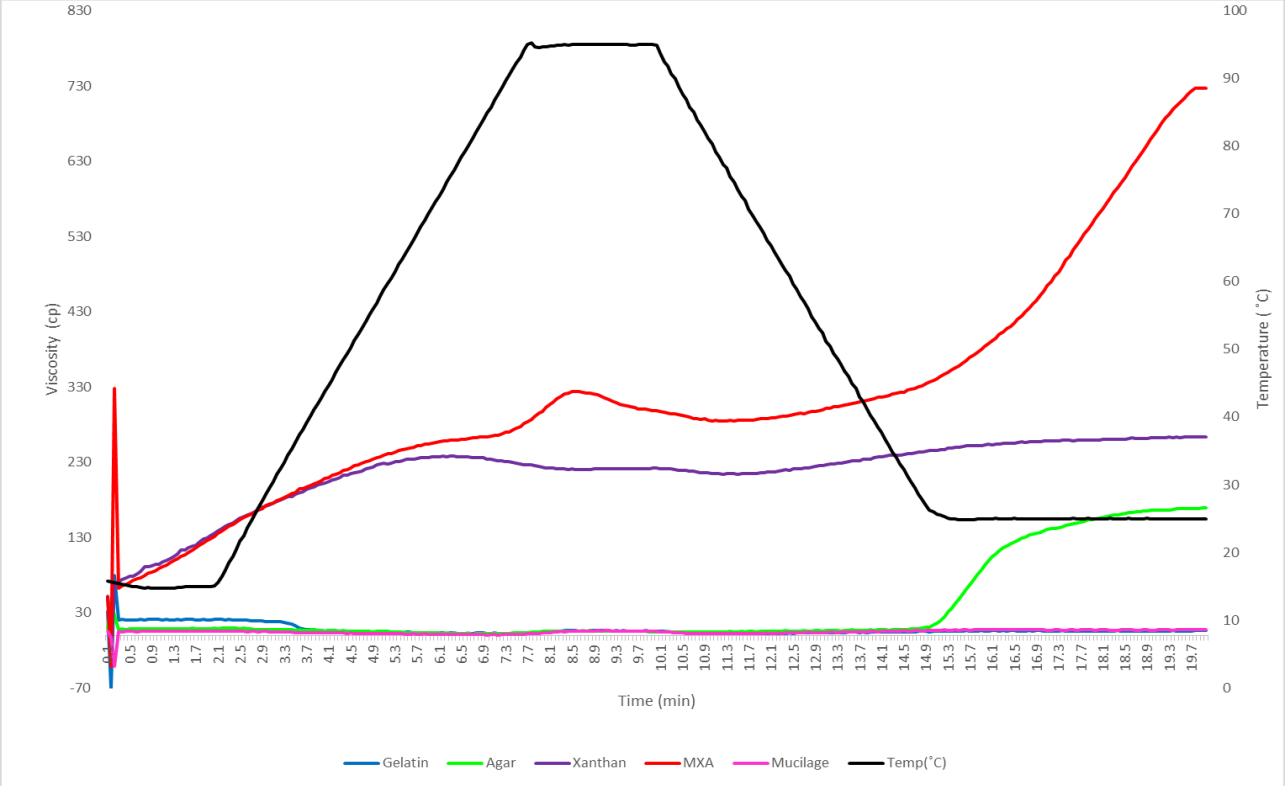

Figure 5.4: Rapid visco analyser heating-cooling cycle displaying the gelling and melting temperatures of gelatin, agar, mucilage, xanthan and MXA in marshmallow samples

a maximum of 17 209 cP for mucilage (Figure 5.1) to 730 cP for MXA marshmallows (Figure 5.4). The gelatin-containing marshmallow sample remained at a low viscosity (6.67 cP), i.e., fluid, for the whole 20 min, up to the end of the period at a temperature of ±45ºC / 113 ºF. The same trend was observed when the 100% gelatin sample was run, as gelling only occurs at around 35ºC / 95 ºF (Bennet, *et al*., 2007).

In the making of gelatin-containing marshmallows, a liquid phase (supersaturated sugar syrup) is forced around external gas (air) by whipping, with proteins (collagen in the gelatin and albumin in the egg whites) acting as stabilisation mechanisms (Campbell and Mougeot, 1999). According to Chavez-Montes *et al.* (2007), on the other hand, egg albumin is the component responsible for the viscosity of the system, while gelatin contributes to the stabilisation of the air bubble structure. None the less, bubbles, that have bursted or coalesced when the interface thins excessively, are stabilised by the Gibbs-Marangoni effect. If local thinning occurs, the proteins diffuse into the depleted area, sweeping with it liquid and re-thicken the thinned region. In addition, proteins also have low rates of surface lateral diffusion and stabilise

bubbles, primarily by forming a rigid layer of interlinked proteins at the interface (Campbell and Mougeot, 1999).

For the agar-containing marshmallow sample, viscosity started to increase for the first time at around 14 min, to reach a maximum viscosity (169 cP) after 20 min at 25 ºC / 77 ºF. A 100% agar sample only increases in viscosity after 40 min (Figure 5.2) to 500 cP. This indicated that the addition of a supersaturated sugar syrup accelerated gelling time, since sucrose has a stabilising effect in polysaccharide gelation and within 0-50% sucrose, it accelerates the gelling ability of agar (Nishinari, 1997); the amount of sugar that was used in the present formulation was 46% (Table 5.1). A mixed system of protein (albumin in egg whites) and agar can be unstable, as the polymer dilutes the proteins and prevents their interlinking. The concentration at which the agar was used in the present recipe (2.3%) is far higher than the concentration of less than 1% that is usually required to form a gel (Armisen and Galatas, 1987). The xanthan-containing marshmallow profile showed a gradual increase in viscosity, reaching a viscosity of 237.67 cP at 6.2 min and 73.65 °C / 164.57 ºF. A very shallow trough is formed upon cooling, with the lowest point (viscosity 214.67 cP) at a temperature of 70 °C / 158 ºF. Upon further cooling, to 25 ºC / 77 ºF, viscosity slightly increased again to 237cP at 20 min. The 100% xanthan sample showed a clear decrease in viscosity upon cooling (Figure 5.1). It should be emphasised that all solutions of xanthan gum at 1% or higher concentration appear gel-like at rest, because of the highly ordered network of entangled, stiff molecules. Shear thinning pseudo plasticity results from disaggregation of this network and alignment of individual polymer molecules in the direction of shear force (Kelco, 2008).

In the making of xanthan-containing marshmallows, the supersaturated sugar syrup is forced around air bubbles by whipping, with only the albumin in the egg whites that could act as stabiliser at the air-liquid interface. Xanthan has a rigid structure and large side chains, which have complex effects on the mobility of an amorphous, hydrogen-bonded sugar matrix (You and Ludescher, 2009). Furthermore, its viscosity is built through inter-molecular association, as well as molecular size, thus having a gel-like appearance at rest, yet, pours like a liquid when moved.

For all practical purposes there was no change in the viscosity of the mucilage-containing marshmallow sample during the complete RVA run from 15 ºC to 95 ºC / 59 ºF to 203 ºF over a period of 20 min. (Figure 5.4). This is in stark contrast to the mucilage sample's profile,

which showed an increase in viscosity, even during the cooling stage of the RVA run (Figure 5.1). It is, thus, clear that not even the sugar syrup and / or egg white foam could assist in increasing the viscosity of the mixture, i.e., no substantial bonds were formed between any of the components in this mixture.

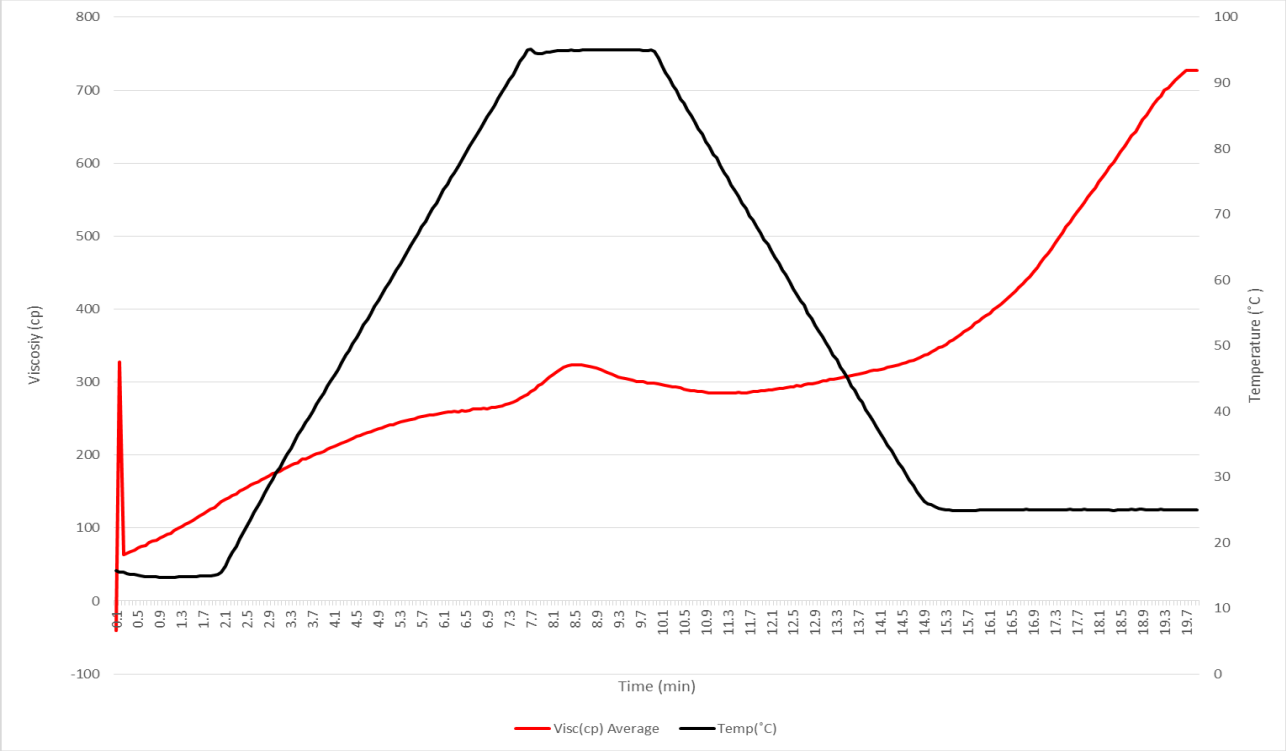

Figure 5.5: Rapid visco analyser heating-cooling cycle displaying the gelling and melting temperatures of the MXA-containing marshmallow sample.

During the heating stage, the MXA-containing marshmallow sample showed a gradual increase in viscosity, from 0.4 to 6.5 min, when a slight shoulder was formed at 40 ºC / 104 °F and 230cP. The viscosity increased for the next 1.5min, reaching a viscosity of 323.6 cP at 94 °C / 201.2 °F. During the holding stage at 95 ºC / 203 °F for about 2 min, the viscosity decreased to form a slight trough, with the lowest viscosity of 284.6 cP at 77 °C / 170.6 °F and 11.26 min. Upon further cooling to 25 ºC / 77 °F , there was an increase in viscosity to the end of the profile, with a viscosity of 727 cP at 20 min. This value was the highest for all the marshmallow samples in the study (Figures 5.4 and 5.5).

The first four minutes of the MXA-containing marshmallow profile followed the same pattern as that of xanthan-containing marshmallow image (Figure 5.4). Thereafter, the viscosity

increased steadily over the next 21 min, until a maximum viscosity of 727 cP was reached, which followed the trend shown by the agar (Figure 5.2) and MXA images (Figure 5.3). Only the albumin in the egg white foam could stabilise the sugar syrup around the air bubbles, with little aid from the weak gel that was formed by the combination of agar, xanthan and mucilage, which loosely bound the water molecules.

5.3.2 Differential Scanning Calorimeter (DSC)

Thermograms, energy axis enlarged inserts of selected thermograms, indicating onset and peak temperatures, as well as enthalpies of thermal events, are shown in Figure 5.6. Table 5.2 summarizes the data that could be extracted from these DSC's. Each thermal event presents a phase change of the sample studied, in particular the conversion of an isotropic liquid state (water with a substance dissolved into it) to a gel, determining the degree of conversion of gelation and gel time at gel point.

From a 100% mucilage stock solution, containing 6 mg sample dissolved in 14 mg of H_2O, 3.18, 5.92 and 9.99 mg samples were analysed. The thermograms obtained showed no thermal events (Figure 5.6a), indicating that no gelation occurred and no gel was formed.

A 100% agar stock (6 mg/14 mg water) solution was prepared. No observable thermal events were noted with 3.35 or 5.92 mg sample sizes, due to the low enthalpies involved. However, when 10.09 mg of solution was analysed, a weak thermal event was observed on the heating cycle at 55.83 °C / 132.49 °F (DSC not shown). By utilising 21.75 mg of sample of a 6 mg sample/10 ml water stock solution, clear thermal events were observed on the heating cycle, but no thermal event on the cooling cycle. The first thermal event was observed at an onset temperature of 35.73 °C / 96.31 °F and the second at 71.06 °C / 159.91 °F (Figure 5.6b and Table 5.2). The first exothermic peak, accompanying the gel-sol transition, appeared at 35 ºC / 95 °F and could be attributed to the increase in ordered regions through arrangement of molecular chains during heating. A second, smaller exothermic peak, accompanying the start of the sol-gel transition, commenced at about 71 ºC / 159.8 °F and was observed as the polymeric crystals started to melt, which accompanied a crystal transition, or recrystallisation or a chemical reaction. The enthalpy value for the second peak (-0.036 mJ/g) was much smaller than the value for the first peak (-0.26 mJ/g) (Table 5.2), indicating that less energy was released during the second peak, albeit both being very low. Melting usually occurs at 85 ºC /

185 ℉ for agar, while gel formation occurs at 32 to 39 ºC / 89.6 to 102.2 ℉ (Clark, *et al*., 1982), implying that at 71 ºC / 159.8 ℉ melting has only started.

For 100% xanthan, a stock solution of 6 mg/14 mg water was prepared. The best thermal results were obtained from a 9.02 mg sample, which exhibited thermal events in the heating cycle at onset temperatures of 81.98 °C and 87.45 °C/ 179.56 ℉ and 189.41 ℉ (Figure 5.6c and Table 5.2). The high temperature thermal event required an extrapolation estimate of the DSC curve to higher temperatures than 95 °C / 203 ℉, to obtain the phase change enthalpy (see Materials and Methods, section 5.2.5). Enthalpy values ranged from – 108.1 to -278.4 mJ/g (Table 5.2), indicating the energy that was released during these exothermic reactions. Eighty five degrees Celsius / 185 ℉ is a critical temperature for xanthan, as heat treatment at this temperature reduces the molecular weight of xanthan to half its initial value. Mass recovery measurements indicate that it completely overcomes its associative nature, leading to order-disorder transition (Fitzpatrick, *et al*., 2013).

Utilising a 19.56 mg sample of 100% gelatin, thermal analysis showed two relatively weak thermal events at onset temperatures of 55.04 °C and 74.50 °C / 131.07 ℉ and 166.1 ℉ on the heating cycle (Figure 5.6d and Table 5.2). At a temperature of 50 to 60 °C / 122 to 140 ℉, syneresis is in process, which is the release of moisture contained within the gelatin molecules, usually caused by excessive heat. Moisture inside expands upon heating and the sample is now in sol state and the macro molecular conformation has changed from helixes to coils (74 ºC / 165.2 ℉). The DSC results showed that samples with lower values of enthalpy (-7.11 mJ/g and -66.17 mJ/g) reflected lower degree of re-organisation (crystallinity), which is correlated with an amorphous structure.

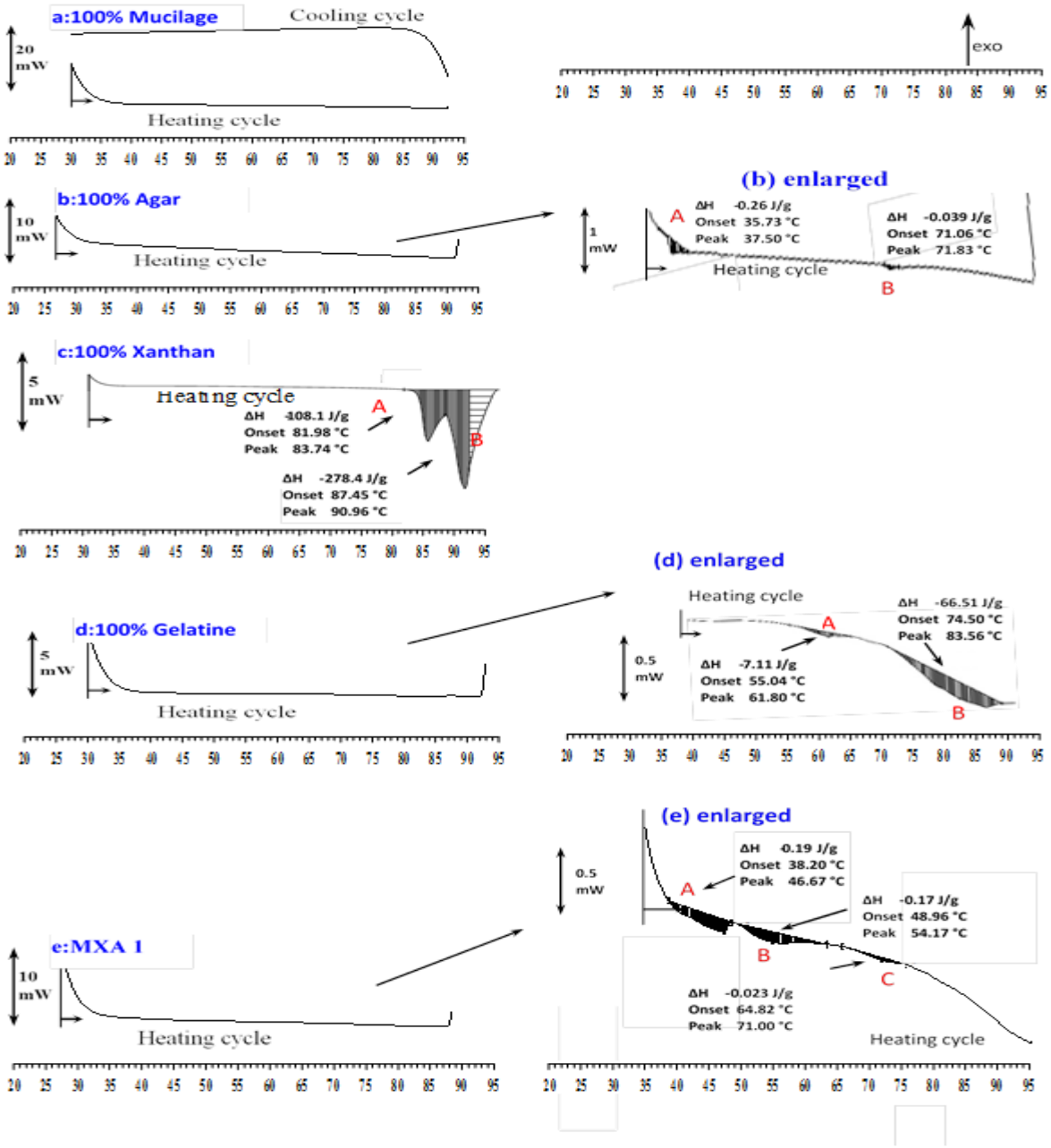

Figure 5.6: Thermograms, energy axis enlarged inserts of selected thermograms, indicating onset and peak temperatures, as well as enthalpies of thermal events for a) 100% mucilage, b) 100% agar; c) 100% xanthan; d) 100% gelatin and e) MXA.

Table 5.2: Thermal data of the thermal events on a heating cycle (10 °C min^{-1} / 50 °F min^{-1}) of the indicated samples.

Compound Thermal event	**[Stock solution] (mg/mg water); sample size (mg)**	**ΔH (mJ/g)**	**Onset Temp. (°C)**	**Peak Temp. (°C)**	**End Temp. (°C)**	**Event temp. range** (°C)**
100% Mucilage	14/6; 9.99					
No thermal event		-	-	-	-	-
100% Agar	6/10; 21.75					
Thermal event A*		-0.26	35.73	37.50	39.82	4.09
Thermal event B*		-0.036	71.06	71.83	73.61	2.55
100% Xanthan	6/14; 9.02					
Thermal event A*		-108.1	81.98	83.74	87.45	5.47
Thermal event B*		-278.4	87.45	90.96	98.72	11.27
100% Gelatin	6/10;19.56					
Thermal event A*		-7.11	55.04	61.80	67.21	12.17
Thermal event B*		-66.51	74.50	83.56	91.67	17.17
MXA	6/28; 19.85					
Thermal event A*		-0.19	38.20	46.67	47.98	9.78
Thermal event B*		-0.17	48.96	54.17	61.27	12.31
Thermal event C*		-0.023	64.82	71.00	74.08	9.26

*Thermal events labels A-C are used for identification purposes see also Figure 5.6.

**Event temperature range = End temperature– Onset temperature

Even for stock solutions consisting of 6.00 mg sample in 28.00 mg H_2O, it was visibly apparent that the MXA sample started to gel in the 40 µl aluminum pan before thermal analysis was possible. However, by initiating the DSC experiment as quickly as possible, after swiftly preparing a 6.00 mg sample in 28.00 mg H_2O stock solution, the DSC thermogram of a 19.85 mg sample of this partially gelled mixture still exhibited three thermal events on the heating cycle (Figure 5.6e and Table 5.2). The onset temperatures of these events were 38.20 °C / 100.76 °F, 48.96 °C / 120.13 °F and 64.82 °C / 148.68 °F, respectively. The low onset temperature of the first encountered thermal event on the heating cycle could be explained in that gelation started even before the DSC experiment could commence. The three peaks represent the formation of a weak gel, followed by a slight melting of the gel and another formation of a weak gel. As mentioned previously, agar molecules are relatively flexible, while xanthan molecules are comparably stiff. Agar is able to form gels by helices, as where xanthan undergoes jamming transition at the appropriate concentration. The gel formation of the agar chain then takes place in a restricted space left, since the jammed rod-like xanthan molecules hinder the free diffusion of the agar molecules. This results in a looser gel, with slightly lower modulus. With an increase in temperature, the existing gel starts to melt, while the third

hydrocolloid in the blend, mucilage, increases in viscosity. In the presence of water, hydrogen atoms are freed and the negatively charged groups along the chain are left open. The fact that the long mucilage molecule repels itself, uncoils and stretches out, results in a slight increase in the viscosity of the melted gel. Figure 5.7 is a schematic representation of the mechanism.

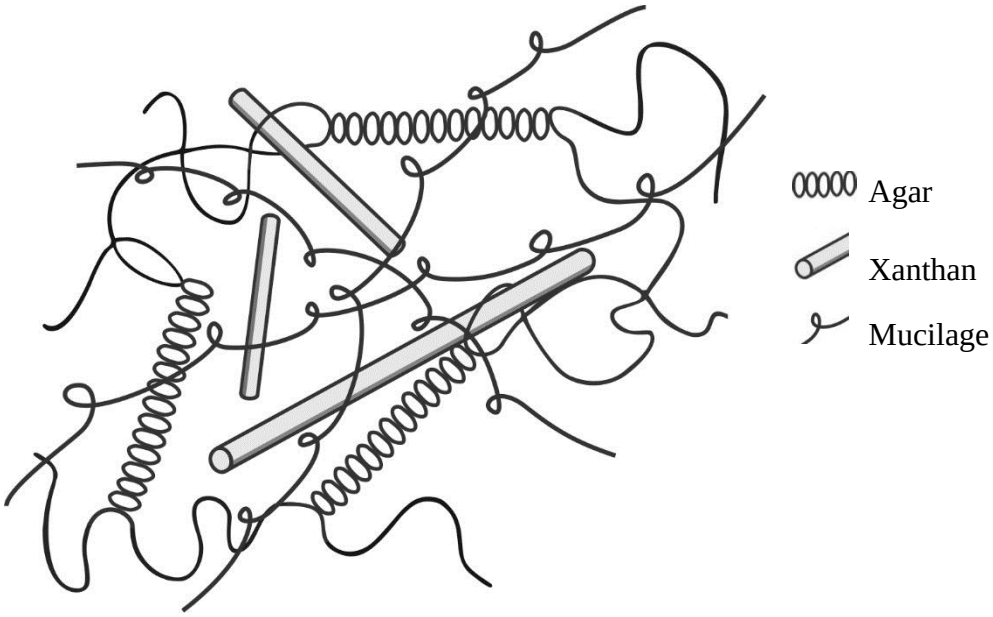

Figure 5.7: The interaction between xanthan (stiff rods), agar (flexible chains) and mucilage (long threads). Upon gelation, the jamming transition of the xanthan molecules prevents the aggregation of the helices - the gel remains softer. The long uncoiled mucilage molecules bind to this structure and increase viscosity slightly (adapted from Nordqvist and Vilgis, 2011).

5.3.3 Scanning electron microscopy (SEM)

Figure 5.8 is SEM images of the hydrocolloids samples. It should be noted that they are not at the same magnification, as images where chosen on the basis of structures visible. Gelatin is presented at magnification x80 in Figure 5.8a and reveals well-defined circular pores, with thin walls. The agar sample, at magnification x400 (Figure 5.8b), shows arrays of oblong-shaped interconnected hollow cells, separated by thin walls. The pores were not homogeneous and exhibit wider pore radii than those of gelatin, despite being at a high magnification. At magnification x80, Figure 5.8c shows the thin flaky layers of xanthan, with no visible pores / cells and no walls. The mucilage sample is shown in Figure 5.8d at x200 magnification, exhibiting layers with thin walls, amongst areas with thick-walled pores, varying in size, amongst branched sections. In Figure 5.8e, at magnification x80, the effect of xanthan can be clearly seen in the image of the MXA sample. The characteristic thin, flaky layers of xanthan

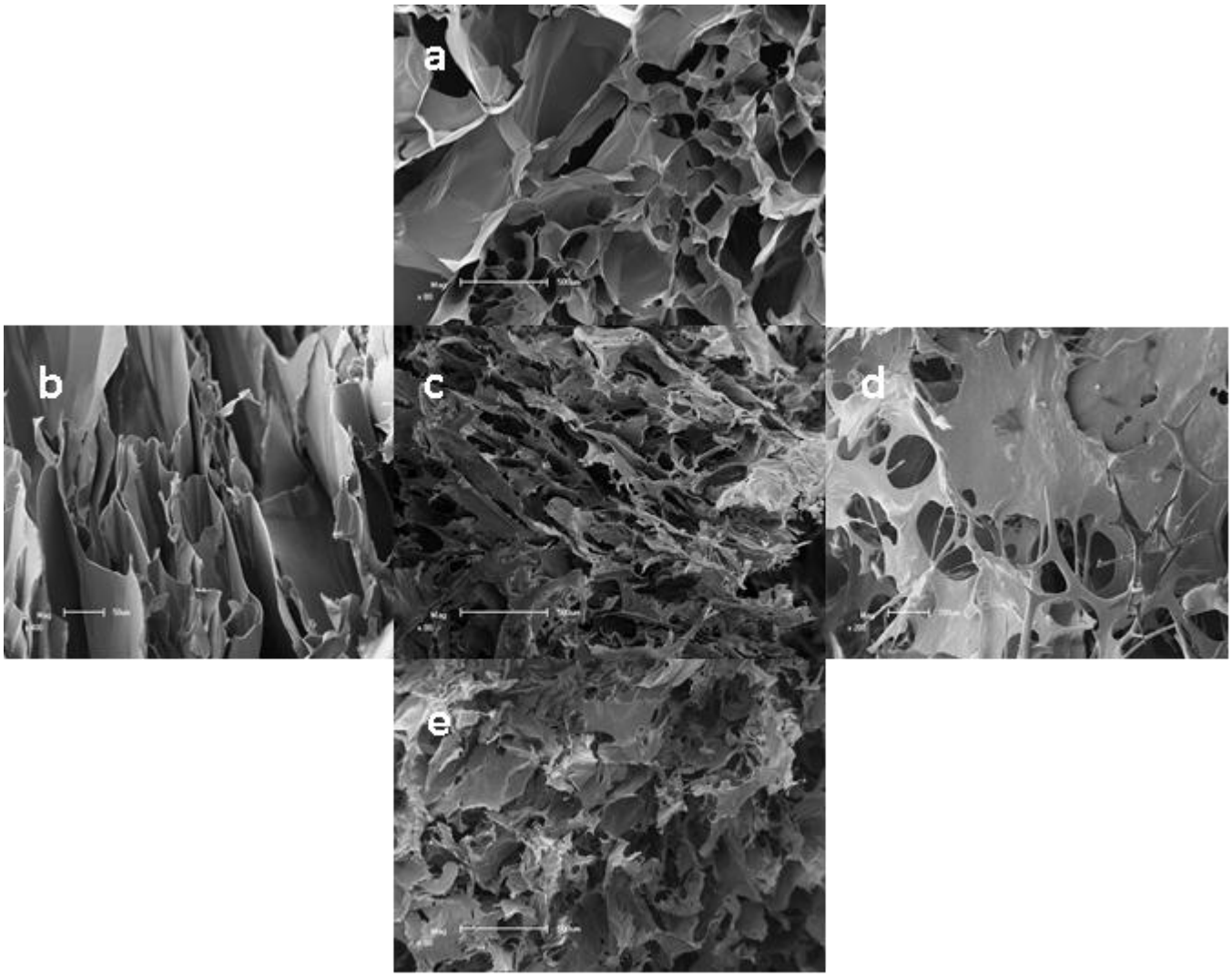

Figure 5.8: Scanning electron microscopy images of the hydrocolloids samples: a).gelatin x80; b) agar x400; c) xanthan x80; d) mucilage x200; and MXA x80.

were visible and arranged in a disorderly fashion, displaying a far lower degree of network than in the case of agar. The branching and large pores associated with the mucilage were no longer visible.

Figure 5.9 includes an array of SEM images from the marshmallow samples: Figures 5.9a-d are the microstructures of the control marshmallows, containing gelatin. All the images show the homogenous structure of the mixture, without any crystals, because gelatin is known for controlling crystal growth of sugar. Marshmallows consist of gas (air) incorporated into a sugar mixture (supersaturated sugar syrup), which is stabilised by proteins (albumin in egg

white foam and collagen in gelatin) before the air can escape. When air is introduced into the system, tiny bubbles are created (Christian and Vaclavik, 1996).

All the other images clearly show the formation of sugar crystals, in various degrees of size. Figure 5.9e-h is the microstructures of agar, used as gelling agent in marshmallows. From the x80 magnification a granular structure with large dark holes can be seen. As the magnification increases to x600, the presence of sugar crystals can be detected, suspended in a thin dispersion medium. Figure 5.9i-l displays the same tendency with xanthan as gelling agent. In this case the sugar crystals were the same size as for agar, again suspended in a thin dispersion medium. For the mucilage marshmallow sample (Figure 5.9m-p), the sugar crystals were substantially bigger, but they were still suspended in a thin dispersion medium. The MXA sample appeared to have smaller crystals, partially suspended in a dispersion medium. There were also clear zones apparent, only consisting of the dispersion medium, seeming to have the appearance of the gelatin-containing sample in Figure 5.9a-d.

As a whipping agent, gelatin binds large amounts of water into marshmallows. However, even marshmallows made with gelatin can undergo changes, due to sucrose crystallisation, cross-linking of gelatin molecules, and glassy formation of sucrose and gelatin during processing, resulting in crystallised, "grained" marshmallow products. This explains the crystals that were seen in the micrographs of the agar, xanthan, mucilage and MXA-containing marshmallows. Agar is known to form very rigid gels at high concentrations and in the case of the marshmallows, a concentration of 2.3% was used, enough to absorb all the liquid from the sugar syrup, transforming the super saturated sugar syrup into crystals in the final product. In the case of xanthan and mucilage, no true gel could be formed and the liquid leached out, leaving behind sugar crystals of varying sizes. The pH of mucilage is 4.5 (Du Toit, 2017), which aids in the formation of large sugar crystals. With the addition of agar in the MXA sample, a very weak gel was formed, again not being able to bind the water. On losing the water, sugar crystals were formed, resulting in a grainy texture and seepage of liquid.

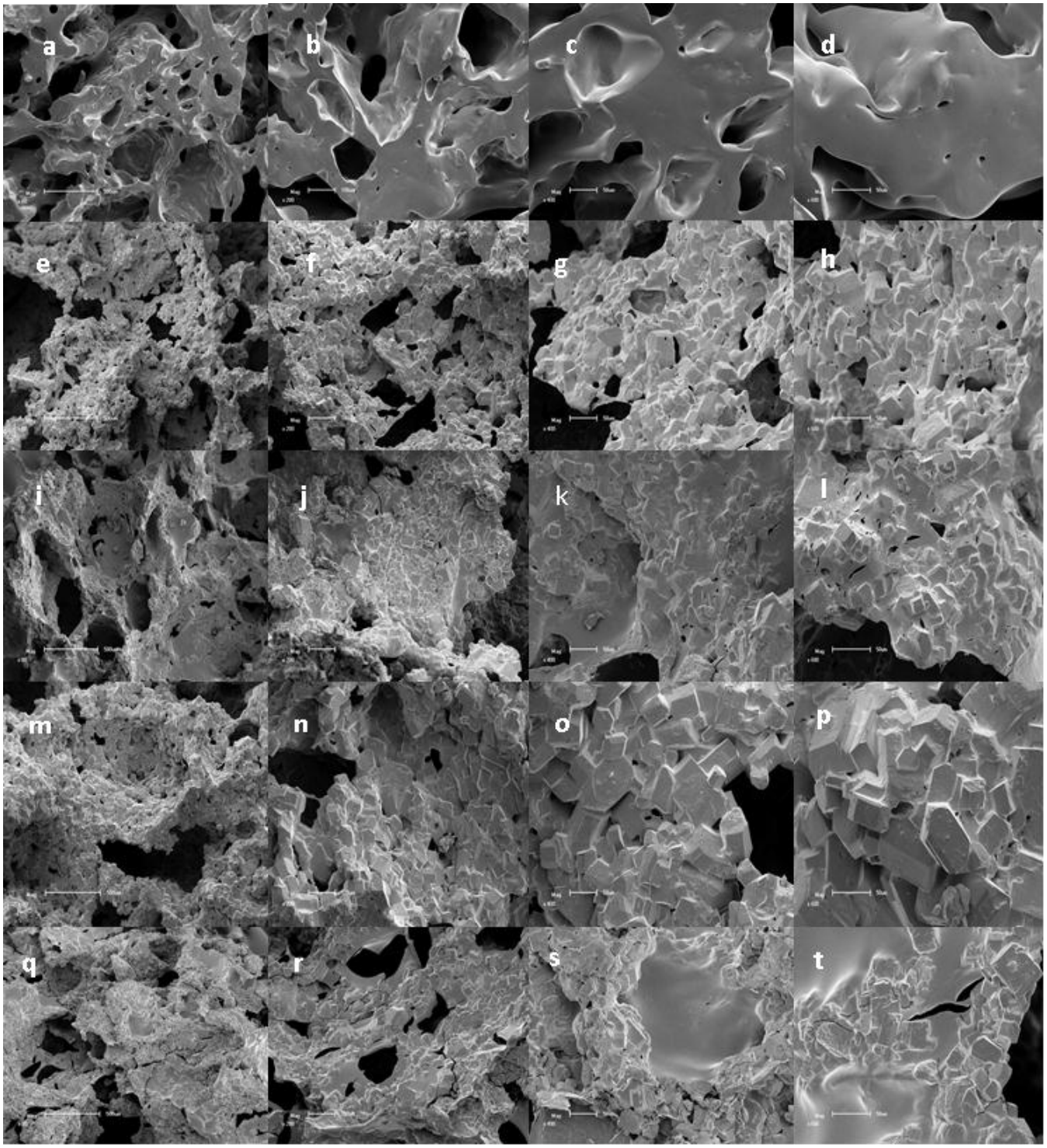

Figure 5.9: Scanning electron microscopy images of the marshmallow samples: a) gelatin x80; b) gelatin x200; c) gelatin x400; d) gelatin x600; e) agar x80; f) agar x200; g) agar x400; h) agar x600; i) xanthan x80; j) xanthan x200; k) xanthan x400; l) xanthan x600; m) mucilage x80; n) mucilage x200; o) mucilage x400; p) mucilage x600; q) MXA x80; r) MXA x200; s) MXA x400; and t) MXA x600.

5.3.4 Light photography

Figure 5.10 represents light photography photographs of freeze-dried hydrocolloid samples. Gelatin (figure 5.10a) has a shiny appearance, and looks like plastic fibers. Figure 5.10b represent the agar sample. It is more yellow in colour than gelatin, but displays the same shinyness and plastic fibre appearance. The xanthan sample (figure 5.10c) has the appearance of a cottonwool bud when it is peeled apart. The mucilage sample (figure 5.10d) has the distinctive greenish colour that was discussed in chapter 3. It also has a shiny appearance, almost like cottoncandy. The MXA sample (figure 5.10 e) is a good repersentation of the three hydrocolloids that it is made up of. It is darker in colour than gelatin, agar and xanthan and has the plastic fiber appearance of agar and the greenish hue of mucilage.

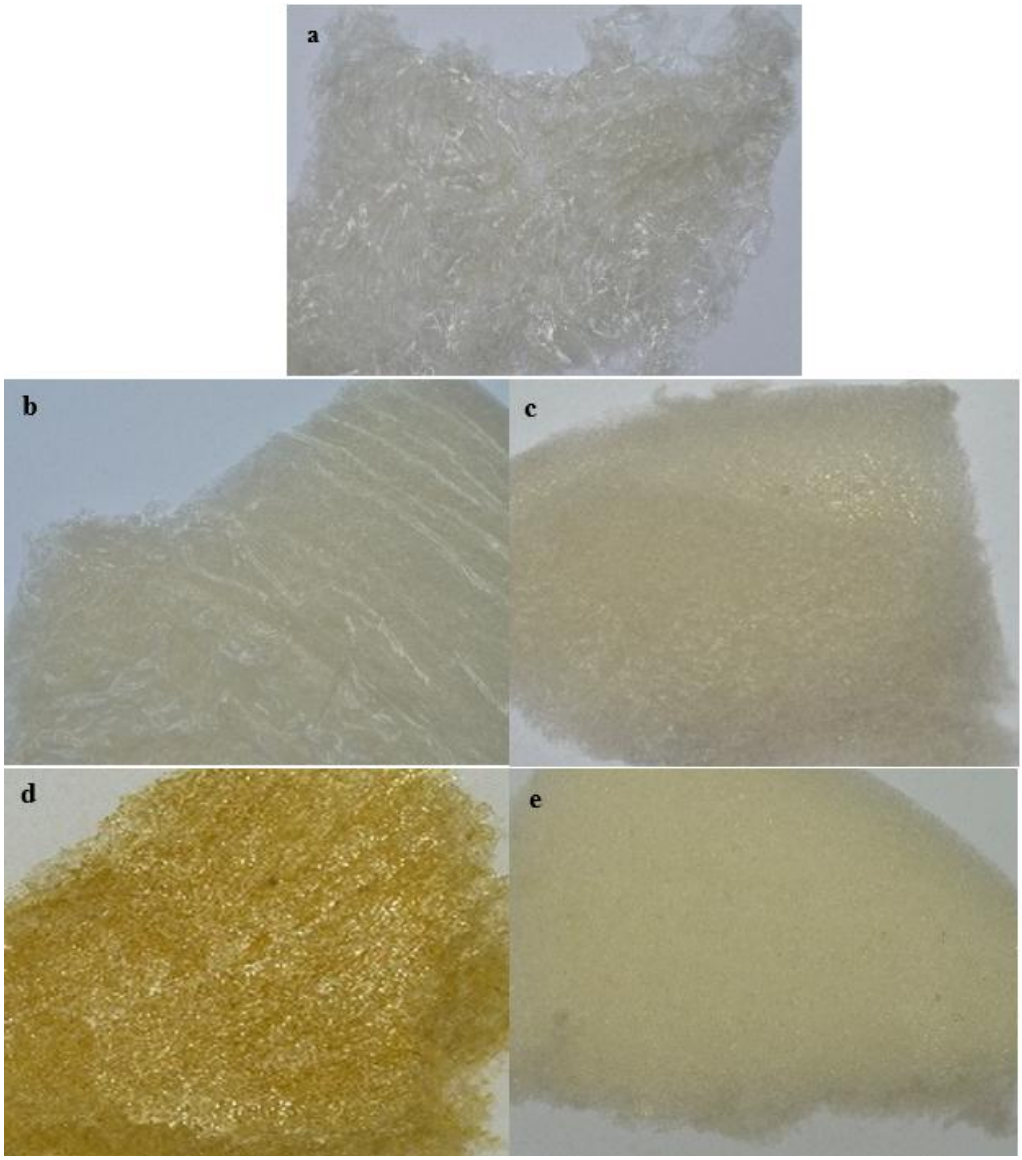

Figure 5.10: Photo images of freeze-dried hydrocolloid samples: a) gelatin; b) agar; c) xanthan; d) mucilage; e) MXA

Figure 5.11, represents light photography photographs of freeze-dried hydrocolloid fiber samples. In the photograph for the gelatin fiber sample (Figure 5.11a), the shiny texture of the fibres, as well as the plastic appearance were visible. Figure 5.11b represents the agar fiber sample, appearing like yellow silk fibres. The xanthan fibres (Figure 5.11c) resembled a bud of cotton wool that has been torn appart, being fluffy with an off-white colour. The mucilage fibres (Figure 5.11d) had a flaky appearance and a very fine structure. The MXA fiber sample (Figure 5.11e) was a good representation of its combined hydrocolloids: a fluffy and flaky appearance with a yellowish hue.

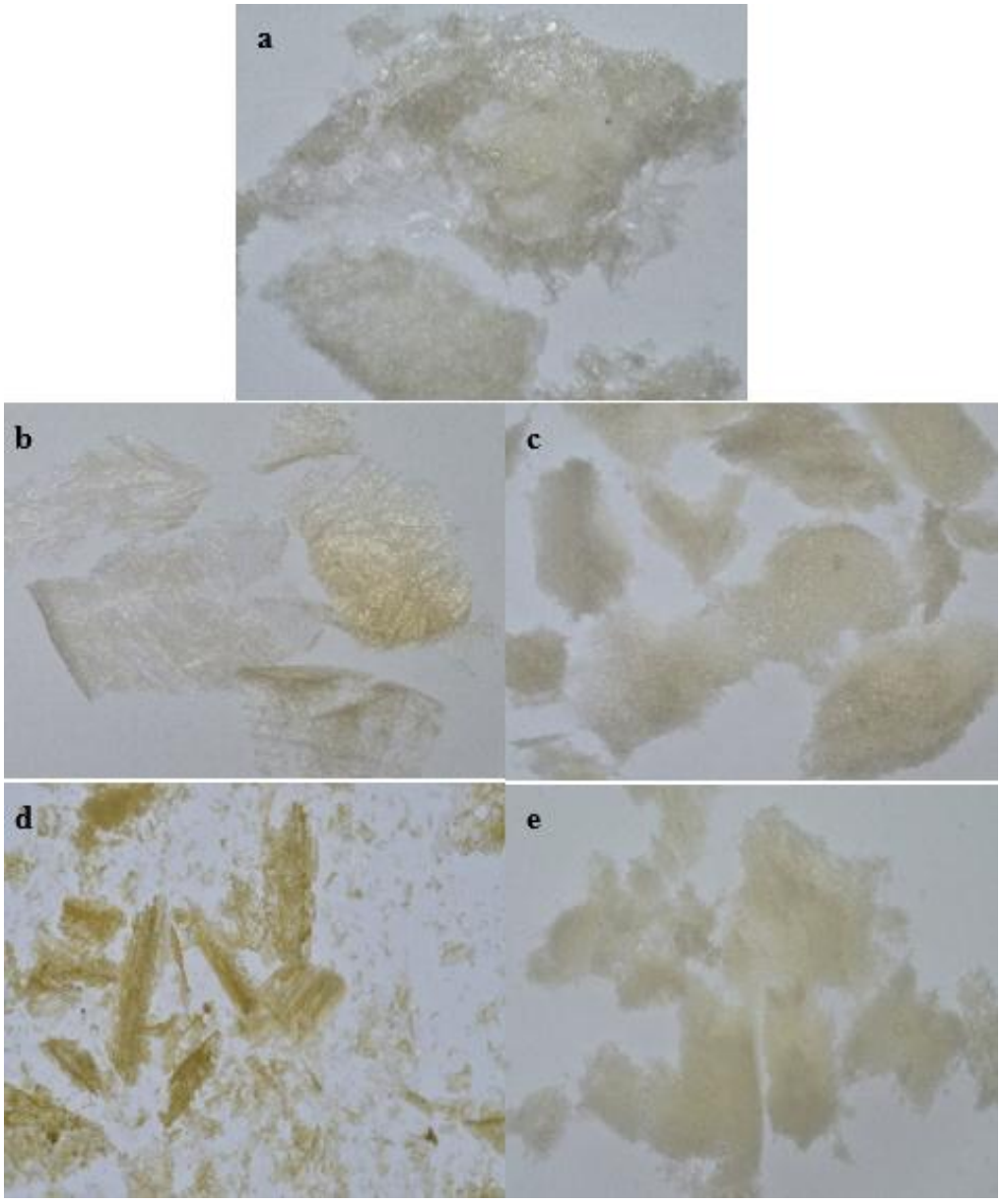

Figure 5.11: Photographs of freeze-dried hydrocolloid fiber samples: a) gelatin; b) agar; c) xanthan; d) mucilage; e) MXA

5.4 Conclusions

It was possible to determine, by means of various techniques, such as RVA, DSC and SEM whether mucilage, alone or in combination with other hydrocolloids, could be used successfully in the making of high sugar and high protein aerated products, such as marshmallows. The mucilage-containing marshmallow sample showed no viscosity during runs on the RVA, while the MXA-containing marshmallow reached a final viscosity, upon cooling, of 730 cP. No thermal events were noted on the DSC for 100% mucilage, while the MXA sample showed very weak exothermic events during the heating cycle, suggesting the formation of a weak gel. Scanning electron micrographs of 100 % mucilage showed irregular large pores with branching and thick walls, while the images of the 100% MXA had a flaky appearance, characteristic of the xanthan part of the combination. Images of the mucilage-containing marshmallows showed huge sugar crystals, thinly covered with a continuous phase, while the MXA-containing marshmallow had fewer sugar crystals, with areas of smooth continuous phase.

It is concluded that mucilage on its own cannot be used successfully in the making of high sugar and high protein aerated products, such as marshmallows. Some success was achieved when mucilage was combined with xanthan and agar; however, this combination could not completely prevent leeching out of moisture and the formation of sugar crystals.

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CHAPTER 6

CONCLUSIONS

Many of foods' functional characteristics depend on their macromolecular composition. The ability that these macromolecules possess in being part of the structural function of foods is known as functional property, and it is expressed among others as gelation capacity, viscosity modification, and stabilisation of suspensions, emulsification, and ability to retain water (Medina-Torres *et al*., 2002). In the food industry hydrocolloids are often used as pure compounds or as mixtures of different compounds. The latter is motivated by a constant search for new functional properties in the final product. In recent years, the use of mixtures of hydrocolloids has opened the possibility of obtaining a wide range of textures and rheological properties (BeMiller, 2014). The recently studied gum, or mucilage from *O. ficus-indica*, displays elastic properties, but it does not form a gel. A mixture, including this hydrocolloid, may result in interesting properties from a functional point of view.

Marshmallows were selected as the potential vehicle for the incorporation of cactus pear mucilage in different formulations, using a variety of hydrocolloids.

The first aim of this study was to replace gelatin in marshmallows with different formulations of liquid mucilage, in combination with different concentrations of powdered hydrocolloids, to see if gelatin could be substituted with mucilage or mucilage-blend. The MXA sample, containing 75% mucilage, 12.5% xanthan, and 12.5% agar, obtained the best results for physical parameters such as consistency, texture and tenderness of gel. It only differed significantly ($p<0.05$) from the control (100% gelatin) sample in regard to shear, as measured by the Warner Bratzler Shear. It was significantly ($p<0.05$) less tender and resembled the shear of commercially available marshmallows in SA. All samples had a light, greyish yellow colour. Marshmallows containing mucilage, in combination with agar and xanthan, might also be a good alternative for similar products, where only one hydrocolloid, e.g. agar is used as a gelling agent, resulting in a very tough texture. Furthermore, gelatin-less marshmallows might also open up a market which was restricted by the use of gelatin, namely for Halaal and Kosher consumers.

The second aim was to determine the sensory acceptability of the mucilage / mucilage-blend marshmallows and to compare it to the liking of commercially available marshmallows. Ninety-two consumers tasted the following six samples: white commercial (*Manhattan*); white control (gelatin); white mucilage (75% mucilage + 12.5% agar + 12.5% xanthan); pink commercial (*Manhattan*); pink control (gelatin); and pink mucilage (75% mucilage + 12.5% agar + 12.5% xanthan). The white mucilage marshmallows had the lowest ranking for taste, aftertaste, texture and overall acceptability, and differed significantly ($p<0.05$) from all the other samples. What is significant is that the pink mucilage marshmallow did not differ from either the pink commercial (which had the highest rankings for taste, aftertaste, texture, and overall acceptability) or pink control marshmallow. The differences between the white and pink mucilage marshmallows ranged between 2.75 and 2.89 on the hedonic scale. The use of a flavouring and colouring masked the distinctive aroma of the mucilage in the marshmallows, thereby also increasing scores for texture and overall acceptability.

Up to this part of the study liquid mucilage was used. However, it has been found that the quality and quantity of the mucilage are influenced by climate, temperature and rainfall. To bring it on the same level as the other hydrocolloids used in the study, liquid mucilage was dried and ground to a powder form.

The third aim was to determine the effect of mucilage / mucilage-blend on the formation of the various food systems in the making of marshmallows. To do this, sophisticated techniques, such as RVA, DSC and SEM were used to detect whether gelation did in fact occur and what the structure of the gel, if formed, would look like. The mucilage-containing marshmallow sample registered almost no viscosity values during runs on the RVA, while the MXA-containing marshmallow reached a final viscosity, upon cooling, of 730 cP. No thermal events were noted on the DSC for 100% mucilage, which confirmed the previous result, as no gel was formed. The MXA sample showed very weak exothermic events during the heating cycle, suggesting the formation of a weak gel, which was in accordance of other results noted during this study. Scanning electron micrographs of 100 % mucilage showed irregular large pores with branching and thick walls, while the images of the 100% MXA had a flaky appearance, characteristic of the xanthan part of the combination. Images of the mucilage-containing marshmallows showed huge sugar crystals, thinly covered with a continuous phase, while the MXA-containing marshmallow had fewer sugar crystals, with areas of smooth continuous phase.

It was concluded that mucilage on its own could not be used successfully in the making of high sugar and high protein aerated products, such as marshmallows. Some success was achieved when mucilage was combined with xanthan and agar; however, this combination could not completely prevent leeching out of moisture and the formation of sugar crystals, over a relatively short period of time. Thus, this combination would not be able to guarantee a shelf life what so ever. A possible solution to the problem would be the addition of additional proteins, such as soy, to increase the strength of the three-dimensional network to hold the sugar syrup intact. It is doubtful if any of the other hydrocolloids would be able to exert a synergistic effect in a mucilage blend, as the fundamental problem is the way in which mucilage behaves under heated conditions, making it impossible to create a three-dimensional network.

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CHAPTER 7

SUMMARY

Mucilage from the cladodes of *O. ficus-indica* is under investigation for application in several foodstuffs. The first objective was to replace gelatin in marshmallows with different concentrations of fluid mucilage, combined with different concentrations of powdered hydrocolloids. Nine different formulations were prepared: control (100% gelatin); 75% mucilage + 25% xanthan (MX); 75% mucilage + 25% agar (MA); 75% mucilage + 25% guar (MG); 80% mucilage + 20% xanthan (8M2X); 80% mucilage + 20% agar (8M2A); 80% mucilage + 20% guar (8M2G); 75% mucilage + 12.5% xanthan + 12.5% agar (MXA); 75% mucilage + 12.5% xanthan + 12.5% guar (MXG); and 75% mucilage + 12.5% agar + 12.5% guar (MAG). Consistency, texture, tenderness of gel and shear measurements were determined, along with colour (L* values, as well as C* and H^o values) and a_w. There were significant ($p<0.05$) differences between the different samples for all measurements. The best formulation for gelatin replacement was found to be the MXA combination, as it only differed significantly ($p<0.05$) from the control sample in regard to shear. It was significantly ($p<0.05$) less tender and resembled the shear of commercially available marshmallows in South Africa. All samples had a light, greyish yellow colour.

The second aim was to compare consumer liking of flavoured and unflavoured marshmallows made with liquid mucilage, to that of a flavoured and unflavoured control sample (with 100 % gelatin), as well as a flavoured and unflavoured commercial brand. Ninety-two consumers tasted the following six samples: white commercial (Manhattan); white control (100 % gelatin); white MXA; pink commercial (Manhattan); pink control (100% gelatin); and pink MXA. The white MXA marshmallows had the lowest ranking for taste, aftertaste, texture and overall acceptability, and differed significantly ($p<0.05$) from all the other samples. The pink MXA marshmallow did not differ from the pink Manhattan (which had the highest rankings for taste, aftertaste, texture, and overall acceptability) and pink control marshmallows. The differences between the white and pink MXA marshmallows ranged between 2.75 and 2.89 on the nine point hedonic scale. Flavouring successfully masked the distinctive aroma of the mucilage in the marshmallows, thereby also increasing scores for texture and overall acceptability.

Rapid visco analyser, differential scanning calorimetry and scanning electron microscopy studies were done on gelatin, mucilage (dry), xanthan, agar and MXA marshmallow samples, as well as individual hydrocolloid samples. Rapid visco analyser profiles showed very high viscosities for xanthan (< 15 000 cP) and mucilage (> 20 000 cP) samples, in contrast to gelatin (± 5 000 cP) and MXA (< 5 000 cP) samples. The addition of ingredients, such as sugar and egg whites, resulted in lowering of the general RVA viscosity, from ± 20 000 cP for mucilage-containing to 730 cP for MXA-containing marshmallows. Xanthan- and MXA-containing marshmallows had final viscosities of 237 cP and 727 cP, respectively, while almost no viscosity was measured for mucilage-containing marshmallows. A 100% mucilage sample showed no thermal events during DSC, indicating that no significant gelation occurred and no gel was formed. The four other samples showed very weak exothermal events, indicating that some phase change took place; in some cases gels were even formed before samples were placed in the DSC. For the hydrocolloid samples, gelatin and agar showed characteristic scaffolding on SEM micrographs, while xanthan appeared flaky. Mucilage had irregular pores, with thick walls and branching. Images showed sugar crystals suspended in the continuous phase for agar-, xanthan-, mucilage- and MXA-containing marshmallow samples; the crystals were the biggest for the mucilage-containing sample, while the MXA–containing sample also had clear zones like the gelatin-containing sample.

Keywords: mucilage; gel formation; texture; consumer acceptability; rapid visco analyser; differential scanning calorimetry; scanning electron microscopy

OPSOMMING

Slymgom uit die blaaie van *Opuntia ficus-indica* word bestudeer vir moontlike toepassings in verskeie voedselsoorte. Die eerste doelstelling was om gelatien in malvalekkers te vervang met verskillende konsentrasies vloeibare slymgom, in kombinasie met verskillende konsentrasies hidrokolloïedpoeiers. Nege verskillende formulasies is voorberei: kontrole (100% gelatien); 75% slym + 25% xanthan (MX); 75% slym + 25% agar (MA); 75% slym + 25% guar (MG); 80% slym + 20% xanthan (8M2X); 80% slym + 20% agar (8M2A); 80% slym + 20% guar (8M2G); 75% slym + 12.5% xanthan + 12.5% agar (MXA); 75% slym + 12.5% xanthan + 12.5% guar (MXG); en 75% slym + 12.5% agar + 12.5% guar (MAG). Konsistensie-, tekstuur-, sagtheid van gel en taaiheidsmetings is bepaal, asook kleur (L*-waardes, asook C*-en Hº-waardes) and a_w. Daar was betekenisvolle ($p<0.05$) verskille tussen die monsters, vir al die bepalings. Die beste formulasie vir gelatien-vervanging was die MXA-kombinasie, want dit het net betekenisvol ($p<0.05$) verskil van die kontrole ten opsigte van taaiheid. Dit was betekenisvol ($p<0.05$) sagter en het ooreengestem met die taaiheid van kommersieel-beskikbare malvalekkers in Suid Afrika. Al die monsters het ‘n ligte grysgeel kleur gehad.

Die tweede doelstelling was om die verbruikers se ‘hou van’ gegeurde en ongegeurde malvalekkers, wat slym bevat, te vergelyk met ‘n gegeurde en ongegeurde kontrole monster (met 100% gelatien), asook ‘n gegeurde en ongegeurde kommersiële handelsmerk. Twee-en-negentig verbruikers het die volgende ses monsters geproe: wit kommersieel (*Manhattan*); wit kontrole (gelatien); wit slym (75% slym + 12.5% agar + 12.5% xanthan); pienk kommersieel (*Manhattan*); pienk kontrole (gelatien); and pienk slym (75% slym + 12.5% agar + 12.5% xanthan). Die wit slymbevattende malvalekkers het die laagste posisie gehad vir smaak, nasmaak, tekstuur en algehele aanvaarbaarheid en het betekenisvol ($p<0.05$) verskil van al die ander monsters. Die pienk slym-bevattende malvalekker het nie verskil van die pienk kommersiële (wat die hoogste posisie gehad het vir smaak, nasmaak, tekstuur en algehele aanvaarbaarheid) en pienk kontrole malvalekkers nie. Die verskille tussen die wit en pienk slym-bevattende malvalekkers het gewissel van 2.75 en 2.89 op die hedoniese skaal. Geurbyvoeging het die kenmerkende reuk van die slym in die malvalekkers suksesvol gemasker en sodoende ook die posisies vir tekstuur en algehele aanvaarbaarheid verhoog.

Rapid visco analyser, *differential scanning calorimetry* en *scanning electron microscopy* studies is gedoen op gelatien, slym (droog), xanthan, agar and MXA malvalekker monsters,

sowel as individuele hidrokolloïed monsters. Rapid visco analyser profiele het baie hoë viskositeite vir xanthan (< 15 000 cP) en slym (> 20 000 cP) monsters gewys, in teenstelling met gelatien (± 5 000 cP) en MXA (< 5 000 cP) monsters. Die byvoeging van bestanddele, soos suiker en eierwitte, het gelei tot verlaging in algemene RVA viskositeit, van ± 20 000 cP vir die slym-bevattende tot 730 cP vir die MXA-bevattende malvalekkers. Xanthan-en MXA-bevattende malvalekkers het finale viskositeite van onderskeidelik 237 cP en 727 cP gehad, terwyl feitlik geen viskositeit gemeet is vir die slym-bevattende malvalekkers nie. ‘n 100% slym-monster het geen termiese gebeurtenis gewys tydens DSC nie, wat daarop dui dat geen betekenisvolle gelvorming plaasgevind het nie. Die ander vier monsters het baie swak eksotermiese gebeurtenisse getoon, wat ‘n aanduiding is dat veranderinge wel plaasgevind het; in sekere gevalle het gels gevorm selfs voordat die monsters in die DSC geplaas is. Vir die hidrokolloïed monsters, het gelatien en agar kenmerkende ‘stellasies’ getoon op die SEM mikrograwe, terwyl xanthan vlokkerig vertoon het. Slym het oneweredige porieë, met dik wande en vertakking. Mikrograwe het ook suikerkristalle gewys vir agar-, xanthan-, slym- en MXA-bevattende malvalekkers, wat gesuspendeer is die aaneenlopende fase. Die suikerkristalle in die slym-bevattende monster was die grootste, terwyl die MXA–bevattende monster ook gladde sones gehad het, soos die gelatien-bevattende monster.

Sleutelwoorde: slym; gelvorming; tekstuur; vebruikers-aanvaarbaarheid; *rapid visco analyser*; *differential scanning calorimetry; scanning electron microscopy*

ANNEXTURE 1

Replacement of gelatin with liquid *Opuntia ficus-indica* mucilage in marshmallows. Part 1: Physical parameters

L. Du Toit, C. Bothma, M. De Wit*, and A. Hugo

Department of Microbial, Biochemical and Food Biotechnology, University of the Free State, PO Box 339, Bloemfontein, 9300, South Africa.

*Corresponding author, email: dewitm@ufs.ac.za

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ABSTRACT

The objective of this study was to replace gelatin in marshmallows with different concentrations of fluid mucilage, combined with different concentrations of powdered hydrocolloids. Nine different formulations were prepared: control (100% gelatin); 75% mucilage + 25% xanthan (MX); 75% mucilage + 25% agar (MA); 75% mucilage + 25% guar (MG); 80% mucilage + 20% xanthan (8M2X); 80% mucilage + 20% agar (8M2A); 80% mucilage + 20% guar (8M2G); 75% mucilage + 12.5% xanthan + 12.5% agar (MXA); 75% mucilage + 12.5% xanthan + 12.5% guar (MXG); and 75% mucilage + 12.5% agar + 12.5% guar (MAG). Consistency, texture, tenderness of gel and shear measurements were determined, along with color (L* values, as well as C* and H° values) and a_w. There were significant ($p<0.05$) differences between the different samples for all measurements. The best formulation for gelatin replacement was found to be the 75% mucilage + 12.5% xanthan + 12.5% agar combination (MXA), as it only differed significantly ($p<0.05$) from the control (100% gelatin) sample in regard to shear, as measured by the Warner Bratzler Shear. It was significantly ($p<0.05$) less tender and resembled the shear of commercially available marshmallows in South Africa. All samples had a light, greyish yellow color.

***Keywords*:** mucilage; xanthan; agar-agar; gelatin; marshmallows; *Opuntia ficus-indica*

INTRODUCTION

Marshmallows are one of the earliest confections known to man (Olver, 2000) and were originally made from the root sap of the marshmallow (*Althaea officinalis)* plant. The first marshmallows were made by boiling pieces of the marshmallow root pulp with sugar until it thickened, whereafter the mixture was strained and left to cool. In 2000 B.C., Egyptians combined the marshmallow root with honey, and made a candy which was reserved for gods and royalty (Groves, 1995; Olver, 2000).

Modern marshmallow confections were first made in France around 1850 when marshmallows were cast and molded individually. When mass production became possible, by 1900,

marshmallows were made by implementing the starch mogul system. By 1955, Alex Doumak of Doumak, Inc. patented a new manufacturing method, called the extrusion process, which changed the history of marshmallow production and is still used today (Groves, 1995).

Marshmallows are made by using sweeteners and emulsifying agents. Proportionally, there is more corn syrup than sugar, because it increases the ability of the sugar to dissolve and retards crystallization. Corn starch, modified food starch, water, hydrocolloids, gelatin, and/or whipped egg whites are used in various formulations, resulting in a specific texture. Hydrocolloids act as emulsifiers in marshmallows and provide the aeration that makes marshmallows puffy; however, they also act as gelling agents. Most marshmallows also contain natural and/or artificial flavoring (Groves, 1991; Olver, 2000).

Opuntia spp. is a genus of < 200 species of readily recognizable cacti, growing from small ground-hugging plants to massive shrubs. The fiber content has the capacity to absorb large amounts of water, forming viscous or gelatinous colloids and absorbing organic molecules (Saenz *et al.*, 2004; Anderson, 2008).

The Cactaceae family, of which *O. ficus-indica* is a part, is characterized by the production of slime, which mainly consists of mucilage. When cutting through a cladode, mucilage is the soluble fiber found in the thick slimy fluid, where they are stored in cells, commonly referred to as "nopal dribbles". Thus, these complex hydrophilic polysaccharide carbohydrates molecules, with a highly branched structure and containing varying proportions of L-arabinose, D-galactose, L-rhamnose, and D-xylose, as well as galacturonic acid, may not only bind water as they increase in volume, but also sugars and fats, which are caught within the matrix of mucilage and water (Goycoolea and Cardenas., 2003; Saenz *et al.*, 2004; Ramirez-Tobias *et al*., 2012).

Mucilage is, therefore, commonly referred to as a gum or hydrocolloid. Hydrocolloids are water-soluble dietary fiber that can be used as healthy additives for profitable food products. None of the mucilage found in cladodes is hydrolyzed or absorbed by the human digestive system, but they can make up alimentary fiber (Sepulveda *et al.*, 2007; Osuna-Martinez *et al*., 2014).

Mucilage has a strong emulsifying capability in that it reduces surface interfacial tensions, stabilizes oil-in-water emulsions and does not contribute to the viscosity of the system. Its stabilizing properties are similar to those of xanthan and guar gums. It can also form part of gelled dessert and powdered drinks, to be reconstituted with water or milk (Bensadon *et al*., 2010).

Another possible function for mucilage could be as a fat mimic, as it has the required tendency to increase the viscosity of the water phase. With increased concentration, it will exhibit gelling capabilities. Mucilage can, therefore, be used to emulsify systems, but as it is not a true emulsifier, processing costs would be reduced, while providing the final product with a natural and healthy ingredient as a replacement for fat (Bensadon *et al.,* 2010; Milani and Maleki, 2012).

Today, new facts at the molecular level of chemistry, as well as on functional properties, allow improved knowledge on the selection of a suitable hydrocolloid for a specific product. Cactus

mucilage definitely has the advantages of being natural, healthy and cheap. However, despite having potential uses, it is not yet an industrial hydrocolloid (Saenz *et al.*, 2004).

The aim of this study was to replace gelatin in marshmallows with different concentrations of liquid mucilage, in combination with different concentrations of powdered hydrocolloids. Various physical parameters, such as consistency, percentage sag, shear, color and water activity (a_w), were determined.

MATERIALS AND METHODS

Extraction of liquid mucilage

Mucilage, from the *Opuntia* cultivar Algerian, was extracted according to the patented method of Du Toit and De Wit (2011). The cladodes (one-year-old with an average mass of ± 700 g, delivering a wet mucilage yield of ± 34% per cladode) were scrubbed and disinfected with chlorinated water to lengthen the shelf life period of the mucilage, and to eliminate impurities and thorns present. The green outside peel was removed with a vegetable peeler. The cladodes were then sliced into squares and cooked in a microwave oven (900 W) for 3 min at 100% power, until soft. The cooked, soft cladode pieces were pulverized in a food processor (300W) to break the skin, which aids in the extraction of the mucilage. The pulp was centrifuged in a Beckman centrifuge, model J2-21, (Beckman Coulter Inc. 2505 Kraemer Blvd. Brea, CA 92817 USA) for 15 min at 8000 rpm, to separate the liquid mucilage from the solids.

Preparation of marshmallows

The formulation for the marshmallows that were used as the control sample is given in Table 1. The gelatin was soaked in cold water. The sugar, salt, syrup and the remaining water were heated and stirred to dissolve the sugar. After the mixture reached a temperature of 118 °C, the gelatin was added. The stiffly beaten egg whites were added to the hot mixture, whilst continuously mixing it until thick and creamy. The mixture was then poured into a greased pan, cooled overnight and cut into 25-mm squares. The squares were rolled in a mixture of corn flour and icing sugar, and stored in an airtight container (Foods and Cookery, 1991).

Table 1. Formulation of control marshmallow (Foods and Cookery, 1991).

Ingredient	Percentage (%)
Gelatin	2.30
Water cold, to hydrate gelatin	11.52
Sugar	46.09
Water	28.81
Egg whites	9.22
Syrup	1.93
Salt	0.13

Replacement of gelatin with liquid mucilage and powdered hydrocolloids

Nine different formulations were prepared according to Table 2. The three hydrocolloids that

were chosen to be used in combination with the fluid mucilage, to replace gelatin in the formulation, were gum guar, agar, and xanthan. Guar was chosen because of the synergistic effect that it displayed when used in combination with xanthan (Pangborn, *et al*. 1978). According to Bensadón *et al*. (2010), the stabilizing properties of mucilage are similar to those of xanthan and guar gums. Agar was also chosen, because it is commercially used in South Africa in chocolate candies to stabilize the marshmallow layer, such as *Cadburys Chocolate log* (Made in South Africa by Mondelez South Africa (PTY) LTD. 18 Harrowdene Office Park, Kelvin Drive, Woodmead Sandton, 2129, SA.).

The control sample contained 100% gelatin and one of the experimental samples 100% liquid mucilage. The lowest concentration at which the mucilage was used in the formulations was 75%. The lowest concentration at which the other hydrocolloids were added, was 12.5 %. The sample containing 100% liquid mucilage did not gel and could not be used for any of the physical texture analysis tests.

Physical texture analysis of marshmallow samples Consistency

A glass plate, placed on paper with concentric circles drawn on it, at intervals of 0.5 cm was used. An open-ended tube, 2 cm x 2 cm in diameter, filled with 5 mL of the liquid sample, was placed in the center of the circles. After the tube was lifted, the liquid sample was allowed to flow for 2 min. The distance, traveled by the liquid, was then measured at each 90º section of the circle and the average calculated; the line spread value (LSV) was the mean of the four values obtained (Kim, 2007) and expressed in cm.

Texture

The penetrometer was used to determine the comparative tenderness and penetration properties of semi-solid substances (Mohos, 2010). The cone penetration test (ASTM D217) was used and the depth was measured in mm. The larger the reading, the more tender the product. The more tender the sample, the deeper the penetrometer will sink into the sample and, thus, the higher the penetration number will be. The flat attachment tests resistance against pressure; thus, the higher the value the more compression is achieved, indicating a more tender softer product. The results were expressed in mm.

Tenderness of gel

The percentage sag test was used to determine the gel tenderness and was expressed as percentage of the height, before unmolding. For this test, the depth of the marshmallow sample was measured in its container, by using a probe. After the sample was unmolded onto a flat surface (McWilliams, 1989), the depth was measured again. Percentage sag was then calculated as the change in height of the sample measured in the container or mold, compared to that of the freestanding gel placed on a flat surface. The greater the percentage sag value, the more tender the gel (McWilliams, 1989).

Table 2. Substitution hydrocolloids, combinations of hydrocolloids, substitution percentages and weights used in the preparation of marshmallows samples.

% substitution replacement	Actual weight (g)	Gelatin substitution Code	Formulation
Control	143.27g	gelatin	G
100%	143.27g	mucilage*	M
75% + 25%	107.45g+35.82g	mucilage* and xanthan	MX
75% + 25%	107.45g+35.82g	mucilage* and agar	MA
75% + 25%	107.45g+35.82g	mucilage* and gum guar	MG
80% + 20%	114.62g+28.65g	mucilage* and xanthan	8M2X
80% + 20%	114.62g+28.65g	mucilage* and agar	8M2A
80% + 20%	114.62g+28.65g	mucilage* and gum guar	8M2G
75%+12.5%+12.5%	107.45g+17.9g+17.9g	mucilage* and xanthan and agar	MXA
75%+12.5%+12.5%	107.45g+17.9g+17.9g	mucilage* and xanthan and gum guar	MXG
75%+12.5%+12.5%	107.45g+17.9g+17.9g	mucilage* and agar and gum guar	MAG

* Liquid mucilage, while other hydrocolloids were powdered.

Shear

Shear analysis was performed with the Instron Universal Testing Machine (UTM Model 3344). The samples from each of the treatments were cooled down to room temperature (19-25°C) for at least 24 hours, before shear force measurements were taken and for each sample six repetitions were done. Samples were prepared, with a core diameter of more or less 12.7 mm and a length of 10 mm, as advised by the prescribed method. The load cell size was 1 kilo Newton. A Warner Bratzler shear device, mounted on the Instron Universal Testing Machine, was used and the reported value in kg represented the average of the peak force measurements of each sample. Sample compression data was generated by the Instron Bluehill software (Instron, 2004).

Color analysis

Color measurements were performed on the marshmallow samples, 24 hours after preparation, using a Minolta CR 400 Chroma Meter, with an 8 mm measuring area at a 0º viewing angle. Calibration was done by using the white calibration plate and measurements were done in sextuplicate. The CIELAB color space (CIE, 1986) was used for measurements, with L* representing lightness. Chroma (C*), as well as Hue Angle (Hº), was calculated by using a* and b* values in the respective formulas: $C^* = \sqrt{(a^*)^2 + (b^*)^2}$ and $H^o = \tan^{-1}\left[\frac{b^*}{a^*}\right]$

(Ripoll *et al*. 2011; Tapp *et al*., 2011), where C* represents saturation and Hº the tone, which indicates color variation in the plane formed by the coordinates a* and b* (Chervin *et al*., 1996).

Water activity (a_w)

The water activity of the marshmallow samples in a_w containers (height of 5 mm and diameter of 39 mm) was measured with a Novasina Thermoconstanter TH 200 water activity meter, at room temperature (20 ºC). The means of the replications for the marshmallow samples were recorded (Mathenjwa *et al.,* 2012).

Comparison between best experimental formulation and commercial marshmallows

The best experimental formulation sample was then tested against four commercial marshmallow brands in South Africa: Woolworths white (WW); Woolworths traditional white (WTW); Beacon white (BW); and Manhattan white (MW). Only Woolworths white is made according to the traditional process, while the other three are extruded. The texture of the extruded marshmallows is tougher than marshmallows made by the traditional way. For this comparison, the line spread test was omitted, because the commercial samples could not flow upon melting. All the other textural tests were performed, as described above.

Statistical Analysis

All results were captured in multiple spreadsheets in Microsoft Excel 2007. A one-way analysis of variance (ANOVA) procedure (NCSS, 2007) was used to determine the effect of mucilage/ hydrocolloid formulations on the physical parameters of the marshmallow samples. Differences were considered statistically significant at $p<0.05$ level or lower. Treatment means were then compared by means of the Tukey-Kramer multiple comparison test at $\alpha = 0.05$.

RESULTS AND DISCUSSION

The replacement of gelatin with mucilage, in combination with guar, agar agar and xanthan, had a significant ($p<0.05$) effect on all the evaluated physical parameters of the marshmallows (Table 3).

Consistency

For the line spread test, the MX sample had the lowest value, signifying that the consistency of this formulation was the highest of the all the samples. This sample differed significantly ($p<0.05$) from all the other samples (Table 3). The consistency of the control sample (G = gelatin) did not differ from that of 8M2A and MAG; however, it was significantly ($p<0.05$) higher than MA, MG, 8M2X and 8M2G, and significantly ($p<0.05$) more fluid than MX, MXA, and MXG. Sample 8M2G had the highest value for the line spread test, indicating that it was the most fluid and was significantly ($p<0.05$) more fluid than all the other samples (Table 3).

Since the control sample contained 100% gelatin and resulted in a successful product, a line spread test value of around 19.72 mm could result in a successful product. The combinations 8M2A and MAG did not differ from the consistency of the control sample (Table 3). In both of these samples, agar was used in combination with mucilage, which could be responsible for the satisfying results. Agar is used in confectionaries, because of its solubility and the strength it confers upon the product (Nussinovitch, 1997). It was also found that, as soon as the percentage mucilage increased, so did consistency (Table 3).

Texture

The sample with the highest measurement for the flat attachment was the control sample (G). According to McWilliams (1989), the larger the reading, the tenderer is the product, as the attachment will sink deeper into a tender sample, resulting in a higher penetration value. The samples with the lowest reading were MA, 8M2X, and MAG, indicating that these samples were less tender (Table 3). Sample MX did not differ from MG and 8M2G, but was significantly ($p<0.05$) harder than G, 8M2A, MXA, and MXG. Although MXA was also significantly ($p<0.05$) harder than G, it was the mucilage combination closest to the tenderness achieved by the control sample, containing 100% gelatin. Agar is known as a ‘gelling type’ hydrocolloid, like

gelatin (Saha and Bhattacharya, 2010), but it has stronger setting properties (five times greater), so agar form gels at lower concentrations (Condrasky, 2014). Xanthan, on the other hand, is used for thickening (Saha and Bhattacharya, 2010) and adds elasticity (Condrasky, 2014). The more crumbly texture caused by agar (Condrasky, 2014) may have been softened by the xanthan, resulting in a tenderer texture.

For the cone attachment, the lowest value was measured for the 8M2A sample, which did not differ from MA, MAG, and 8M2G; these samples were the tenderest and differed significantly ($p<0.05$) from all the other samples (Table 3). The MG sample was significantly ($p<0.05$) less tender than MXG, MXA and G, and significantly ($p<0.05$) tenderer than all the other samples (Table 3). Finally, MXA was significantly ($p<0.05$) less tender than the control sample G (197.00), which contained 100% gelatin.

Tenderness of gel

The sample that scored the lowest percentage sag was the control sample (G), indicating a strong gel texture, as the greater the percentage sag value, the more tender the gel is (McWilliams, 1989); this sample differed significantly ($p<0.05$) from all the other samples (Table 3). The 8M2G and MAG samples had the highest percentage sag, meaning that they were the tenderest of all the samples. The sample closest to the control sample (G), again, was MXA, which was also the best sample in the penetrometer tests. Although it did not differ from MX and 8M2X, it had the lowest numerical percentage sag.

Shear

For the physical attribute shear, the lowest value was scored by three samples, 8M2A, 8M2G, and MAG. These did not differ from the values recorded by the control, MA and MG. MXA, the toughest of all the samples, differed significantly ($p<0.05$) from all the other samples. This sample, which is a combination of 75 % mucilage + 12.5 % xanthan + 12.5 % agar, was the most promising hydrocolloid formulation thus far. For this attribute, it measured at the opposite end of the scale, compared to the control sample G. As mentioned earlier, xanthan does not form a gel when used alone. In combination with certain hydrocolloids, such as guar and locust bean gum (LBG), gels are formed, because of synergistic interactions. Xanthan shows quite spectacular synergistic interactions with other non-gelling polysaccharides of the galactomannan family (Saha and Bhattacharya, 2010), giving an increase in consistency (Casas and García-Ochoa, 1999) and gel formation (Rodriguez - Hernández and Tecante, 1999). It might be possible that there may also be an interaction between xanthan and mucilage, which requires further investigation.

The MA combination had a low score which did not differ from that of the control sample. This indicated that the shear increase was introduced by the 12.5% xanthan in the MXA combination. This is in agreement with the findings of Al-Assaf *et al*. (2006), who stated that xanthan shows a very high consistency at low shear rate and is thus preferred as a thickener or stabilizer for dispersed solids or emulsions.

Table 3. Physical and color properties of different formulations of hydrocolloids, in the making of marshmallows.

Property	G	MX	MA	MG	8M2X	8M2A	8M2G	MXA	MXG	MAG
Consistency (cm)	1.97±0.02^{d}	1.42±0.06^{a}	2.14±0.10^{e}	2.32±0.07^{f}	2.27±0.12^{f}	2.05±0.01^{de}	2.33±0.07^{f}	1.83±0.11^{c}	1.66±0.15^{b}	1.98±0.05^{d}
Texture (flat)* (mm)	0.23±0.33^{f}	0.77±0.22^{ab}	0.53±0.20^{a}	0.90±0.19^{bc}	0.53±0.03^{a}	1.33±0.15^{d}	0.90±0.09^{bc}	1.90±0.05^{e}	1.10±0.21^{cd}	0.53±0.07^{a}
Texture (cone)* (mm)	19.70±0.83^{f}	14.87±0.80^{b}	0.15±0.08^{a}	16.03±1.00^{c}	14.13±0.17^{b}	0.14±0.02^{a}	0.19±0.06^{a}	18.47±0.56^{e}	17.27±0.28^{d}	0.17±0.00^{a}
Tenderness of gel (%)	0.96±1.11^{a}	9.18±1.10^{bc}	19.84±1.76^{d}	16.38±3.18^{d}	8.85±1.65^{bc}	9.74±1.50^{c}	29.78±3.63^{e}	5.41±0.86^{b}	9.70±3.18^{c}	27.90±6.51^{e}
Shear(kg)	0.07±0.03^{a}	0.45±0.20^{bc}	0.06±0.02^{a}	0.05±0.03^{a}	0.33±0.15^{b}	0.02±0.01^{a}	0.02±0.01^{a}	0.69±0.10^{d}	0.56±0.22^{cd}	0.02±0.01^{a}
a*	-0.12±0.09^{f}	-0.57±0.25^{de}	-1.08±0.18^{c}	-2.39±0.07^{a}	-0.64±0.13^{de}	-1.09±0.24^{c}	-1.86±0.08^{b}	-0.71±0.05^{d}	-0.44±0.11^{e}	-1.13±0.13^{c}
b*	9.29±0.87^{b}	8.16±2.53^{ab}	16.67±0.20^{c}	20.61±0.72^{e}	7.89±2.11^{ab}	16.26±0.31^{c}	18.13±0.42^{cd}	9.64±0.35^{b}	6.34±2.15^{a}	19.20±0.63^{de}
L*	90.03±1.88^{d}	90.63±1.96^{d}	85.67±1.80^{c}	82.52±0.85^{b}	89.25±1.77^{d}	86.40±0.50^{c}	81.27±1.11^{b}	91.33±0.36^{d}	89.47±1.79^{d}	77.59±2.86^{a}
C*	9.29±0.07^{b}	8.18±2.54^{ab}	16.70±0.18^{c}	20.74±0.71^{e}	7.91±2.12^{ab}	16.30±0.30^{c}	18.22±0.41^{cd}	9.67±0.35^{b}	6.35±2.15^{a}	19.23±0.63^{de}
H°	90.27±0.55^{a}	93.82±0.62^{bc}	93.71±0.66^{bc}	96.62±0.42^{e}	94.69±0.34^{d}	93.84±0.89^{bc}	95.87±0.32^{e}	94.24±0.44^{cd}	94.09±0.42^{bcd}	93.37±0.34^{b}
a_w	0.78±0.01^{de}	0.78±0.01^{ef}	0.76±0.01^{bc}	0.77±0.01^{cd}	0.76±0.01^{ab}	0.80±0.01^{g}	0.78±0.01^{de}	0.75±0.01^{a}	0.79±0.01^{fg}	0.78±0.01^{de}

Means with different superscripts in the same row differ significantly ($p<0.05$).

Texture (flat) =penetrometer flat; Texture (cone) =penetrometer cone; a=redness; b*= yellowness; L*=lightness; C*= Chroma (saturation index); H°=Hue angle; a_w=water activity.

Samples:

G Gelatine
MX 75% Mucilage and 25 % Xanthan
MA 75% Mucilage and 25% Agar
MG 75% Mucilage and 25%Guar Gum
8M2X 80% Mucilage and 20% Xanthan 8M2G 80% Mucilage and 20% Guar Gum 8M2A 80% Mucilage and 20% Agar
MXA 75% Mucilage and 12.5% Xanthan and 12.5% Agar
MXG 75% Mucilage and 12.5% Xanthan and 12.5% Guar Gum
MAG 75% Mucilage and 12.5% Agar and 12.5% Guar Gum

Color analysis

Values for a* and b* measurements will not be discussed and is only included in Table 3 for clarification of the C* and Hº calculations. L* values distinguish between bright and dull colors (Konica Minolta, 1998). All the samples tested showed high values for L*, with MXA having the highest value; this sample did not differ from the control sample and appeared to be the best formulation of hydrocolloids to replace gelatin (Table 3). The MAG sample had the lowest score for lightness, indicating that it was significantly ($p<0.05$) darker when compared with the other samples. All the samples containing xanthan had the highest L* values, suggesting that xanthan had a lightening effect on the final product. Except for sample MXG, which contained xanthan, guar had a darkening effect on all the marshmallow samples (Table 3). In a study by Bortnowska and Makiewicz, (2006) on the influence of xanthan and guar on the color of mayonnaise, sensory profiling indicated that both hydrocolloids intensified the creamy color of low-fat mayonnaise. With an increase in the hydrocolloid, the characteristic yellow color disappeared and became a light creamy color, which increased with increasing concentration of the hydrocolloid. Considering that the color of a food is the first contact point of the consumer (Alvarenga *et al*., 2012) and that the consumer may want a bright white marshmallow, the L* value may play an important role.

Chroma is an indication of the saturation of colors, indicating whether they are vivid/strong to dull, to weak/greyish (McGuire, 1992; Konica Minolta, 1998). All the samples had values ranging from
6.35 to 20.74 (Table 3), putting them horizontally closer to the center or greyish/ weak area in the plane formed by the coordinates a* and b* (Chervin *et al*., 1996; Konica Minolta, 1998). The sample with the lowest C* value was MXG, which was the dullest or most greyish sample; it differed significantly ($p<0.05$) from G, MA, MG, 8M2A, 8M2G, MXA and MAG, but not from MX and 8M2X. This could be attributed to xanthan that was used in the formulations. With the L* values, the xanthan-containing samples were also the lightest. Sample 8M2X differed significantly ($p<0.05$) from MA, MG, 8M2A, 8M2G and MAG, but not from G, MX, MXA, and MXG. Sample MA differed significantly ($p<0.05$) from G, MX, MG, 8M2X, MXA, MXG and MAG, but not from 8M2A and 8M2G. Sample 8M2G differed significantly ($p<0.05$) from all the samples, but not from 8M2A and MAG, while MG had the highest value for C* and differed significantly from all the samples, except from MAG. Samples containing guar had thus a stronger saturation of color than the other formulations.

Hue is the term used for the classifications of colors, for example red, yellow, blue, etc. For this attribute, all the samples had values ranging between 90º and < 96º (Table 3), and when the hue angle in this region, the color is yellow (Konica Minolta, 1998; XRite, 2007; Zhang *et al*., 2007). The highest values were found for both samples containing guar, namely MG (75% mucilage and 25% guar gum) and 8M2G (80% mucilage and 20% guar gum), while the lowest Hº value was for the sample containing gelatin (Table 3). For the samples where xanthan was added to the formulations, the hue angle increased significantly ($p<0.05$) for samples 8M2X (80% mucilage and 20% xanthan), MXA (75% mucilage and 12.5% xanthan and 12.5% agar) and MXG (75% mucilage and 12.5% xanthan and 12.5% agar).

Although the various marshmallow formulations differed significantly ($p<0.05$) amongst each other, it could be concluded that all samples had a light greyish yellow color.

Water activity

All the samples fell within the range of intermediate moisture foods (IMF). These are foods with a_w of 0.65 - 0.85, which is largely responsible for its protection from microbial spoilage (Garbutt, 1997). The sample with the lowest a_w value was MXA; this sample differed significantly ($p<0.05$) from all the other samples (Table 3). The sample with the highest a_w of 0.80, namely 8M2A, differed significantly ($p<0.05$) from all the samples, but still fell within the range of IMF. The MXA combination had a significantly ($p<0.05$) lower a_w than the control (G) sample, rendering it even more acceptable as a substitute for gelatin in the marshmallow formulation. This water dispersing property is common to all hydrocolloids; however, the extent of thickening varies with the type and nature of the hydrocolloid (Saha and Bhattacharya, 2010). Nammakuna *et al*. (2009) found that addition of hydrocolloids to rice crackers increased its moisture content, which correlated to significantly higher a_w in treated samples, as compared to the controls.

It can be concluded that the best textural attributes were associated with sample MXA, consisting of 75% mucilage, 12.5% xanthan, and 12.5% agar. This sample showed good results in all the above mentioned physical evaluations and it was the closest sample to the control. It is then clear that in combination with xanthan and agar, mucilage can be used as a substitute for gelatin in the production of marshmallows.

Comparison between best experimental formulation and commercial marshmallows

Texture

The sample with the highest measurement for the penetrometer with the flat attachment was the MXA. As noted earlier, the larger the reading, the tenderer is the product, as the attachment will sink deeper into a soft sample, resulting in a higher penetration value. The sample with the lowest reading was BW, indicating that this sample was not tender (Table 4). The two Woolworths samples did not differ, although the one was made in the traditional way and the other one was extruded. All the other samples differed significantly ($p<0.05$). The gelling agent for all four commercial samples was gelatin. For this test, the MXA sample resembled the tenderness of the MW sample the most, although they differed significantly ($p<0.05$).

When the cone attachment was used on the penetrometer, the samples were divided into three groups: WW and BW, with the lowest scores, which differed significantly ($p<0.05$) from the next group, MXA and MW, which in turn differed significantly ($p<0.05$) from WTW, which had the highest score (Table 4), indicating that this sample was the tenderest. For these two measurements, the BW samples scored the lowest values in each instance, confirming that it was the least tender sample. It should be noted that again the measurements for MXA and MW did not differ for this attachment.

Tenderness of the gel

Both MXA and MW had the lowest values for percentage sag and both samples differed significantly ($p<0.05$) from BW and WTW, but not from WW; this sample differed significantly ($p<0.05$) from WTW (Table 4). Samples MXA and MW, thus, had tough gel textures. The greater the percentage sag value, the more tender is the gel (McWilliams, 1989); therefore, the WTW sample was the tenderest gel.

Shear

For the physical attribute shear, the lowest value was scored by MXA, indicating least shear. Interestingly, all the commercial samples differed significantly ($p<0.05$) from this sample, as well as from one another. From these samples, the sample with the least shear was WTW. Sample MW was the toughest sample, with BW second toughest, followed by WW. This confirms the belief that traditionally made marshmallows are not as tough as the extruded ones. For extrusion to be successful, the mixture contains modified corn starch, in addition to gelatin, the temperature of extrusion has to be just right so that the mixture will flow under pressure, but hold its shape after leaving the pipe (Mulvaney, 2015).

Color analysis

Values for a* and b* measurements will not be discussed and is only included in Table 4 for clarification of the C* and Hº calculations. All the samples, again, showed high values for L* (lightness). The sample with the lowest L* value was MXA and it differed significantly ($p<0.05$) from all the other samples, the reason for this being the greenish color of the fluid mucilage, which lowered the L* value. The BW sample differed significantly ($p<0.05$) from MXA, WW, and MW, but not from WTW; this sample differed significantly ($p<0.05$) from MXA and WW. The commercial sample with the highest value for lightness was WW (Table 4). In a study by Periche *et al.* (2015), it was found that the factor that had the greatest influence on lightness, was the percentage of gelatin; the higher the level of gelatin, the higher the lightness. Although there were significant differences ($p<0.05$) between the samples for this color attribute, all samples were light, which is of importance for the consumer looking for a white marshmallow.

The C* values were significantly ($p<0.05$) different amongst the samples, except for samples BW and MW, which did not differ from each other. The coordinates, again, placed the samples in the greyish / weak yellow spectrum (McGuire, 1992; Konica Minolta, 1998; Sepulveda, *et al*., 2007). Sample MXA had significantly ($p<0.05$) the highest value for C*, while sample WW had significantly ($p<0.05$) the lowest value. Despite these significant ($p<0.05$) differences, the experimental sample and the commercial samples had values in the same range, and the use of mucilage, along with agar and xanthan, would have a favorable impact on the acceptability of the consumer.

For Hº, all the values were between 90.71º and 99.22º (Table 4), placing the color again in the yellow region (McGuire, 1992; Konica Minolta, 1998; Zhang *et al*., 2007). The highest value was found for the WW and WTW samples and the lowest Hº value was calculated for the BW sample. The mucilage sample, MXA, differed significantly ($p<0.05$) from the three previously mentioned samples, but not from the commercial brand MW.

Although the samples differed significantly ($p<0.05$) amongst each other for the color analysis, they all had a light greyish yellow color.

Table 4. Physical and color properties for MXA and four commercial marshmallows brands.

Property	MXA	WW	WTW	BW	MW
Texture (flat)* (mm)	1.90 ±0.06^{d}	0.78 ± 0.15^{b}	1.00 ± 0.14^{b}	0.52 ± 0.13^{a}	1.43 ± 0.16^{c}
Texture (cone)* (mm)	18.47 ± 0.76^{b}	17.38 ± 0.73^{a}	20.10 ± 0.26^{c}	17.13 ± 0.51^{a}	18.92 ± 0.75^{b}
Tenderness of gel (%)	5.41 ± 1.15^{a}	9.18 ± 1.48^{ab}	16.38 ± 4.27^{c}	9.74 ± 2.01^{b}	5.41 ± 1.15^{a}
Shear (kg)	0.69 ± 0.14^{a}	25.36 ± 6.55^{c}	16.47 ± 4.01^{b}	41.00 ± 5.54^{d}	67.42 ± 6.11^{e}
a*	-0.71 ± 0.07^{b}	-0.98 ± 0.10^{a}	-1.13 ± 0.12^{a}	-0.07 ± 0.12^{c}	-0.77 ± 0.09^{b}
b*	9.64 ± 0.47^{d}	5.81 ± 0.58^{a}	6.97 ± 0.85^{b}	8.31 ± 0.94^{c}	8.25 ± 0.15^{c}
L *	91.33 ± 0.48^{a}	96.39 ± 0.21^{d}	94.40 ± 1.02^{bc}	93.61 ± 0.36^{b}	94.90 ± 0.54^{c}
C*	9.67 ± 0.47^{d}	5.89 ± 0.59^{a}	7.06 ± 0.85^{b}	8.31 ± 0.94^{c}	8.29 ± 0.15^{c}
H(°)	94.25 ± 0.59^{b}	99.61 ± 0.72^{c}	99.22 ± 0.65^{c}	90.71 ± 0.89^{a}	95.30 ± 0.61^{b}
a_w	0.75 ± 0.01^{e}	0.52 ± 0.01^{a}	0.65 ± 0.01^{d}	0.63 ± 0.00^{c}	0.60 ± 0.01^{b}

Means with different superscripts in the same row differ significantly ($p<0.05$). *Texture (flat) =penetrometer flat; *Texture (cone) =penetrometer cone; a*=redness; b*= yellowness; L*=lightness; C*= Chroma (saturation index); H^{o}=Hue angle; a_w=water activity.
Samples:

MXA=75% Mucilage and 12.5% Xanthan and 12.5% Agar WW=Woolworths white

WTW=Woolworths traditional white

BW=Beacon white

MW=Manhattan white

Water activity

Again, all the samples fell within the range of intermediate moisture foods (IMF) and even lower. The sample with the highest a_w value was MXA, which differed significantly ($p<0.05$) from all the other samples (Table 4). The liquid mucilage has a high a_w of 0.96 (De Wit, 2015), which contributed to the high a_w of the MXA sample. It is interesting to note that all the commercial samples had values that differed significantly ($p<0.05$). Furthermore, the traditional commercial sample, WTW, was the only commercial sample with a_w of 0.65, while the other commercial samples had lower values. Sample BW, an extruded commercial brand, had a_w of 0.63, which is lower than the recommended range of 0.65 and 0.85. This low a_w ensures the microbial stability of the product and lengthens the shelf life. Sample MW had an even lower a_w of 0.60, qualifying it to be a low moisture food. The lowest a_w was achieved by WW, with a value of 0.52, also qualifying as a low moisture food.

CONCLUSIONS

Marshmallows were selected as the potential vehicle for the incorporation of cactus pear mucilage in different formulations, using a variety of hydrocolloids. The MXA sample, containing 75% mucilage, 12.5% xanthan, and 12.5% agar, obtained the best results for all the physical parameters tested and could be a potential formula requiring further research. Marshmallows containing mucilage in combination with agar and xanthan might also be a good alternative for similar products, where only one hydrocolloid, for example, agar is used as a gelling agent, resulting in a tough texture. Furthermore, gelatin-less marshmallows might also open up a market which was restricted by the use of gelatin, namely for Halaal and Kosher consumers.

Since the consumer has the final decision in whether a newly developed product is acceptable, it is necessary to submit it to a sensory panel of regular marshmallow consumers to determine its overall acceptability, taste, aftertaste, as well as the two most important attributes in the case of this aerated gelled product, namely appearance and texture.

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Replacement of gelatin with *Opuntia ficus-indica* mucilage in flavored pink and unflavored white marshmallows. Part 2: Consumer liking

L. Du Toit, C. Bothma, M. De Wit, and A. Hugo

Department of Microbial, Biochemical and Food Biotechnology, University of the Free State, PO Box 339, Bloemfontein, 9300, South Africa.

Corresponding author: dewitm@ufs.ac.za

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ABSTRACT

Mucilage from the cladodes of *Opuntia ficus-indica* is under investigation for application in several foodstuffs. Since it can not form gels on its own, it is advised to be used in combination with other hydrocolloids. The aim of this study was to compare consumer liking of flavored and unflavored marshmallows made with wet mucilage, to that of a flavored and unflavored control sample (with 100% gelatin), as well as a flavored and unflavored commercial brand. Ninety-two consumers tasted the following six samples: white commercial (*Manhattan*); white control (gelatin); white mucilage (75% mucilage + 12.5% agar + 12.5% xanthan); pink commercial (*Manhattan*); pink control (gelatin); and pink mucilage (75% mucilage + 12.5% agar + 12.5% xanthan). Consumer liking was tested for taste, aftertaste, texture, as well as an overall acceptability of liking. The white mucilage marshmallows had the lowest ranking for taste, aftertaste, texture and overall acceptability, and differed significantly ($p<0.05$) from all the other samples. However, the pink mucilage marshmallow did not differ from the pink commercial one (which had the highest rankings for taste, aftertaste, texture, and overall acceptability) and pink control marshmallow. The differences between the white and pink mucilage marshmallows ranged between 2.75 and
2.89 on the hedonic scale. It could be concluded that flavoring successfully masked the distinctive aroma of the mucilage in the marshmallows, thereby also increasing scores for texture and overall acceptability.

Keywords: *Opuntia ficus-indica,* consumer liking, marshmallows, mucilage, gelling agent.

INTRODUCTION

Marshmallows are one of the earliest confections known to humankind (Olver, 2000). Originally, they were made from the root sap of the marshmallow (*Althaea officinalis*) plant, a genus that is native to parts of Europe, North Africa and Asia. The first marshmallows were prepared by boiling pieces of the marshmallow root pulp with sugar until thick, whereafter the mixture was strained and cooled. As far back as 2000 B.C., Egyptians combined the marshmallow root with honey and reserved this candy for gods and royalty (Petrusso, 2014).

Gelatin is used in whipped confections, such as marshmallows, where it serves to lower the surface tension of the syrup, stabilize the foam through increased viscosity, set the foam via gelation and prevent sugar crystallization (GMIA, 2012). Furthermore, it is also responsible for the chewy, bouncy texture of marshmallows (Karim and Bhat, 2008).

Gelatin is unique, because when mixed with water, it forms a thermally-reversible, elastic and clear gel (De Vries *et al*., 2004). These gelatin gels have a melting temperature just below body temperature (< 35ºC), so the gel product literally melts in the mouth and releases intense flavor immediately as it dissolves, which is a difficult quality to replicate with other hydrocolloids (Karim and Bhat, 2008).

Gelatin makes marshmallows chewy by forming a tangled three-dimensional network of polymer chains (Glicksman, 1982). Once gelatin is dissolved in warm water (dubbed the 'blooming stage'), it forms a dispersion, which results in a cross-linking of its helix-shaped chains. The linkage in the gelatin protein network, called 'junction zones', trap air in the marshmallow mixture and immobilize the water molecules in the network (Burey *et al*., 2008). As mentioned previously, gelatin melts at temperatures < 35ºC, because the junctions zones are only bound by weak hydrogen bonds (De Vries *et al*., 2004). Gum-on- gum interaction tends to produce synergistic textures and there are many combinations to choose from. One of the most used is when guar gum is often added to xanthan gum to reduce cost, increase viscosity and improve texture, while xanthan gum prevents syneresis (Sakharam and Meschi, 2008).

The genus *Opuntia* is characterized by the production of a hydrocolloid, commonly known as mucilage, which forms molecular networks that are able to retain large amounts of water, resulting in colloidal and very viscous suspensions or jellied masses (Sepulveda *et al*., 2007). Mucilage is present in the characteristic slimy fluid secreted by cladodes and fruits. Two distinctive, water-soluble, high-molecular-weight, pectic polysaccharide materials occur in *Opuntia* cladodes and fruits, namely mucilage and a calcium-sensitive gelling fraction. Mucilage do not gel in the presence of calcium. Pectin with a low degree of methoxylation occurs in the cell wall and can be extracted using a mild alkali process, aided by a chelating agent; this pectin shows remarkably good gelling properties in the presence of $CaCl_2$, by a cooperative Ca^{2+} "egg-box" binding mechanism (Goycoolea and Cardenas, 2003).

Furthermore, mucilage is a complex polymeric carbohydrate, with a highly branched structure, which contains varying proportions of L-arabinose, D- galactose, L-rhamnose, and D-xylose, as well as the galacturonic acid in different proportions. There are also minerals present, such as Ca^{2+} and K^{+}, carbohydrates and dietary fiber (Sepulveda *et al*., 2007). Although both materials share similarities in the composition profile of their neutral constituent sugar residues, pectin has a significantly greater amount of linear polygalacturonic acid. This difference causes very different physicochemical and functional properties, underlying the potential applications of these polysaccharides in a wide variety of fields (e.g., foods, biotechnology and medicine).

Many popular uses in different countries shed light on the numerous mucilage properties, making this an interesting ingredient for the food industry, because of its viscosity properties.

In South Africa, mucilage has been included into various products in various research studies: Turkish delight (confectionary); carrot cake; seed bread; mayonnaise; ice cream; and yogurt to replace various ingredients (De Wit and Fouche, 2015). Nowadays,

consumers are becoming more aware of their own health and health foods. Thus, the demand for health foods is increasing, because of their functions of lowering the risks of chronic health diseases. Also, some religions exclude the use of certain animal by-products, such as gelatin. By replacing such a product with an ingredient of plant origin, a huge range of products will be made available to a broader consumer market.

The aim of this study was, therefore, to evaluate consumer liking of the 75% mucilage + 12.5% xanthan + 12.5% agar formulation (Du Toit *et al*., 2016), with that of the control marshmallows (containing gelatin) and one commercial brand. Three marshmallow samples were left uncolored and unflavored, and were compared to the liking of three pink- colored, strawberry-flavored samples.

MATERIALS AND METHODS

Marshmallows

Six marshmallow samples were prepared: 75% mucilage + 12.5% xanthan + 12.5% agar; 100% gelatin (control); commercial brand white (*Manhattan*); strawberry flavored and pink colored 75% mucilage + 12.5% xanthan + 12.5% agar; strawberry flavored and pink colored 100% gelatin; and commercial brand pink (*Manhattan*).

The formulation of the recipe for the control marshmallows (white and pink) are given in Table 1. The gelatin was soaked in cold water. The sugar, salt, syrup and rest of the water were heated and stirred to dissolve the sugar. After the mixture reached a temperature of 118°C (sea level), the gelatin was added. The stiffly beaten egg whites were added to the hot mixture, whilst continuously mixing, until thick and creamy. The mixture was then poured into a greased pan, cooled overnight and cut into 25-mm squares. The squares were rolled in a mixture of corn flour and icing sugar to prevent it from sticking together, and stored in an airtight container (Foods and Cookery, 1991).

Table 1. Formulation of marshmallow used as control (Foods and Cookery, 1991).

Ingredient	Percentage (%)
Gelatin	2.29
Water, cold, to soak gelatin	11.45
Egg whites	9.16
Sugar	45.78
Water	28.62
Syrup*	2.00
Salt	0.13
Flavoring, strawberry**	0.57
Coloring, pink, commercial**	2-3 drops

*Golden syrup is a thick, amber-colored form of inverted sugar syrup made in the process of refining sugar cane or sugar beet juice into sugar, or by treatment of a sugar solution with acid. It is used in a variety of baking recipes and desserts.

**Pink control; white control has no flavoring and coloring.

For the mucilage-containing marshmallows, 75% mucilage was combined with 12.5% agar and 12.5% xanthan, and the same method was used as with the control sample. Mucilage was extracted from the cladodes, by using the method of Du Toit and De Wit (2011). As mentioned earlier (Bensadón *et al.*, 2010), the stabilizing properties of mucilage are similar to those of xanthan and guar gums. Agar was also chosen, because it is commercially used in South Africa in chocolate candies to stabilize the marshmallow layer, such as *Cadburys Chocolate log* (Made in South Africa by Mondelez South Africa (PTY) LTD. 18 Harrowdene Office Park, Kelvin Drive, Woodmead Sandton, 2129, SA.). This combination was chosen, as it showed the most desired textural attributes of all the combinations evaluated in previous studies (Du Toit *et al*., 2016). Half of the samples from each treatment were left unflavored and uncolored, (representing the white mucilage formulation), while the other half were colored pink and flavored with strawberry flavoring (representing the pink formulation).

The commercial brand *Manhattan* (Manufactured by Premier Foods, South Africa 1 Joist Street, Islando, 1609, South Africa) also showed textural properties similar to the control and 75% mucilage + 12.5% xanthan + 12.5% agar combination (Du Toit *et al.*, 2016). The marshmallows were melted on the stove in a double cooker and poured into containers, to resemble the shape of the other samples.

Samples were cut into cubes of 1x1x1cm and rolled in a mixture of icing sugar and corn flour (Foods and Cookery, 1991). Samples were evaluated simultaneously, under white lights and served on white polystyrene trays. Bottled water was used as a palate cleanser and between samples. The liking of taste, aftertaste, texture and overall acceptability were evaluated on a structured line scale, ranging from 1 (dislike extremely) to 9 (like extremely) (Figure 1). The nine-point hedonic scale has been used routinely in food science research for the past 60 years and is a scale of liking. It should be emphasized that the numbers on the scale are alternative names for the categories. For example, if the category 'like extremely' is assigned the number '9', the category has not become a number; it has merely been given a new name 'nine' and is no more numerical than ranks (Wichchukit and O'Mahony, 2014).

1	2	3	4	5	6	7	8	9
Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Figure 1. Nine-point hedonic scale of liking (Stone and Sidel, 2004).

Consumer panel

Ninety-two consumers, 21 male and 71 female panelists, aged 18 to older than 60 years, were sourced from staff and students from the Bloemfontein campus of the University of the Free State, Bloemfontein, South Africa (Table 2). All had to be regular consumers of marshmallows, indicating that they consume it at least once every two weeks. The percentage split for the age profile was as follows: younger than 18 years (4%); 20-29 years (74%); 30-39 years (3%); 40 – 49 years (6%); 50-59 years (6%); and older than 60 years (7%). The majority of the panel members were, thus, mainly students, being in the younger

age group from 18 to 39 years old (81%), while only 19% were in the age group of 40 years to older than 60 years, representing students and staff members.

Table 2. Age and gender profile of consumer panel.

Gender panelists	Number of	Age (Years) number of panelists	Percentage split of total
Female	71	18-19	4
Male	21	20-29	74
30-39			3
40-49			6
50-59			6
Older than 60			7

Statistical analysis

The significance for the liking of taste, aftertaste and texture, as well as overall acceptability, measured for each treatment, was tested by means of a one-way analysis of variance (ANOVA). When applicable, Fisher's LSD-test ($p<0.05$) was applied to determine the direction of the differences between treatment mean values (NCSS, 2007). The two sample *t*-test was performed to determine the effect of age (NCSS, 2007).

RESULTS AND DISCUSSION

The ANOVA on the effect of gender, age and marshmallow type on the liking of sensory properties of the samples is summarized in Table 3. It was decided that p-values smaller than 0.05 would be considered as a significant difference. For age, there was a significant ($p<0.05$) effect for the liking of aftertaste, while all the marshmallow types showed significant ($p<0.05$) effects for liking of taste, aftertaste, texture and overall acceptability.

Table 3. ANOVA on the effect of gender, age and marshmallow type on the liking of the sensory properties of marshmallows.

Sensory property	Age	Gender	Marshmallow type	Age X Marshmallow type	Gender X Marshmallow type	Age X Gender X Marshmallow type
Taste	p = 0.0889	p = 0.4163	p = 0.0123	p = 0.3542	p = 0.6397	p = 0.5706
Aftertaste	p = 0.0124	p = 0.0901	p = 0.0234	p = 0.4028	p = 0.7754	p = 0.3435
Texture	p = 0.0576	p = 0.9846	p = 0.0145	p = 0.6699	p = 0.8227	p = 0.1346
Overall acceptability	p = 0.0752	p = 0.6602	p = 0.0165	p = 0.2987	p = 0.7375	p = 0.1943

For age group, there was a significant ($p=0.0124$) difference between the liking for aftertaste, meaning that the older group (40 to older than 60 years old) of consumers ranked the liking of the aftertaste higher than the younger group (18 to 39 years old) (Table 4). Both rankings were situated in the 'like slightly' region of the hedonic scale, with the rank of the older group situated more to the 'like moderately' side of the scale. Liking of aftertaste was included to determine whether the consumers would detect the grassy aroma of the mucilage (Rothman *et al*., 2012). In especially the pink samples the aftertaste was predominantly sweet due to the addition of strawberry flavoring, which is one of the top six

preferred food-related flavors (Hoffman, *et al*., 2016). Although only 19 % of the panelists in the present study fell into the age group 40 years to older than 60 years, it is still a valid result, confirming Hoffman and co-workers' (2016) findings that older adults (older than 55 years) find sugar more pleasant at higher concentrations, such as in confectionaries, compared with young adults (18 to 35 years) and middle-aged adults (36 – 55 years) (Petry, 2002).

Table 4. Effect of age group on the liking of sensory properties of marshmallows types.

Sensory property	Younger than 18 to 39 years	40 to older than 60 years	Significance level ($p < 0.05$)
Taste	6.43 ± 2.15	6.80 ± 1.64	p = 0.0889
Sweet aftertaste	6.26^{a} ± 2.08	6.78^{b} ± 1.63	p = 0.0124
Texture	6.11 ± 2.32	6.55 ± 1.78	p = 0.0576
Overall acceptability	6.39 ± 2.09	6.76 ± 1.59	p = 0.0752

Means with different superscripts in the same row differed significantly, according to two sample *t*- test.

In Table 5 the effect of the mucilage inclusion on the liking of taste, aftertaste, texture and overall acceptability is shown. The lowest ranking for the liking of taste was given to the mucilage white sample, representing 'dislike slightly' on the hedonic scale. This sample differed significantly ($p<0.05$) from the control white and the *Manhattan* white samples, as well as all the pink samples. The control white sample differed significantly ($p<0.05$) from all the samples, except the *Manhattan* white, while the *Manhattan* white sample differed significantly ($p<0.05$) from the mucilage white, control pink and *Manhattan* pink. The mucilage pink differed significantly ($p<0.05$) from the mucilage white and control white samples. For the liking of taste, the *Manhattan* pink sample had the highest ranking and did not differ from the control pink and mucilage pink samples. It was interesting to note that all the pink samples, including the mucilage pink sample, were ranked higher than 7 on the hedonic scale, indicating that the panel liked these samples 'moderately' to 'very much' (Figure 1). The strawberry flavoring was thus successful in masking the grassy flavor of the mucilage (Rothman *et al*., 2012), as there was an increase of almost three categories in ranking, on the hedonic scale, from the white unflavored to the pink strawberry flavored mucilage sample.

Table 5. Effect of marshmallow type on the liking of the sensory properties of the samples.

Sample	Taste	Aftertaste	Texture	Overall Acceptability
Mucilage White	4.26^{a} ± 2.30	4.20^{a} ± 2.23	3.93^{a} ± 2.28	4.21^{a} ± 2.18
Control White	6.34^{b} ± 1.89	6.38^{bc} ± 1.65	6.08^{bc} ± 1.98	6.38^{b} ± 1.76
Control Pink	7.32^{d} ± 1.41	7.08^{d} ± 1.59	7.40^{d} ± 1.65	7.38^{c} ± 1.32
Manhattan White	6.47^{bc} ± 1.84	6.10^{b} ± 1.89	5.66^{b} ± 2.00	6.22^{b} ± 1.81
Mucilage Pink	7.11^{cd} ± 1.58	7.01^{cd} ± 1.51	6.68^{cd} ± 1.72	7.10^{c} ± 1.48
Manhattan Pink	7.51^{d} ± 1.42	7.37^{d} ± 1.30	7.42^{d} ± 1.64	7.48^{c} ± 1.38

Means with different superscripts in the same column differed significantly ($p<0.05$), according to Fisher's LSD-test.

The mucilage white sample scored the lowest ranking for the liking of aftertaste, representing 'dislike slightly' on the hedonic scale. This low rank may be due to the grassy flavor of the mucilage (Rothman *et al*., 2012). This sample was significantly ($p<0.05$) less liked than all the other samples. The control white sample differed significantly ($p<0.05$) from the mucilage white, control pink and *Manhattan* pink, the latter having the highest rank of all the samples. The *Manhattan* white sample differed significantly ($p<0.05$) from the mucilage white, mucilage pink, control pink and Manhattan pink. The mucilage pink sample was ranked significantly ($p<0.05$) higher for aftertaste than mucilage white and *Manhattan* white. There was no difference amongst the mucilage pink, control pink, and Manhattan pink samples. Mucilage pink differed significantly ($p<0.05$) from mucilage white and Manhattan white (Table 5).

The mucilage white sample had the lowest rank for the liking of texture ('dislike moderately') and differed significantly ($p<0.05$) from all the other samples. The control white sample differed significantly ($p<0.05$) from mucilage white, control pink and *Manhattan* pink. *Manhattan* white differed significantly ($p<0.05$) from mucilage white, mucilage pink, control pink and *Manhattan* pink. Texture liking was the only attribute where mucilage pink could not be ranked higher than seven on the hedonic scale. This sample only differed significantly ($p<0.05$) from mucilage white and *Manhattan* white. Although the mucilage pink sample did not score higher than seven, the ranking for its liking of texture did not differ from that of the control pink and *Manhattan* pink samples; the latter scored the highest numerical ranking for the liking of texture (Table 5). From these results, it is clear that the addition of strawberry flavoring positively influenced the flavor/taste of the mucilage marshmallow and also changed the consumer's perception of the texture. There was, again, an increase of almost three categories in the ranking on the hedonic scale, from the white unflavored mucilage marshmallow to the pink, strawberry flavored mucilage marshmallow (Table 5). A positive correlation was found for 'intention of buying and – texture' in a study where isomaltulose replaced sucrose in pink strawberry-flavored marshmallows. Different combinations of sugars were used, as well as different levels of gelatin. Authors concluded that texture defines acceptability and intention of buying this type of product (Periche *et al*., 2015).

In a study by Gress *et al*. (2009), the use of agar alone to replace gelatin produced marshmallows of inferior quality, as determined by a panel of ten trained judges. It is also known that agar gels can not melt in the mouth, as its melting temperature is 85ºC (Condrasky, 2014) and thus needs to be chewed. On the other hand, xanthan gum is a general thickener, which can have a somewhat slimy texture at higher levels, when used on its own. For this reason, it is often used in combination with other hydrocolloids and starches, such as konjac flour, where it produces an extremely elastic gel, suitable for chewy candy (Sakharam and Meschi, 2008). In a previous study by Du Toit *et al*. (2016), it was concluded that the best combination to replace gelatin was 75% mucilage (liquid) + 12.5% xanthan (powder) + 12.5% agar (powder), as it only differed significantly from the control (100% gelatin) sample in regard to shear value, as measured by the Warner Bratzler Shear Force.

In a study by Saint-Eve *et al*. (2004), it was confirmed that a single aroma complex, such as strawberry, led to low-fat yogurts being perceived as thicker and stickier, by using three sensory methodologies, namely sorting, free-choice profiling and descriptive analysis.

Kostyra and Barylko-Pikielna (2007) studied the effect of fat levels and guar gum addition in mayonnaise-type emulsions on the sensory perception of smoke-curing and salty taste. They found that it was the addition of guar gum that led to intensity differences in flavor and taste, rather than the different fat levels. Thus, it can be speculated that there could be an interaction between the hydrocolloids and flavorings, resulting in a better sensory perception of the textural properties in the present study. Gelatin, being a protein, already aids in the distribution of flavorings and colorings, despite being responsible for the characteristic foam-like texture of marshmallows (De Vries *et al*., 2004; Karim and Bhat, 2008; GMIA, 2012). Periche *et al*. (2015) used 20 trained panelists and a nine-point hedonic scale to evaluate various attributes, including strawberry flavor of five pink marshmallows formulations, consisting of different sugar mixtures, levels of gelatin and amounts of strawberry flavoring. The marshmallow formulation which had double the amount of flavoring was rated the best, as panelists liked a more intense aroma in this kind of product.

In the present study, three distinct groupings of marshmallows were noted for the liking of overall acceptability, namely, the lowest ranking mucilage white sample, then the other two white samples, and finally the cluster of three pink samples. The mucilage white sample had the lowest ranking for overall acceptability, representing 'dislike slightly' on the hedonic scale; this sample differed significantly ($p<0.05$) from all the other samples. The control white and *Manhattan* white samples both differed significantly ($p<0.05$) from the mucilage white and three pink samples. The three pink samples did not differ, again stressing the importance of flavoring to mask any unwanted flavors, such as the grassy flavor associated with mucilage. The sample that had the highest numerical ranking for overall acceptability was the *Manhattan* pink sample.

It is not possible for the authors to comment on the significant differences between the white and pink *Manhattan* marshmallow samples, as the formulation of these marshmallows is not of public knowledge. It was found in a previous study (Du Toit *et al*., 2016), that the shear values of three brands of pink commercial marshmallows were significantly ($p<0.05$) lower than that of the corresponding white commercial marshmallows. It, thus, appears that the South African consumer favors a softer, flavored and colored marshmallow to a white, unflavored and uncolored marshmallow.

It should again be stressed that ranking on the hedonic scale, is a representation of a category on the hedonic scale. The simplest illustration that the assigned number is merely a new label is that if 'eight' is subtracted from 'nine', the answer is 'one'. Yet, if 'like very much' is subtracted from 'like extremely', the answer is hardly likely to be 'dislike extremely' (Wichchukit and O'Mahony, 2014). Furthermore, the fact that commercial marshmallows were ranked as 'liked moderately', can be attributed to the fact that it may not have been the best tasting marshmallows in South Africa. The main reason for its inclusion was the similarity it showed to the mucilage-containing marshmallow and the control sample (gelatin), in regard to textural properties (Du Toit *et al.*, 2016). From the study done by Du Toit *et al.* (2016), it was concluded that texture was the sensory property mostly affected by the reformulations.

Color is an important quality attribute in the food industry and it influences consumer's choice and preferences. The correlation between color and other sensory quality attributes is well established (Pathare *et al*., 2013). In a study by Spence *et al*. (2015), they reviewed

the growing amount of scientific research showing that people steadily associate specific colors with particular tastes. The color white is widely associated with salty, because of the whiteness of sodium chloride, while pink and/or red colors are associated with sweetness, because sweetness is more often present in red foodstuffs, such as berries and cherries, than in yellow, white, brown or orange foodstuffs (Dematte *et al.*, 2006). These cross-modal correspondences are consistent across different cultures and at least over the last three decades, despite the wide cultural differences in the use of colors across cuisines (Zhou *et al.*, 2015). In the present study the same conclusions could have been drawn from the consumers, when presented with pink and white samples, awarding higher rankings to sweet taste for the pink samples than to the white samples, when in fact at least four of the samples had exactly the same sugar content (Table 1). In a study by Periche *et al*. (2015), a positive correlation was found for 'colour and overall appearance' of pink strawberry flavored marshmallows, containing different sugars and levels of gelatin.

To illustrate the positive effect of pink coloring and strawberry flavoring on the perception of the consumer, the frequencies of the hedonic scale rankings per marshmallow sample, for the liking of the four sensory properties were determined (Figure 2). Each of the graphs can be divided into two parts: i) bars pointing upwards, indicating the positive or "like" side of the hedonic scale, ranging from "like extremely" to "neither like nor dislike"; and ii) bars pointing downwards, indicating the negative or "dislike" side of the hedonic scale, categorized as between "dislike slightly" to "dislike extremely". The first three graphs on the left side of each of the figures represent the frequency of use of the hedonic tags for the white samples, while the pink samples are situated on the right side of each figure. In general, the positive hedonic ratings were assigned more regularly than the negative rankings. It is clear that the negative indicators were used more frequently for the white marshmallow samples, with high frequencies being assigned to the white mucilage marshmallow, for all attributes tested. The highest frequency of negative hedonic ranking was recorded for the texture of the mucilage white sample (Figure 2C). However, for the liking of this attribute, all three white marshmallow samples showed an increase in the use of negative hedonic rankings. For the pink samples, the liking of texture had the most frequent usage of negative hedonic ranking by the panel members (Figure 2C). All the pink samples also had the highest frequency of use for the positive hedonic rankings.

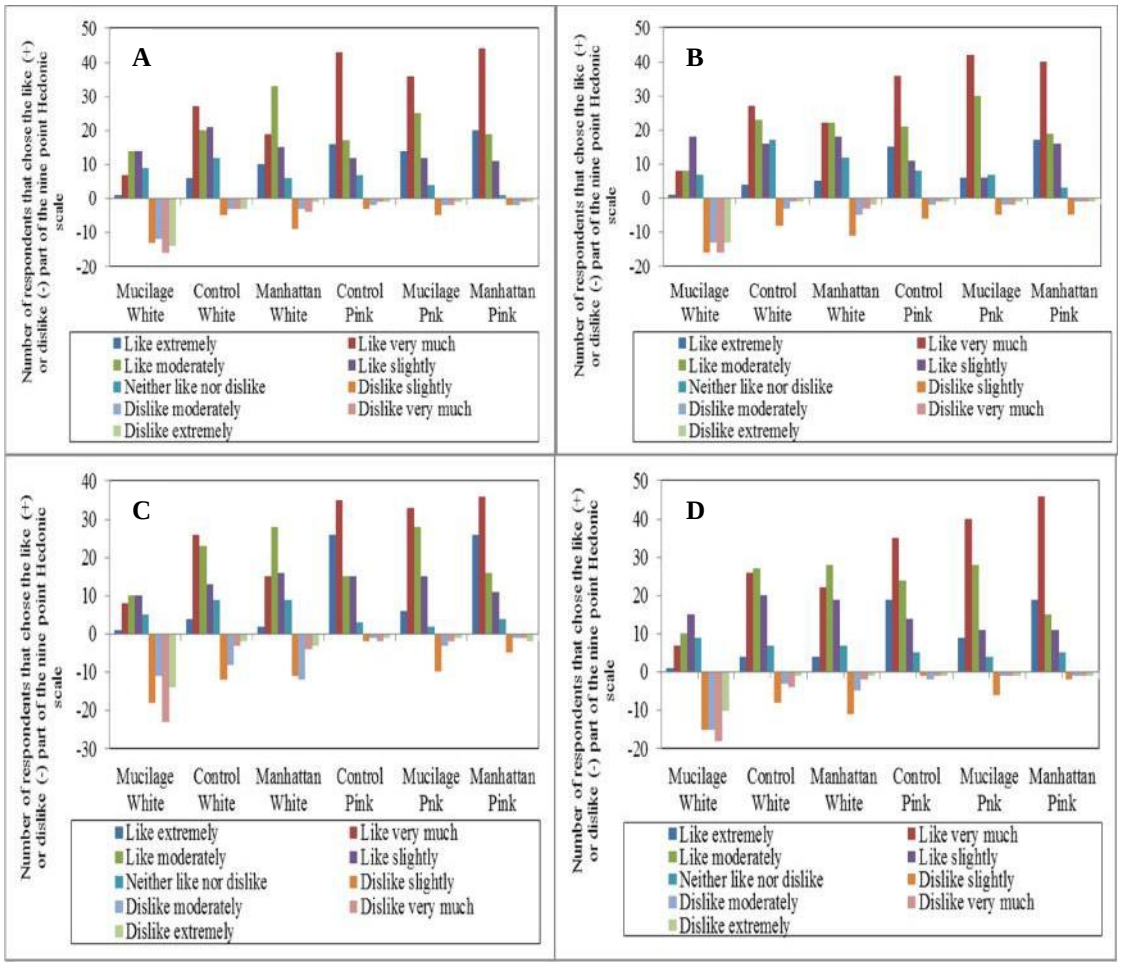

Figure 2. Frequency of the hedonic scale rankings per marshmallow sample, for the liking of A) taste, B) aftertaste, C) texture and D) overall acceptability.

CONCLUSIONS

Mucilage from the cladodes of *Opuntia ficus-indica* is under investigation for application in several foodstuffs. The results from the present study showed that flavoring successfully masked the distinctive taste and aftertaste of mucilage in marshmallows, thereby also increasing scores for texture and overall acceptability. It was thus concluded that the inclusion of mucilage in a colored and flavored confectionary product is a possibility.

The usage of wet mucilage, however, is questionable, since the quality and quantity of the mucilage are influenced by climate, temperature, and rainfall, as noted in literature. Therefore, research should continue on mucilage as a dried powder, as all the other hydrocolloids are dried. At first, the brown color, produced by heat during the drying process, and grassy flavor of the mucilage powder should be ignored in favor of developing a desirable combination with other hydrocolloids, to find a practical formulation. Furthermore, techniques, such as scanning electron microscopy, might shine a light on whether there is a synergistic effect when mucilage is combined with other hydrocolloids in a sugar-based confectionary.

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