Masters Integrated in Bioengineering

Biological Engineering specialization

Development of vegan, sugar-free gummy candies - applicability to formulations with paracetamol

Master's Thesis

of

Teresa Isabel Araújo Gouveia

June 2018

Developed within the subject of Dissertation

Conducted with

DoctorGummy

The Healthier Candy Gurus

FEUP supervisors: Prof. Margarida Bastos, Prof. Fernando Rocha

DoctorGummy supervisor: Eng. Carmo Agostinho

Chemical Engineering Department of Faculty of Engineering of University of Porto

U.PORTO

FEUP FACULDADE DE ENGENHARIA UNIVERSIDADE DO PORTO

June 2018

"Strive not to be a success, but rather to be of value."

Albert Einstein

Acknowledgements

First of all, I owe a big thank you to my parents. Apart from the distance, they are always present and have always been by my side.

To my sister, Cristina Gouveia, who understands me better than anyone and that is always there supporting me and giving me the best advices.

A big thank you to Simão Chaves, the only one who was physically present during my 5 years' course, with whom I shared my achievements and frustrations, the one who gave me strength and motivation at the end of each day.

To Joana Fernandes, someone I see as a sister, someone with whom I passed the funnier moments of my life. Thanks for always making me laugh!

To all my friends, specially Margarida Simas, Carolina Rocha, Teresa Moleiro, Lussinga Costa, Laura Carvalho, Joana Silva, Mariana Rodrigues and Marina Coelho, with whom I went through the best moments of the last 5 years.

I would like to express my gratefulness to everyone that helped me doing this thesis:
To my advisors Dr. Margarida Bastos, Dr. Fernando Rocha and Dr. Carmo Agostinho, that were always ready to help with anything I needed; To Paulo Silva (Formulab), for giving us some samples of the ingredients and providing the prices of some reagents; To Inês Cardoso for helping me with the use of TGA/DSC equipment's; To Tânia de Carvalho for teaching me how to use of the vacuum oven; To Diana Paiva for teaching me how to use the DSC and the mechanical properties equipment's; To Fátima Ferreira for helping me with the use of the UV-VIS equipment; To Sofia Loureiro, my laboratory companion, the one who tested all the gummies before everyone.

A special thank you to DoctorGummy and FEUP for providing me the opportunity to realize this thesis, giving me everything that was needed both in terms of reagents, equipment and space to work.

Professors Margarida Bastos and Fernando Rocha, supervisors of this dissertation as an integrated member of LEPABE – Laboratory for Process Engineering, Environment, Biotechnology and Energy, financially supported by: project POCI-01-0145-FEDER-006939 (Laboratory for Process Engineering, Environment, Biotechnology and Energy – UID/EQU/00511/2013), funded by European Regional Development Fund (ERDF) through COMPETE2020 – Programa Operacional Competitividade e Internacionalização (POCI), and by national funds through FCT – Fundação para a Ciência e a Tecnologia; project "LEPABE-2-ECO-INNOVATION" – NORTE-01-0145-FEDER-000005, funded by Norte Portugal Regional Operational Programme (NORTE 2020), under PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF).

Resumo

Os mercados vegetarianos e vegano estão a crescer exponencialmente e cria-se cada vez mais uma necessidade de oferecer produtos de origem vegetal. As gomas são geralmente feitas à base de gelatina, uma proteína de origem animal, e em Portugal são muito limitadas as ofertas de gomas veganas, sendo que nenhuma delas é isenta de açúcar. O objetivo deste estudo consistiu no desenvolvimento de uma fórmula de gomas veganas e sem açúcar, tentando mimetizar da melhor forma possível a textura das gomas à base de gelatina.

Para isso, foram testados diferentes ingredientes e concentrações dos mesmos de forma a obter a textura pretendida, perfazendo um total de 69 fórmulas testadas. A seleção das melhores fórmulas baseou-se em testes sensoriais onde caraterísticas como a dureza, facilidade de quebra, opacidade, amargueza, travo e presença de grumos foram analisadas. Depois de escolhidas as três melhores fórmulas (C29, C40 e C41), foram estudadas as propriedades mecânicas e de o teor de humidade. As maiores diferenças entre as fórmulas selecionadas residiam não só no facto de terem concentrações diferentes dos ingredientes, mas no facto de apenas as formulações C40 e C41 conterem citrato trissódico. Todas elas utilizam carragenano como agente de gelificação. O teor de água foi determinado por secagem a 120 ºC e foi feito um estudo da evolução do mesmo numa estufa de vácuo a 55 e 65 ºC com medições frequentes da massa das gomas até massa constante. Em termos de resultados, as gomas apresentaram um teor de água de 27±1% em base húmida (wb) para a fórmula C41, 28±2% wb para a fórmula C40 e 52±2% wb para a fórmula C29. Através de testes de compressão e tensão foram avaliadas as propriedades: resistência à tração, elongação, módulo de elasticidade, rigidez, elasticidade, coesão e gomosidade. Os testes de tensão indicaram que a fórmula C29 é a mais resistente, suportando uma tensão de 0,13±0,03 MPa, com maior percentagem de elongação, 214±13% e módulo de elasticidade mais elevado ($4{,}6\times10^{-4}\pm8{,}0\times10^{-5}$ MPa). Esta diferença foi comprovada estatisticamente ($p>0{,}05$). Os testes de compressão indicaram que não há diferenças significativas entre amostras para a elasticidade (com valores a variar entre 0,76±0,12 e 0,90±0,08), coesão (com valores a variar entre 1,0±0,3 e 1,3±0,4), e gomosidade (com valores a variar entre 0,9±0,4 e 1,2±0,2), mas existe para a rigidez, sendo a C29 a goma mais rígida, com 1,2±0,2 N.

A estimativa de preço para a produção de 1 kg destas gomas foi de €1,76 para C29, €2,79 para C40 e €2,82 para C41, tendo em conta apenas os reagentes de cada formulação.

Foi possível a inclusão e quantificação de paracetamol nas gomas através da medição de absorção em UV-VIS a 247 nm de uma solução de goma com paracetamol em etanol: água (1:1, v/v), tendo sido obtido um valor final de 16,6±0,8 miligramas de paracetamol por grama de goma.

Palavras chave: gomas, vegan, sem açúcar, carragenano, paracetamol.

Abstract

The vegetarian and vegan markets are growing exponentially and there is an increasing need to offer products of plant origin. Gummies are usually made from gelatine, an animal origin protein, and in Portugal the offer of vegan gummies are very limited and none of which are sugar-free. The objective of this study was to develop a formula of vegan and sugar-free gummies, trying to mimic the texture of gelatine gummies as best as possible.

Different ingredients and concentrations of the same were tested in order to obtain the desired texture, making a total of 69 formulas tested. The selection of the best formulas was based on sensory tests where characteristics such as hardness, breakability, opacity, bitterness, aftertaste and presence of grumps were analysed and, after choosing the three best formulas (C29, C40 and C41), the mechanical properties and the moisture content were evaluated. The major differences among the selected formulas resided not only in the different concentrations of the ingredients, but also in the fact that only C40 and C41 formulations contain trisodium citrate. All of them use carrageenan as the binding agent. The water content was determined by drying the gummies at 120 °C and the evolution of the drying process was performed in a vacuum oven at 55 and 65 ºC with frequent measurements of the mass of the gummies until constant weight. In terms of results, formula C41 presented a water content value of 27±1% wet basis wb, formula C40 28±2% wb and formula C29 52±2% wb. Through tensile and compression tests, the following properties were evaluated: Tensile Strength, Elongation, Young's Modulus, Hardness, Springiness, Cohesiveness and Gumminess. The tensile tests indicated that C29 is the most resistant gummy, with a tensile strength of 0.13±0.03 MPa, with a higher percentage of elongation, 214±13% and a higher Young's modulus ($4.6\times10^{-4}\pm8.0\times10^{-5}$ MPa). This difference was statistically proven ($p>0.05$). The compression tests indicated that there were no significant differences between samples for springiness (with values varying between 0.76±0.12 and 0.90±0.08), cohesiveness (with values varying between 1.0 ± 0.3 and 1.3 ± 0.4), and gumminess (from 0.9±0.4 to 1.2±0.2), but exists for hardness, with C29 being the most rigid gummy, with 1.2±0.2 N.

The estimated price for the production of 1 kg of these gummies was €1.76 for C29, €2.79 for C40 and € 2.82 for C41, taking into account only the reagents of each formulation.

It was possible to include and quantify paracetamol in the gummies through the measurement of UV-VIS absorption at 247 nm of a gummy solution with paracetamol in ethanol: water (1:1, v/v), and a final value of 16.6±0.8 milligrams of paracetamol per gram of gum was obtained.

Key words: gummy candies, vegan, sugar-free, carrageenan, paracetamol.

Declaration

I declare, under an honour pledge, that this work is original and that all non-original contributions were duly referenced with source identification.

Date: 20-07-2018

Signature: Teresa Gouveia

Index

Figures list

Tables list

List of acronyms

a	Slope
Abs	Absorbance
ANJE	Associação Nacional de Jovens Empreendedores (National Association of Young Entrepreneurs)
b	Intercept
BMI	Body Mass Index
db	Dry basis
DSC	Differential Scanning Calorimetry
DTG	Differential thermogravimetric curve
FDA	Food and Drugs Administration
FTIR	Fourier-transform Infrared Spectroscopy
GAB	Guggenheim-Anderson-de Boer model
GLP-1	Glucagon-like Peptide 1
HM Pectin	High metoxyl pectin
I-carrageenan	Iota-carrageenan
IgE	Immunoglobulin E
K-carrageenan	Kappa-carrageenan
K-F	Karl-Fischer
LBG	Locust Bean Gum
LDL	Low-density lipoprotein
LM Pectin	Low metoxyl pectin
MC	Moisture Content
Sa	Standard Deviation of the Slope
Sb	Standard Deviation of the Intercept
TGA	Thermogravimetric analysis
UPTEC	Parque Científico e Tecnológico da Universidade do Porto (Science and Technology Park of the University of Porto)
UV-VIS	Ultraviolet-Visible
VLDL	Very-low-density lipoprotein
wb	Wet basis
xLD	Detection limit (concentration, mg/L)
xLQ	Quantification limit (concentration, mg/L)
yLD	Detection limit (Abs)
yLQ	Quantification limit (Abs)

1. Introduction

1.1. Theory of the enterprise and presentation of the project

The first gummy candies ever were created by Hans Riegel. In 1920, he was frustrated with his job and decided to create his own business, starting with hard, colourless candies. Some years after, he produced a line of soft, gelatine-based, fruit-flavoured treats in the shape of dancing bears. That was when Haribo brand was born (Burt, 2014).

DoctorGummy is an enterprise, born in Aveiro by Nuno Santos, a chemical engineer. It started in Shark Tank TV program, in 2015, where they got three investors, being able to start their business (Castro, 2018). Nowadays, they got two new investors, Sergio Silvestre, new executive president and Eduardo Rocha, owing 55% of the capital of the start-up (Nunes, 2016).

In July 2018, the executive administration of DoctorGummy started to be fully managed by Imperial- Produtos Alimentares S.A.

DoctorGummy started being commercialized in the final of August 2016 through internet and currently they sell through pharmacies, supermarkets and hypermarkets. In 2017 they sold more than 250 thousand units (Castro, 2018). They have already won some prizes and recognition and are currently getting bigger year-by-year. At the end of 2015 they received both the honourable mention under the Young Entrepreneur Award, promoted by the National Association of Young Entrepreneurs (ANJE) and the Creative Business Cup health innovation award in Copenhagen, Denmark (Nunes, 2016). In 2017 they were finalists of the innovation prize of NOS (Castro, 2018).

Part of DoctorGummy's profits is applied in support of institutions and projects in the social sector in areas such as health, education, entrepreneurship, innovation and sustainability (Castro, 2018). Nowadays, the company headquarters is situated in a research centre in Porto city, UPTEC. DoctorGummy sell healthy gummies in a fun and pedagogic context, without sugar, gluten or lactose.

Miyazaki et al. (2011) studied the possibility of delivering paracetamol in carrageenan gels to help patients with swallowing difficulties and they concluded that those gels have suitable rheological and sustained release characteristics for using as vehicles for oral delivering.

The main objective of this thesis is to develop both vegan, non-sugared gummies and study the possibility of adding an active agent, paracetamol, in the gummy formula. In this sense, the first step is to develop a vegan gummy formula, maintaining the characteristics of DoctorGummie’s actual formulas (without lactose, gluten or sugar, only with natural ingredients). After having a vegan formula, it will be tested the incorporation of paracetamol in the gummy.

1.2. The necessity of vegan gummies in the market

Gelatine is an ingredient which price is getting higher for two main reasons: the concern that the higher feed prices in some countries may lead to less availability of animals for gelatine production, and the new severer production standards in China that have increased the production costs. Besides that, the vegetarian and vegan markets are growing and there is a need to offer alternatives to gelatine based products (Gómez, 2013).

Still, gelatine can be an allergen since it can induce specific IgE antibodies (Wahl and Kleinhans, 1989) and modified gelatine used in some plasma substitutes can cause histamine release from blood basophils (Vervloet et al., 1983).

1.3. Components of a gummy candy

Gummy candies are composed by one or more binding agents (≅ 7%), sugars or/and sweeteners (≅ 88%), acids (≅ 2%), glazing agents (≅ 0.5%), additives (colouring and flavouring, ≅ 2.5%) and, in some cases, additional ingredients (such as vitamins, active ingredients, etc.) (Judy, 2010). The percentage of each ingredient varies with the type of the ingredient and the pretended final texture of the product (Burg, 1998; Yang and Oh, 1998).

1.3.1. Sugars and sweeteners used in the food industry

The research of the worldwide widespread of obesity continues and the relation between consumption of sugar-sweetened foods and body weight has become a problem nowadays (Malik et al, 2013).

In 2004, about 10% of all children were estimated to be overweight or obese (Johnson et al, 2007). There are many studies, such as Malik et al. (2013), or Jones et al. (1995), that reveal negative effects of sugar in children (overweight, bad behaviours due to an increase of their energy, feeling shaky and sweaty, fall in the plasma glucose concentration sufficient to induce hormonal, symptomatic, and neurophysiologic changes in healthy children, tooth decay, etc.).

A product is considered “high sugared” when sugar appears in one of the first 3 positions in the label ingredients of a food product. Table 1 presents a list with different types of sugars, that helps identifying sugar in the labels of the food products (Clifford et al., 2016). There are some sugars that may have different designations, although they refer to the same compound (e.g. dextrose and glucose). The compounds referred in “others” column, are usually an attempt to disguise the sugars through a more socially accepted designation, associated with “healthy foods”.

Table 1 Representation of sugars in product labels (Clifford et al., 2016)

Powders		Syrups	Others
Lactose	Anhydrous dextrose	Molasses	Fruit juice concentrate
Dextrose	Confectioner's sugar	Malt Suryp	Cane juice
Glucose	Date sugar	High Fructose Corn syrups	Corn juice
Beet sugar	Brown sugar	Corn syrups	Honey
Sucrose	Galactose	Brown rice syrup	Nectar
Maltose	Turbinado	Invert sugar	
Cane Sugar	Granulated sugar	Corn sweetener	
Fructose		Maple syrup	

Some sugars are naturally found in many foods such as fruits, vegetables, cereals, etc. Its existence is crucial in human body since certain tissues exclusively use sugar for obtaining energy (Clifford et al., 2016). However, sugar is a cariogenic food, which means that it induces the formation of dental caries. Besides that, Lustig et al., 2012 and other authors stated that sugars, when ate in excess, can have more serious consequences such as hypertension, diabetes, insulin resistance, ageing process caused by damaged lipids and liver toxicity.

Each year, about 15,000 tons of sweeteners are used worldwide, replacing about 13 million tons of white sugar (Hui, 2006). The Directive of Sweeteners says that a product is considered "with no sugar added" when it does not have any mono- or disaccharides used for its sweetening properties. In the other hand, a considered "energy reduced" product must have an energy value 30% lower compared with the original foodstuff (European Parliament and Council Directive on Sweeteners in Foodstuffs, 2003).

Polyols are carbohydrates that are not considered sugars but sugar alcohols and they are produced by the hydrogenation of simple to more-complex sugars (Deis, 2000). As polyols are known by their low calories, most of them are non-absorbed or slowly absorbed by human intestines (low glycaemic response, low caloric density) and therefore have laxative effects (Daly et al., 1993). Artificial sweeteners and sugar substitutes are natural or synthetic alternatives to sugar with a variable sweetener index. They usually have low or no calories however its high consumption may lead to some problems such as diarrhoea or headaches (Clifford et al., 2016). When some of them reach the colon, its microflora ferments them, reducing their energy value (Bornet, 1994).

Other properties of polyols include no cariogenic properties (they do not promote tooth decay); they can be less or sweeter than sucrose and are chemically stable. They do not contribute to Maillard browning or similar oxidative reactions (Deis, 2000).

Erythritol, for example, does not have side effects such as gas, bloating and diarrhoea (Bush, 2017; Gonze, 2000), as other polyols have. This small (four-carbon, tetritol) molecule is absorbed readily by diffusion, with approximately 10% escaping to the large intestine in men. Absorbed erythritol distributes widely through the tissues but its metabolism is minimal and, being poorly reabsorbed via the kidneys, it is essentially excreted in urine, which gives it almost 0 kcal.

(Livesey, 2003). Hiele et al., 1993 showed that after 24h of ingesting erythritol, 80% was recovered in urine.

Sweeteners can be divided into two categories: nutritive or non-nutritive. Basically, nutritive sweeteners are those which confers calories to the food product where it will be applied, and non-nutritive sweeteners have 0 kcal. The glycaemic index is related to the glucose release in the body (Carlson and Woo, 2004) and the Relative Sweetness is a comparation of the degree of sweetness of sugars and sweeteners, usually relative to sucrose (Relative Sweetness=1) (Biester et al., 1925). The chemical structures of the sweeteners are presented in Figure 1 and Table 2 shows some properties (Calories, Relative Sweetness and Glycaemic Index) of the main sweeteners used in the food industry.

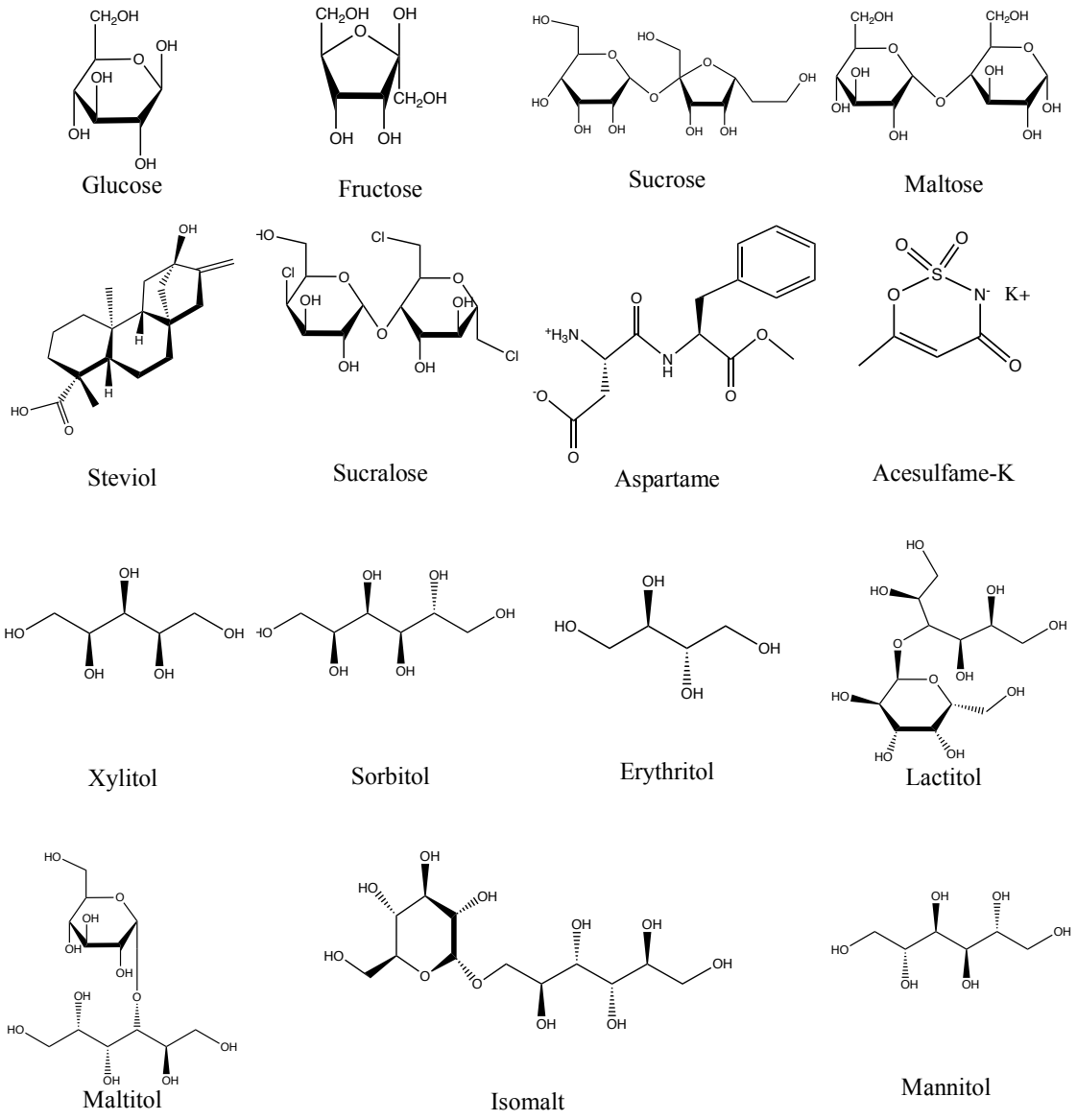


Figure 1 Sugars and sweeteners chemical structures (ChemDraw Professional, 2018)

As can be seen, sucralose presents the highest Relative Sweetness value, with no calories or glycaemic index. However, it is an artificial sweetener which has associated negative effects in human body, as will be described after. Stevia also presents very high Relative Sweetness (200-350), with no calories or glycaemic index. It is natural and there are no negative effects reported in human body.

The sugars/sweeteners with lower Relative Sweetness than sucrose is glucose, tapioca syrup, maltose, sorbitol, erythritol, lactitol, mannitol, maltitol and isomalt.

Table 2 Properties of some sugars/sweeteners

Name	Calories (kcal/gram)	Relative Sweetness	Glycaemic Index
Glucose	4.0	0.75	100
Fructose	4.0	1.70	23
Sucrose	4.0	1.00	65
Tapioca syrup	3.1	0.69	85
Maltose	4.0	0.30	105
Stevia	0.0	200-350	0
Sucralose	0.0	600	0
Aspartame	4.0	80.0	0
Acesulfame-K	0.0	200	0
Xylitol	2.4	1.00	12
Sorbitol	2.6	0.55	4
Erythritol	4.0	0.75	100
Lactitol	2.0	0.40	3
D-mannitol	1.6	0.50	2
Maltitol	2.4	0.90	35
Isomalt	2.1	0.50	2

The following sections present a summary of the main characteristics of these sweeteners and sugars.

- **Glucose/dextrose and polydextrose**

Glucose or dextrose ($C_6H_{12}O_6$), is a naturally occurring sugar in fruits and other parts of plants in its free state (National Toxicology Information, 2018).

Polydextrose is a polysaccharide synthesized by random polymerization of glucose, sorbitol, and a suitable acid catalyst. It is widely used in many countries as a bulking agent and as a lower-energy ingredient in a variety of prepared foods (Jie et al., 2000).

A study made with rats by Figdor and Rennhard (1981) demonstrated that polydextrose is not absorbed after oral ingestion. Approximately 30% of fed polydextrose is fermented in the lower gut by the intestinal microflora and the major portion (≈60%) is excreted in the faeces. Jie et al. (2000) also concluded that polydextrose ingestion had significant dietary fibber–like effects with no laxative problems in Chinese people.

An analysis of this compound showed that the typical composition of polydextrose includes at least 90% of polydextrose polymer and the rest of the components are glucose, sorbitol, levoglucosan, water and citric acid (Allingham, 1982) which means that a food composition with this ingredient may show a residual value of sugar due to glucose. However, FDA (Foods and Drugs Administration) approves it as a non-sugar sweetener and its safety has been carefully established (Allingham, 1982).

- **Fructose**

Fructose ($C_6H_{12}O_6$) is a natural sugar found in many fruits. It is sweeter than glucose or sucrose (Relative Sweetness of 1.7) and is therefore commonly used as a bulk sweetener (Rizkalla, 2010). Fructose is metabolized primarily in the liver, unlike glucose and so it does not lead to a significant increase in blood sugar levels (Clifford et al., 2016).

- **Sucrose**

Sucrose ($C_{12}H_{22}O_{11}$) is a nonreducing disaccharide composed of glucose and fructose linked via their anomeric carbons. Also known as "table sugar", it can be obtained either from sugar cane or from sugar beets. When eaten, the body hydrolyses sucrose into glucose and fructose and use them to obtain energy or to store as fat in the body (McCooey, 2016).

- **High Fructose Corn Syrups**

Syrups are sweeteners in the liquid form. One example is high fructose corn-based syrups, a concentrated, aqueous solution of nutritive saccharides, where some of its glucose is converted into fructose by glucose isomerase in many formulations (Clarke, 2003).

A high consumption of fructose syrups may increase triglyceride levels, decrease insulin sensitivity, and promote visceral adiposity (Ibarra et al., 2017). In terms of formulations, it prevents crystallization of the sucrose in the high solids (80% to 83%) content of the finished product. Another advantage of the syrups compared to the powdered sweeteners is that some of them go through an ion-exchange chromatography in order to concentrate the fructose content, removing minerals, catalytic metals, proteins or colours from the syrup that could lead to Maillard browning from powders (Clarke, 2003; Dow Chemical Company, 2002).

In terms of nutritional information, the composition of the syrup varies with the quantities and is very similar to fructose nutritional information (McCooey, 2016).

- **Tapioca syrup**

Tapioca naturally occurs in a brown skinned tuberous root known as cassava, which grows in Asia, Africa and South America (Nwachukwu and Ahunanya, 1985).

Tapioca syrup, obtained from tapioca starch hydrolysis, has been used more and more in the food industry as it has lower calories than sucrose (see Table 2 below for more details).

- **Maltose**

Maltose ($C_{12}H_{22}O_{11}$), sometimes called malt sugar, comes from the hydrolysis of starch and is a reducing disaccharide composed of two glucose units, only one of which is reducing. The

tendency to Maillard-colouration reactions is reduced by one-third, compared to glucose. Maltose is less sweeter than glucose (Farmer and Reusch, 2013).

Higher-maltose glucose syrups may be used to replace regular glucose syrups with several advantages for food confections, allowing the confection of solutions with less viscosity. Due to these properties, maltose syrup is one of the most suitable enzyme converted products for high-boiled confections (Jackson and Lees, 1973).

- **Stevia**

Stevia, $C_{20}H_{30}O_3$, is a sweetener and the Stevia plant was discovered by indigenous people in South America, through *Stevia rebaudiana* leaves. Its sweet tasting components are called steviol glycosides and are 200-350 times sweeter than sugar (Priscilla et al., 2017).

Initially, FDA did not permit the use of whole leaf Stevia or crude Stevia extracts because these substances were not approved for use as a food additive (Hsieh et al., 2003).

The preclinical and clinical applications of stevia were studied and it was concluded that it is not toxic and exhibit several biological activities such as antidiabetic, anticariogenic, antioxidant, hypotensive, antihypertensive, antimicrobial, anti-inflammatory, antiplaque and antitumor activities (Ruiz et al., 2017). Some other studies with rats administered with crude stevia extracts also demonstrated that Steviol, the sweetener component of stevia, do not poses a reproductive or developmental hazard (Curry and Roberts, 2008).

- **Sucralose**

Sucralose, $C_{12}H_{19}Cl_3O_8$, is one of the most used sweeteners in the food industry nowadays due to its good properties such as a good solubility either in water or alcohol (Schiffman and Rother, 2013; Stroka et al, 2003) and its stability at high temperatures and low pH values (Grotzand Munro, 2009). Sucralose is prepared either from sucrose (Chattopadhyay et al., 2014) or synthetically by various methods (Schiffman and Rother, 2013). Sensorial tests concluded that sucralose does not have the bitter aftertaste that other sweeteners have.

Besides sucralose has been approved in the European Union (EU) in 2004 for food purposes (Schiffman and Rother, 2013), there is a lot of controversy about the health effects of sucralose in human body. Grotz and Munro (2009) gathered the conclusions of several studies that prove that sucralose does not have any side effects in human health. They also corroborate these conclusions with physicochemical properties and pharmacokinetics studies; acute toxicity studies and tests during pregnancy were also done. All these tests concluded that sucralose is safe with no limitations for use (Grotz and Munro, 2009). Bowen et al., 1990 proved that sucralose is non-cariogenic in rats.

However, Schiffman and Rother (2013) stated that sucralose caused a diminish of beneficial bacterial in rats intestines. Several tests of the same authors also showed that one of its hydrolysis products ($C_6H_{10}Cl_2O_4$) is mutagenic and cooking sucralose at high temperatures generate chloropropanols, a potentially toxic class of compounds. Human studies verified that sucralose

may alter glucose, insulin, and glucagon-like peptide 1 (GLP-1) levels (which interferes with insulin production) (Schiffman and Rother, 2013).

- **Aspartame**

Aspartame, $C_{14}H_{18}N_2O_5$, is an artificial sweetener discovered in 1965. Aspartame may generate methanol by hydrolysis with pH variations. It is slightly soluble in water and the solubility increases with higher or lower pH as well as with increased temperatures. Its maximum stability is at pH 4.3. Aspartame is not very stable when heated so it should not be cooked (Chattopadhyay et al., 2014).

As in the case of sucralose, the health safety of aspartame has some controversy. National Toxicology Program, 2005 concluded that aspartame exposure was related with an increase in cancer both in male and female mice. However, some governmental research reviews indicate that aspartame has been found to be safe for human consumption (Chattopadhyay et al., 2014).

- **Acesulfame Potassium**

Acesulfame potassium, also known as Acessulfame-K ($C_4H_4KNO_4S$), is a synthetic sweetener. It is stable at high temperatures and has showed to have synergetic effects when joined with aspartame or sucralose (Chattopadhyay et al., 2014).

According to Chattopadhyay et al. (2014), Acesulfame-K is not metabolized in human organism, so it provides no calories (0 kcal). One breakdown product of Acesulfame-K when hydrolysed in human organism is acetoacetamide, toxic if consumed in high amounts. However, those quantities are barely null and therefore FDA concluded that Acesulfame-K does not have any side effects in terms of health (Chattopadhyay et al., 2014).

However, Bian et al. (2017) stated that Acesulfame-K consumption altered the gut bacterial community composition in mice, enriched the functional bacterial genes related to energy metabolism and showed metabolomic changes in faecal, especially in male mice.

- **Xylitol**

Xylitol, $C_5H_{12}O_5$, is a five-carbon sugar alcohol and is naturally found in many fruits (strawberries, plums, raspberries) and vegetables (cauliflower, for example) (National Center for Biotechnology Information, 2018).

Xylitol has showed to prevent caries in children of mothers who used xylitol gum during pregnancy by reducing maternal transmission of *Streptococcus* mutants for their children, a bacterium responsible for dental decay that is vertically transmitted during and after birth (Silva Bastos et al., 2015). In teens and young adults, it has also proved to be non-cariogenic and anti-cariogenic (Isokangas et al., 2000; Lund, 2008). It has also been proved by Shafer et al., 1987 to decrease food intake and also delay gastric emptying.

- **Sorbitol**

Sorbitol, $C_6H_{14}O_6$, is a six-carbon sugar alcohol that occurs naturally from apples, pears, peaches, etc. and also be produced synthetically from glucose or lactose (National Center for Biotechnology Information, 2018; Kuusisto et al., 2007).

However, its ingestion should not exceed 20 mg a day due to its laxative effects; sorbitol is actually used with this purpose in certain medicines. Another charactecteristics of sorbitol is that some people are allergic to it, which becomes a disadvantage when talking about food formulations (McCooey, 2016).

- **Erythritol**

Erythritol, $C_4H_{10}O_4$, is a four-carbon polyol and it occurs naturally in a wide variety of fruits and vegetables (mushrooms, watermelon, pears, grapes, soy sauce, sake, and beer at levels up to 0.13%). It can be manufactured by fermentation from glucose and sucrose by *Trichosporonoides megachiliensis* (Chattopadhyay et al., 2014; Gonze, 2000) keeping similar taste and functional properties to sucrose.

Erythritol is less soluble in water compared to other sweeteners and sugars talked previously (Corporation, 1998).

Boesten et al. (2015) reviewed several studies about erythritol safety and they concluded that it can be defined as a non-toxic food, with no effects on carcinogenicity or other health problems. In addition, it was verified to be among the best sugar replacer for people with diabetes or pre-diabetes. As stated before, erythritol does not have laxative effects as other sweeteners do.

- **Lactitol**

Lactitol, $C_{12}H_{24}O_{11}$, is obtained from a catalytic hydrogenation of lactose (Bornet, 1994; Kuusisto et al., 2007). It is suitable for development of sugar-free, reduced calorie and low glycaemic index products, with non-cariogenic and prebiotic properties (Hayashibara and Sugimoto, 1976).

Nowadays, lactitol is used in the treatment of chronic constipation and it was found to have better results than lactulose for that purpose (Maydeo, 2010).

- **Mannitol**

Mannitol, $C_6H_{14}O_6$, an isomer of sorbitol, is derived from mannose and is naturally present in plants and some algae but it can also be produced by fungi and bacteria from glucose, fructose or sucrose (Dobbing, 2012). Mannitol is widely used in the pharmaceutical industry (as a tablet excipient, as an osmotic diuretic in the treatment of cerebral edema, for reducing intraocular pressure and as a laxative) (Schiweck et al., 2000).

Abdo et al., 1986 stated that the addition of 2.5 and 5% mannitol to the food of rats and mice, respectively, for a period of two years, exhibited no carcinogenic properties (Abdo et al., 1986; Schiweck et al., 2000).

- **Maltitol**

Maltitol, $C_{12}H_{24}O_{11}$, is produced by hydrogenation of maltose obtained from starch and maltitol syrup is acquired by chromatographic separation on ion-exchange resins from high-maltose glucose corn syrups (Schiweck et al., 2000). It exists as a syrup and in its powdered form (Dobbing, 2012).

Thabuis et al. (2013) concluded that maltitol and xylitol chewing gums ingestion for 30 days in children between 13-15 years old reduced the concentration of 4 cariogenic bacteria species (*S. mutants, S. sobrinus, A. viscosus* and *Lactobacillus*) in dental plaque compared to gum base.

- **Isomalt**

Isomalt, $C_{12}H_{24}O_{11}$, is a substitute for sugar widely used in the food industry as a low-glycaemic and low-energy sweetener (Gostner et al., 2007; Thiébaud et al., 1984).

Isomalt has a low digestibility in the small intestine, so a major fraction of the polyol isomalt reaches the colon. Gostner et al. (2007) concluded that isomalt must be considered a prebiotic carbohydrate that might contribute to a healthy luminal environment of the colonic (prebiotics are no digestible food ingredients that benefit the host by selectively stimulating the growth or activity of one or a limited number of bacteria in the colon (Klingeberg et al., 2004; Roberfroid, 2000).

1.3.2. Binding agents used in the food industry

Binding Agents are any ingredient that can help a mixture hold its shape or remain bound together. Gelatine, an animal origin compound, is the most used in the food industry but there are other alternatives to gelatine called hydrocolloids ("range of polysaccharides and proteins that are nowadays widely used in a variety of industrial sectors to perform a number of functions (…)" (Williams and Phillips, 2009) from different sources (see Table 3).

The total substitution of gelatine has been done with pectin, agar-agar and carrageenan by themselves or combined with modified starches (Grazela and Morrison, 2002; Hui, 2006), but none of these formulations were able to achieve the chewy and elastic structure that gelatine provides (Grazela and Morrison, 2002).

Table 3 Source of commercially important hydrocolloids (Williams and Phillips, 2009)

Type	Font	Hydrocolloid
Botanical	Trees	Cellulose
	Tree gum exudates	Gum Arabic, gum karaya, gum ghatti, gum tragacanth
	Plants	Corn starch, pectin, cellulose
	Seeds	Guar gum, Locust bean gum, tara gum, tamarind gum
	Tubers	Konjac mannan, potatoes and tapioca starches
Algal	Red seaweeds	Agar, carrageenan
	Brown seaweeds	Alginate
Microbial		Xanthan gum, curdlan, dextran, gellan gum, cellulose
Animal		Gelatine, caseinate, whey protein, soy protein, albumin, chitosan

The following sections describe succinctly some of these hydrocolloids.

- **Gelatine**

The most common gelling agent in gummies is a protein, gelatine, which comes from both cow or pork and provides a chewy structure to food confections (Grazela and Morrison, 2002).

Gelatine forms thermally reversible gels, which can front shelf-life problems in hot days. At gelling temperatures, it can re-form helical regions in the chains, trapping water. The larger the relative size of the gelatine molecular chains, the greater the gel strength. Higher concentrations of gelatine also increase gel strength (Burg, 1998). This is an advantage of gelatine: the possibility of making either jellies or gummy candies with it, varying gelatine concentration.

Gel strength is important to achieve and keep a gummy structure, because too high temperatures and very acidic conditions tend to degrade gelatines. Under processing conditions, the gummy formulation must be quickly prepared and promptly cooled to prevent gel-structure loss. There are two types of gelatine (see Table 4):

Table 4. Types of gelatine

	Origin	Observation	Price
Type A	Pork skin	More used	More expensive
Type B	Alkali hydrolysed derived from beef skin or bone	Tendency toward Mailard-reaction browning at cook T>110 ºC	Cheaper

The gelled structure that gelatine forms is able to hold flavours but the addition of other compounds (such as pectin), helps it to release the favour more, making the candies more tasty (Burg, 1998). Also, important to modifying and delivering defined fruit notes are food acids, such as citric acid and malic acid, which also are required to develop gel structures (Burg, 1998).

- **Arabic Gum**

Gum Arabic is derived from exudates of some trees (called Acacia) and a possible representation of its structure is presented in Figure 2. It consists of a mixture of galactose (A), arabinose (B), rhamnose (C) and glucuronic acid (D) liked to an arabinogalactan-protein complex. However, its composition can vary with its source, climate and soil (Williams and Phillips, 2009). There are various types of arabic gum, according to its origin, such as *Acacia senegal* var. *senegal* gum (ASG), *Acacia seyal* var. *seyal* gum (ASY), *Acacia mellifera* gum (AMF) and *Acacia tortilis* var. *raddiana* gum (ATR).

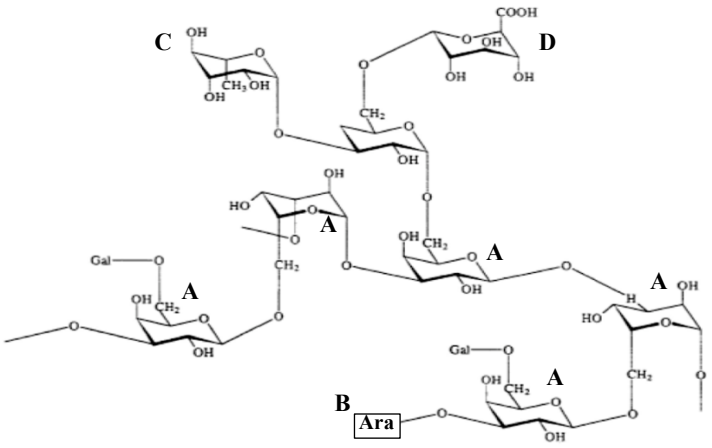


Figure 2 Arabic Gum structure (Hobby Takt, 2005)

Arabic gum is used as an emulsifier, thickening agent and flavour stabilizer in both pharmaceutical and food industries. It is also used in textile, pottery and cosmetic industries (Babiker et al., 2012). As a raw material, arabic gum is available in the form of odorless, brittle, dried sap chunks, without brown appearance. For easy handling and processing, as well as for advantageous storage characteristics, the raw material is generally further processed into powder form (Daneshvari, 2013).

Arabic gum has the ability to retard or inhibit sugar crystallization and is used when the sugar content is very high. It can also reduce fat in a foodstuff when combined with food starch and alginates (Williams and Phillips, 2009).

Babiker et al. (2012) studied the effects of regular gum arabic (GA) ingestion on body mass index (BMI) and body fat percentage among healthy adult females. They concluded that, after ingesting 30 mg/day of gum arabic for six weeks, the value not only of BMI but also of body fat have decreased.

- **Agar-agar**

Agar-agar is a hydrophilic colloid extracted from certain seaweeds of the *Rhodophyceae* group of algae. It is a heterogeneous mixture of two types of polysaccharides: agarose and agaropectin. Agarose, the jellifying fraction, is a neutral, essentially sulphate-free, linear molecule consisting of repeat chains of alternating units of galactose (A) and 3,6-anydro-L-galactose (B). Agaropectin, the non-gelling fraction, is a sulphated polysaccharide (3% to 10% sulphate) composed of agarose and varying percentages of sulphate ester, glucuronic acid and small amounts of pyruvic acid. Agarose represents 70% of the total and its structure is presented in Figure 3 (Armisen and Galatas, 2010; Wankenne, 2008). The type of agar-agar varies according to the seaweed where it comes from, and it confers different characteristics to products (Ármisen, 1987).

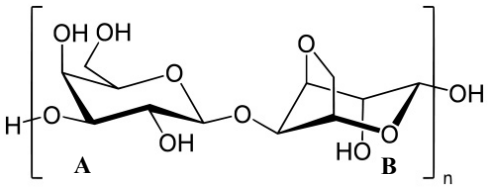


Figure 3 Molecule of agarose from agar (Armisén and Gaiatas, 2009)

The use of agar-agar in foods is based on its inherent properties (high gelling capacities, wide pH working range, resistance to heat treatment, large hysteresis, no effect on flavours and formation of stable reversible gels) (Hispaganar, 2013). A hot solution with 1.5% agar when is cooled to 34-43°C, forms a firm gel which does not melt again below 85°C (Ármisen, 1987).

Relative to the effect of agar on feed, Lee et al., 2014 stated that agar-agar has excellent anti-obesity and anti-diabetes effects, and the administration or intake of a composition with agar can effectively prevent, treat, and remedy obesity and diabetes.

- **Locust Bean Gum**

Locust bean gum (LBG) comes from a tree, called *Ceratonia siliqua* which is a polygamous, thermophilus and typical evergreen species of the leguminous tree. The part of that tree that has more value is the seed of its fruit; that is what origins locust bean gum.

Galactomannan, a compound comprising more than 90% of the seed, is a linear polysaccharide that contains about 20% of D-galactose (A) and 80% of D-mannose (B), organised in a mannose linear backbone and galactose residues (Dakia, 2011). It is represented in Figure 4 (Dionísio and Grenha, 2012).

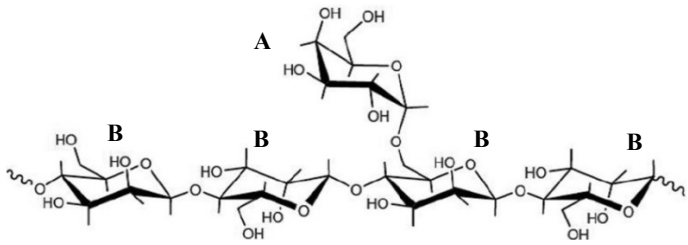


Figure 4 Locust bean gum structure (Dionísio and Grenha, 2012)

This gum is already used in the food industry (as an additive due to its abilities providing high viscosity at low concentrations (0.1-1%), as well as water binding), textile and paper industries (as a strengthening agent) and pharmaceutical industries (controlled release of activity in various pharmaceutical and cosmetic products) (Dakia, 2011; Miles et al., 1984).

The European Commission Scientific Committee for Food indicates that LBG can be used to treat Gastroesophageal reflux very common in young infants, by thickening their food formulas (Savino et al., 1999). A two-year study was realized with rats by Melnick et al. (1983), giving them LBG as 5% of their diet, and they showed that no carcinogenic or other toxic effects were observed.

- **Xanthan Gum**

Xanthan gum is produced by the bacteria *Xanthomonas campestris*, which can be naturally found in the leaves of some vegetables such as cabbage. This gum is synthetically produced from a pure culture of the same bacteria (Sworn, 2009).

Its primary structure consists in a linear glucose backbone (A) with a trisaccharide side chain on every other glucose at C-3, with glucuronic acid (B) and mannose (C) (Sworn, 2009) and its molecular structure is presented in Figure 5 (Muzzarelli and Muzzarelli, 2009).

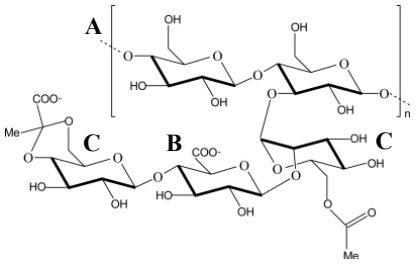


Figure 5 Xanthan gum structure (Muzzarelli and Muzzarelli, 2009)

Xanthan gum is widely used as a food gum because it produces high viscosity solutions at low gum concentrations; there is no apparent change in solution viscosity between 0 to 100 °C and it is stable in acidic systems (Demodaran et al., 2008). This gum is mostly used as a stabilizer of suspensions and emulsions and it gives stability to products exposed to freezing and thawing (Demodaran et al., 2008). To dissolve xanthan gum in water, Scariotto (2013) stated that its dissolution is better at high temperatures, but the most important parameter is the agitation.

In order to study the health effects of Xanthan gum, it was studied the ingestion of muffins with xanthan gum for 6 weeks (12 g a day) to 12 people, 9 of them with diabetes, showing that the total plasma cholesterol, levels of gastrin and gastric inhibitory polypeptide and VLDL (very low-density lipoproteins) triglyceride and cholesterol in VLDL and LDL (low density lipoproteins) fractions lowered (Osilesi et al., 1985).

- **Carrageenan Gum**

Carrageenan is an hydrocolloid derived from red edible seaweeds (from different *Rhodophyta* species), primarily used as a stabilizer and thickener in food (Weiner, 2014). There are many types of carrageenan, such as: Kappa (k), Iota (I), Lambda (λ), Beta (β) and respective hybrids (Weiner, 2014), being its structure based on a repeating disaccharide (2 galactopyranose molecules (A and B) where B residue can be converted into 3,6-anydrogalctose) represented in Figure 6). The residual composition of carrageenan is variable (with the source of the algae and extracts). The helix forming residues are β, K or I units which all contain the 3,6-anhydro form of the B-unit, but differ only in the number of sulphate substituent (R1 and R2- Figure 6) (Phillips, 1996).

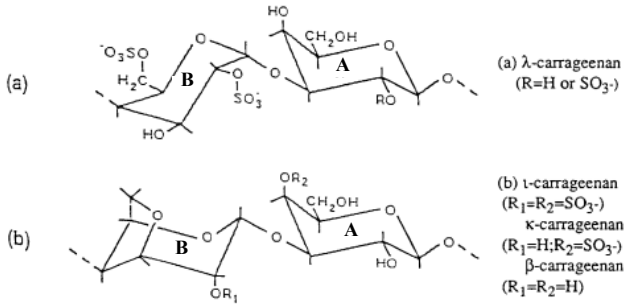


Figure 6 Carrageenan types structure (Phillips, 1996)

Carrageenan type Lambda is the most sulphated one and does not form gels by itself but has good sensorial characteristics (like fat). The mixture of carrageenan kappa-II (k) and Iota (I) is usually used by their jellifying and thickening properties (Deosen Biochemical, 2015) (with at least 50% iota (Grazela and Morrison, 2002)).

Carrageenan can also be used in other non-food industries due to its properties, such as: thickening effect, film forming ability and diffusion rate. E.g. as a thickener in toothpaste, as an excellent conditioner in shampoo, as well as a suitable tablet coating agent (Cpkelco, 2001).

Carrageenan cannot be neither digested by human intestinal enzymes nor broken down (fermented) by beneficial large intestinal bacteria, so it is not likely absorbed and provides no calories (Tobacman, 2001).

- **Pectin**

Pectin is a polysaccharide presented in the cell wall of plants, especially citrus fruits and beet pulp (Nguyen et al., 2011).

Pectin is a polymer containing at least 65% by weight of galacturonic acid units linked to galactosyluronic residues in an galactosidic chain (TutorVista, 2018). These uronic acids have carboxyl groups (A), some of which are naturally present as methyl esters (B) and others as carboxamide groups (C) when are commercially treated with ammonia (see structure in Figure 7, where A, B and C are possible carboxyl substitutes in pectin) (Sundar Raj et al., 2012).

The type of pectin varies according to their degree of esterification (DE) or amidation (DA) of the galacturonic acid residues. High methoxy pectin (HM pectin) have an esterification level above 50% and they are able to form gels when present with sugar and acid. Low methoxy pectin (LM pectin) have esterification levels below 50% (Nguyen et al., 2011).

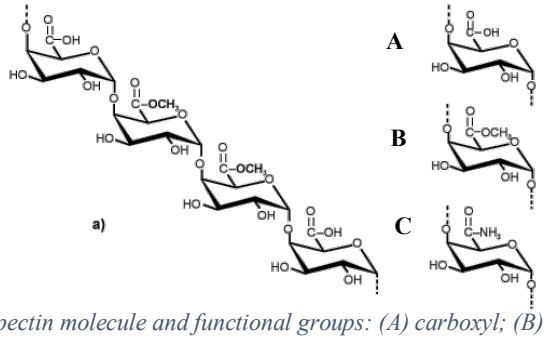


Figure 7 Repeating segment of pectin molecule and functional groups: (A) carboxyl; (B) ester; (C) amide in pectin chain (Sundar Raj et al., 2012)

An advantage of using pectin is that it forms gels that melt only at very high temperatures, what protects the product during shipping when temperatures are high (Burg, 1998).

According to Embrapa (1998), the addition of sweeteners affects the pectin/water balance, destabilizing pectin conglomerates and forming a network of fibbers, which makes up the gel, whose structure is able to withstand liquids. The rigidity of the structure is affected by the concentration of sugar and the acidity.

Pectin has beneficial health effects in human body not only due to the fact that it is a fibber, but there are studies that show that pectin can lower cholesterol levels, serum glucose levels and may also have anti-cancer effects (Yamada et al., 2003).

- **Guar Gum**

Guar gum is derived from the seed of the guar plant, *Cyanaposis tetragonolobus,* and it is composed by linear chains of mannose (A) with galactose (B) attached by (1-6) linkages, owning hydroxyl groups (Dodi et al., 2011). Its structure is presented in Figure 8 (Trivedi et al., 2014).

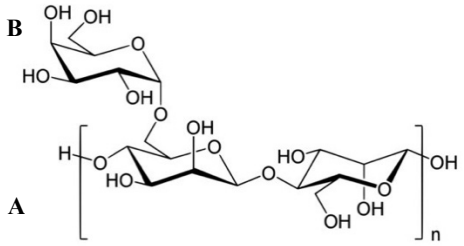


Figure 8 Guar gum structure (Trivedi et al., 2014)

Guar gum is used as an additive in food, textile (it allows the production of sharply printed patterns because it thickens the dye solutions), oil well drilling (fracturing fluids), cosmetics, etc. (Dodi et al., 2011; Mudgil et al., 2014).

Studies suggest that the use of this gum in the food industry may have beneficial effects in human health. In an attempt to verify the health effects of Guar gum in men, it was fed 10 g/3 times a day of guar or placebo granulate for 6 weeks with a 2-week run-in before and a 2-week wash-out period (period without guar gum). The conclusions revealed a decrease in blood sugar, cholesterol, triglycerides and plasminogen activator inhibitor-1 (PA1) activity (elevated PAI-1 is a risk factor for thrombosis and atherosclerosis). Insulin sensitivity increased, adipose tissue-glucose uptake increased, and 24-h urinary excretion of sodium and potassium increased during guar treatment (Landin et al., 1992).

Yamamoto et al. (2000) showed that a mixture of xanthan gum and guar gum has an improved hypolipidemic effect on non-diabetic and diabetic rats.

- **Starch**

Starch is present in vegetables and gives about 70% of the calories consumed by humans, since they can easily be found in roots, tubers and cereals (Rocha, 2016). Nowadays, there are lots of chemically and physically modified starches, adequate to several applications (Peroni,

2003). Chemical modification of starches, for example, includes acetylation, cationization, acid hydrolysis, oxidation and cross linking. However, there are much more modifications that have been extensively reviewed by Din et al., 2015.

From native granules, two components can be extracted: Amylose (A), which is a predominant glucan and Amylopectin (B), a glucan with occasional glyosidic bonds, which are responsible for the branching (Din et al., 2015; Farmer and Reusch, 2016). Native starches consist of about 10%–30% amylose and 70%–90% amylopectin (Farmer and Reusch, 2016). Their structures are presented in Figure 9.

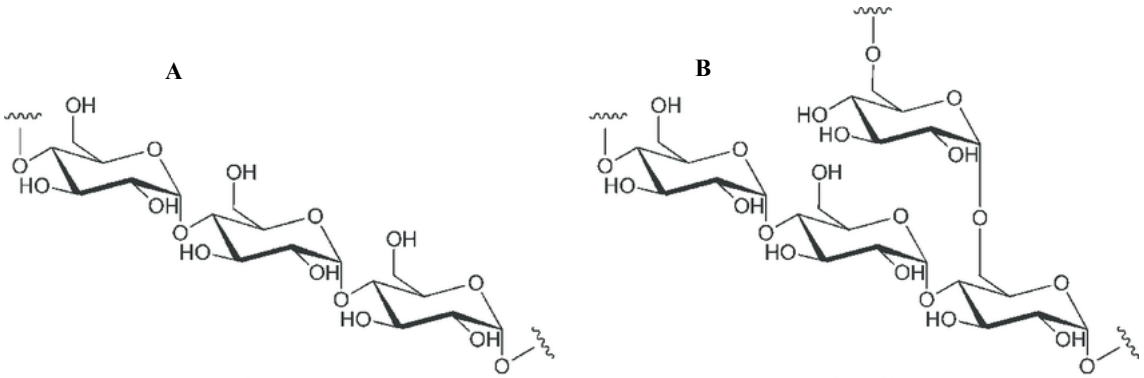


Figure 9 Amylose (A) and Amylopectin (B) structures (Farmer and Reusch, 2016)

In terms of health effects, starches are an important source of energy for humans and they are also known for their beneficial interactions in the digestive system (some chemically modified starches can be used to manipulate the bacteria in the gut) and other starches can even decrease the cariogenesis in the mouth (Dobbing, 2012). The health effects of starch in human body will depend on its modification. For example, there is a very oxidized corn starch that produced a low weight gain in male and female rats that were fed daily 5 g of a balanced diet supplemented with 1 or 2 g of oxidized corn starch compared to native starch or even less modified starch during the same period, concluded Whistler and Belfort (1961).

- **Gellan Gum**

Gellan gum is produced by the bacterium *Pseudomonas elodea* and is constituted by glucose (A), glucuronic acid (B) and rhamnose (C) in the molar ratios of 2:1:1 (Sanderson, 1990) (see Figure 10).

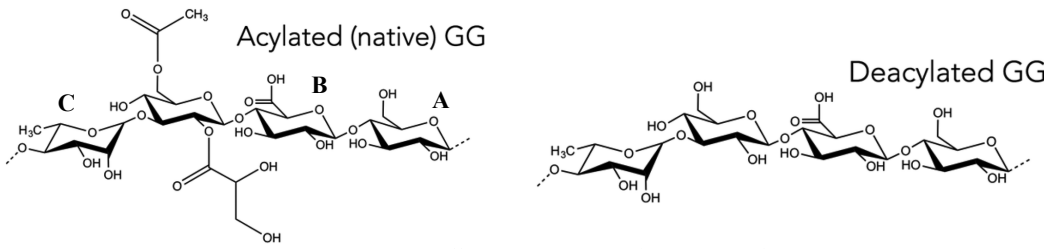


Figure 10 Gellan gum structure (Sworn, 2009)

There are two types of this gum: low acyl and high acyl. High acyl gellan gum is the native form, while low acyl form is a deacylated one (Sworn, 2009). The main differences between these types of gellan gum are presented in Table 5 (Bar-Shalom et al., 2006).

Table 5. Comparison of physical properties of High Acyl and Low Acyl Gellan Gum (Bar-Shalom et al., 2006)

	High Acyl	Low acyl
Molecular Weight	(1-2)x10^6 Daltons	(2-3)x10^5 Daltons
Solubility	Hot water	Hot or cold water
Set temperature	70°- 80 °C	30°- 50 °C
Thermo reversibility	Thermo-reversible	Heat stable

The high acyl form has two substituents: acetate and glycerate. In low acyl gellan gum, there is no acyl groups (Bar-Shalom et al., 2006). The same authors stated that "the high acyl form produces soft, elastic, non-brittle gels, whereas the low acyl form produces firm, non-elastics, and brittle gels". Native gellan gum on heating and cooling in the presence of cations forms cohesive, elastic gels similar to those obtained by heating and cooling mixtures of xanthan gum and carob-bean gum (Sanderson, 1990).

Regarding the health effects of Gellan gum, a 23-day test realized by Anderson at al., 1988, 5 male and 5 female humans ingested gellan gum and it was concluded that no adverse dietary or physiological effects were caused. Another study with rat cells showed that gellan gum can be used as cells encapsulating agents in cartilage regeneration approaches, since the human nasal chondrocytes were efficiently encapsulated in the gellan gels (Oliveira et al., 2010).

1.3.3. Synergies between binding agents

The mixture of some binding agents is known by their synergetic effects, allowing the development of different textures in gels.

When locust bean gum is mixed with k-carrageenan, it creates synergistic interactions, diminishing the polymer concentrations needed to promote jellification compared to k-carrageenan by itself (see interaction model in Figure 11-i) (Miles et al., 1984). Another advantage of this interaction is that the carrageenan gel texture gets additional desirable characteristics such as reduced brittleness and increased softness, when locust bean gum is added (Cheney et al., 1979). At pH values below 5.0, the carrageenan/glucomannan mixture produces thermo-reversible gels and the presence of potassium ions usually produces an increase in the toughness of the gel (Cheney et al., 1979).

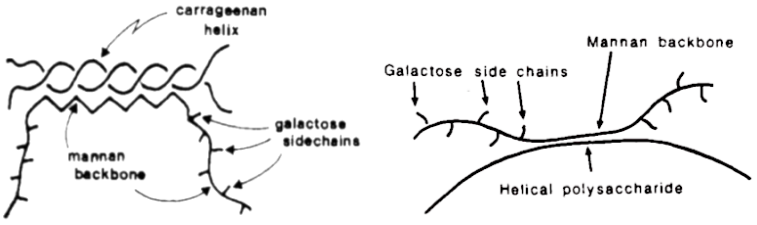


Figure 11 Interaction of LBG/ k-carrageenan (i) and Xanthan gum/LBG (ii) (Cairns et al., 1986; Miles et al., 1984)

Xanthan gum has excellent compatibility with salts and forms synergic gels when used in combination with Locust bean Gum. Cairns et al., 1986 showed that LBG can establish interactions with xanthan gum (see Figure 11-ii) when the mixture is heated above xanthan helix-coil transition temperature (95 ºC) and then re-cooled to room temperature (Cairns et al., 1986).

Grazela and Morrison (2002) suggested the use of iota-carrageenan with other hydrocolloids such as agar-agar, xanthan gum, locust bean gum, gellan gum, gum arabic, pectin, gelatine, kappa-carrageenan, guar gum, or modified or unmodified starches for food products. They also demonstrated that a formulation with carrageenan as a binding agent showed excellent elasticity and cohesiveness but low hardness. Carrageenan gum forms thermo-reversible gels in the presence of potassium (carrageenan I or K) or calcium (carrageenan I). The gel formation starts with the connection of the carrageenan I and K molecules forming a helix as showed in Figure 12.

The US Food and Drug Administration (FDA) allows the use of ammonium, calcium, potassium and sodium carrageenan in foods (Administration, 2013).

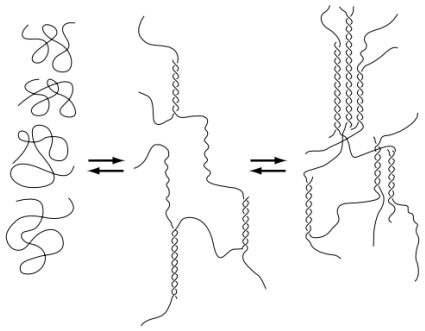


Figure 12 Gelation mechanism of kappa and iota carrageenan's (Demodaran et al., 2008)

Another synergetic effect is found when mixtured gelatine with pectin: Burg (1998) stated that the remelt temperature of a solution with 0.5% of pectin and 6.5% of gelatine, will increase from about 30 ºC to about 40 ºC compared to a 7.0% gelatine solution. In gelatine/pectin formulations, the critical point is to maintain the pH of the product between 4.5 and 5.0, and lowering to be in the range of 3.0 to 3.3 prior to depositing, to avoid co-precipitation of the binding agents (the acid thickens the network fibbers, but the high acidity affects the elasticity, due to the hydrolysis of the pectin) (Embrapa, 1998).

These synergistic interactions have some advantages like: reducing costs when one of the polymers is expensive, they are commercially attractive and mixtures offer the potential for creating new textures or manipulating the rheology and texture of the final gum (Miles et al., 1984).

1.3.4. Acids

Acids are added for numerous purposes in foods where they provide the benefits of many of their natural actions. The main reasons to use acids in gummy formulations are not only to conservate the final product from microorganisms but also for flavouring (Hartel et al., 2018) and

guaranteeing that the final pH is adequate for a gummy product (Demodaran et al., 2008). Ascorbic and citric acids are the most common ones used in the food industry.

The pH of gummy confections is one of the most important parameters since it directly affects the hardness of the final product. For example, HM pectin need an acidic environment so that they can set. When the pH is reduced, HM pectin sets into a gel very quickly (Hartel et al., 2018). However, a pH below 3 will hydrolyse pectin gels, in spite of thickening the network fibbers as it does in a higher pH (Embrapa, 1998). Another example is carrageenan, that hydrolyses at pH below 3.5 (Iglauer et al., 2011). According to several authors the final pH of gummy confections must be around 3.0-4.0, depending on the binding agent used (Minnesota Dental Association, 2017; Lefkowitz, 2010; Yaranossian and Lynch, 2014).

Sometimes the use of a second acid may help building the flavour profile (e.g. the main acid for an apple flavour is malic acid and the second one, tartaric or fumaric acids; for a strawberry taste, it should be used citric acid as the main one and malic or tartaric acids as the second acid) (Jarret, 2012).

The intensity and duration of the flavour also alters with the acid used, being lactic acid the one with more intensity and latest taste duration (Jarret, 2012).

1.3.5. Glazing agents

Glazing agents are usually waxes and oils that are sprayed onto the final product with the aim of detach gummies from each other and make them shinier (Ervin and Ervin, 2011; Yamabe et al., 2017). Carnauba wax, bee wax and vegetable oils (coconut, sunflower and palm oils) are among the most common glazing agents used in the food industry.

1.4. Paracetamol in a gummy

Giving children an oral medicine may be very difficult. Polaha et al., 2008 studied the medication acceptance among children and adolescents; they concluded that 30-40% of them rejected pill or liquid formulations and >50% of them were actually unable to swallow either a standard size pill or a small capsule.

There are some tips such as crushing and mixing with food, dissolving the tablet into a liquid or creating minitablets to make them swallow easier (Gupta, 2015; Schiele et al., 2013; Thomson et al., 2009). Another way to help children to take their medicines is making them good tasting and easy to swallow: in a gummy formulation.

Fever and pain occurs regularly in children (Martino and Chiarugi, 2015). Acetaminophen, also known as paracetamol (structure in Figure 13), is the most frequently used analgesic and antipyretic drug worldwide (Abdel-Daim et al., 2017) for management of fever and pain (Martino and Chiarugi, 2015).

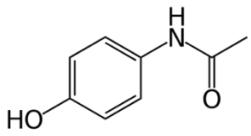


Figure 13. Paracetamol structure

The recommended dose of paracetamol for children, according to their age and body weight, is presented in the Table 6 (Infarmed, 2014).

Table 6 Recommended dosage of paracetamol for children (Infarmed, 2014)

Age	Body Weight	Paracetamol Dosage per take	Maximum dosage in 24 h
3-6 months	Until 7 kg	70-100 mg	350 mg
7-12 months	8-10 kg	100-150 mg	500 mg
2-3- years	11-15 kg	150-200 mg	750 mg
4-6 years	16-22 kg	200-300 mg	1000 mg
7-9 years	23-30 kg	300-500 mg	1500 mg
10-12 years	31-40 kg	400-600 mg	2000 mg
More than 12 years	More than 41 kg	500-1000 mg	3000 mg

As paracetamol is one of the most used drugs in children and DoctorGummy is specialized in gummies for children, it has emerged the idea of creating gummies with a controlled concentration of paracetamol per gummy, facilitating its administration both from parents and hospitals to kids.

Foods that have a physiologic benefit or prevents diseases such as anti-ageing, vitamins, etc. (Nasri et al., 2014) are called nutraceuticals; however, they must be natural and with no drugs included (Hardy, 2000). This means that a gummy candy with paracetamol cannot be called as a nutraceutical but as a medicine.

Paracetamol in a tablet form is rapidly absorbed from the human duodenum with a half-life in blood of 1.5–3.0 h (Abdel-Daim et al., 2017).

One of the most important properties of paracetamol for this project is its solubility in water since it will be solubilized in a solution where water is its major component. Thus, it was verified that paracetamol is barely soluble in cold water (O'Neil, 2001) but 14 g can be dissolved in 1 L of water at 25 ºC (DrugBank, 2018; Yalkowsky et al., 2010). O'Neil, 2001 also stated that paracetamol is soluble in boiling water. According to Medeiros et al., 2007, pure paracetamol melts between 168-172 ºC. Other authors confirmed this value: Hoppu et al., 2007 stating a melting point of 169 ºC and National Toxicology Program, 1993 a range between 169-170 ºC.

There is no evidence that paracetamol will be stable at pH=3.0-4.0. It is written that this compound lacks functional groups that hydrolyse under environmental conditions (pH 5 to 9) (TOXNET). However, it is known that paracetamol is an acidic drug. As its pKa is around 9.38 (Ionescu et al., 2015), at pH around 4 it acquires a protonated form and so it is not ionized.

Described methods to detect the presence of paracetamol include High Performance Liquid Chromatography, Gas Chromatography and Colourimetry (TOXNET, 2015). The method for quantifying paracetamol in this study will be an Ultraviolet Visible measurement.

1.5. Characterization of gummies

The most important parameters that should be measured in gummy candies are the moisture content and the mechanical properties. For that, methodologies such as Karl-Fischer and Vacuum Drying for moisture content and Texture Analyser for mechanical properties are described below. The evaluation of the temperature stability of paracetamol (TGA and DSC) is an important procedure in order to evaluate the possibility of adding it to the gummy formulation at high temperatures. The quantification method of paracetamol will determine if it reacted chemically or disintegrated thermally after being added to the solution.

1.5.1. Water content

One of the most important measurements that must be done in gummy candies is the moisture content. Moisture content in jellies and gummies is used as a quality factor to prevent crystallization and to be sure that the food gets the exact toughness (McClements, 2003).

Drying of food, also called dehydration, is the process of applying heat under controlled conditions in order to remove the free water present in the food by evaporation. Drying is usually the last step of a series of operations and the resulting product usually goes into the final package. According to Marcinkowski (2006), this technique is based on the reduction of the moisture content to a level where there is no microbial growth or there is a minimization of the rate of degradative chemical or enzymatic reactions. Generally, the temperature used is not high, and this process is not enough to inactivate microorganisms and enzymes, but only to inhibit their degradative action.

- **Karl-Fischer method**

The Karl Fischer (K-F) titration is a method of determining the water content of solid, liquid and gaseous samples. K-F titration may be considered the simplest, the most accurate, and reproducible among the methods for water determination and consequently used as a reference method in many studies. It is based on the oxidation of sulphur dioxide by iodine with consumption of water in a buffered solution, through the equation 1:

$$I_2 + 2H_2O + SO_2 \rightarrow 2HI + H_2SO_4 \qquad (1)$$

The endpoint of the titration represents the volume needed for the reaction with the total amount of water present in the sample. In this titration, the endpoint can be determined visually (by colour change) or by electrometry (coulometry or volumetry). More information about both methods and the application of Karl-Fischer to solids is showed in *Appendix 1*.

- **Vacuum Drying**

Vacuum drying consists in placing a sample inside an oven at a determined temperature that is below the boiling temperature of pure water (at a pressure between 0.022 and 0.197 atm, the water boiling temperature varies from 20 to 60 ºC (Toolbox, 2010)), to promote the evaporation

of the water molecules inside the sample. The decrease of the pressure decreases the boiling point of water, making the process faster.

Moisture content can be calculated either in wet basis or dry basis. Generally, the wet basis percentage is used in trade designations and pricing. However, moisture content in dry basis is commonly used in research and in specific calculations (Sousa and Rufato, 2018).

1.5.2. Mechanical properties

As gummy candies are edible products, its mechanical properties are extremely important because they have a direct influence on its texture.

The texturometer is an equipment with a dynamometer that provides mechanical energy at a constant defined rate. The result is a force *versus* time curve (or force versus distance) where the variation of the texture parameters of the material is recorded. This equipment performs several tests, one of the most popular being the two-bite test, in which the probe acts twice on the sample in penetrometry or compression. The objective is to reproduce the action of two teeth, giving some time of recovery to the material (Castro et. al, 2001). According to Periche et al., 2014, the most common parameters that are measured in compression tests to food are Hardness, Springiness, Cohesiveness and Gumminess.

Tensile tests are also important to be measured since they are correlated with the deformation of the material, its tensile strength and its Young Modulus.

Tensile and compression properties vary with the material thickness, method of preparation, speed of testing and with the type of grips used. Therefore, when comparative results are desired, these factors should be carefully controlled (Standards, 2002).

1.5.3. Thermal properties

Thermogravimetric analysis (TGA) is a method for analysing a system by its changes in physico-chemical properties when increasing the temperature (Gornicka and Gorecki, 2010). The results are presented either by weight versus temperature/time curve (thermogravimetric curve) or by rate of loss of weight versus temperature/time curve (DTG- differential thermogravimetric curve)(Gornicka and Gorecki, 2010). The curve obtained varies with the sample being tested, but many cases follow a characteristic path common to several decompositions (Mohammed, 2014).

Differential Scanning Calorimetry (DSC) is a method for analysing the difference on the calorific energy supply between a sample and a reference, measured in function of the temperature increase or decrease (NETZSCH, 2016). The advantages of both techniques are the fast processing, small amount of sample required and easy detection of alterations in the sample (Oliveira et al., 2017).

2. State of the Art

2.1. What is currently sold

Nowadays there are several brands that sell gummy candies. Table 7 presents some of the brands (national and international) that sell them, and their ingredients separated by the main categories (sweeteners, binding agents, acids, buffering agents, flavour/colour and glazing agents).

Table 7 Examples brands that sell gummy candies

	Sugars/ sweeteners	Binding agents	Acids/ buffer agents	Flavour/ Colour	Glazing agents
Haribo	Corn syrup, sugar, dextrose	Gelatine, corn starch	CC, SM, MA CA	Artificial and natural flavours; Artificial colours*	CW
Continente	Glycose syrup, sugar	Gelatine	SC, CA	Colour (curcumin, cochineal, blue), flavours	CW; CO
Fini	Glucose and fructose syrup, Dextrose	Gelatine, Wheat flour, Starch	SL, SC, CA, MA, LA	Vegetable extracts (carrot, black currant), flavours	CO, BW, CW
Pingo Doce	Glycose syrup, sugar	Gelatine, Pectin, Modified starch	CA	Vegetable concentrates; Colour**	CW, BW, CO
NaturSoy	Corn syrup, unrefined cane sugar	Pectin	CA	Fruit juice; Fruit and plant colouring extracts ***	SO, CW
Doctor Gummy	Maltitol syrup, Stevia	Gelatine	CA	Natural flavours And colourants	CO, BW, CW
Annie's	Tapioca syrup, Cane sugar	Tapioca Starch, Citrus Pectin	CA, SC, AA	Natural flavours and Colourants	SO, CW
Organic Candy Factory	Organic sugar	Pectin	CA, TP, ATA	Natural and organic flavours and colourants	
Surf Sweets	Grape juice, cane juice, Tapioca syrup	Starch Blend (Tapioca Starch, Corn Starch)	CA, AA, LA	Natural and organic flavours and colourants	SO, CW
Goody Good Stuff	Glucose syrup, sugar	Modified starch, carrageenan	CA, SM, MA, SC	Natural flavours and colorants	
Gummi King	Maltitol	Pectin	FA, CA	Natural flavours and colorants	

CA=Citric acid; FA=fumaric acid; SC=sodium citrate; CC=calcium citrate; SM= sodium malate; MA=malic acid; SL= Sodium lactate; LA= Lactic acid; AA=ascorbic acid; ATA= DL-Alpha Tocopheryl Acetate; TP= Tricalcium Phosphate; CW= Carnauba wax; BW= Bee wax; SO= Sunflower oil; CO=coconut oil;

*yellow 5, red 40, blue 1;

** Vegetable concentrates: carrot, black currant, lemon, safflower; Colour: copper complexes of chlorophyll and derivatives, caramel, pepper extract, titanium dioxide;

*** Fruit juice concentrate: apple, orange, lemon, black currant, raspberry, pineapple; Fruit and plant colouring extracts: pumpkin, apple, seaweed, safflower, elderberry;

Analysing the ingredients of these gummy brands, it can be seen that almost all of them, except DoctorGummy and Gummi King, have sugars in their formulations. The vegan ones are NaturSoy, Annie's, Organic Candy Factory, Surf Sweets, Goody Good Stuff and Gummi King and the only one that has both requirements (no sugar and vegan), is Gummi King.

Between these brands, only Haribo, Continente, Fini, Pingo Doce, NaturSoy and DoctorGummy are sold in Portugal, which means that between these products there is only one vegan option from Portugal, NaturSoy, however with sugar. The binding agent used in this formulation is pectin, the most common binding agent used in the vegan formulations that already exist. Starch is usually combined with other binding agents due to its low price.

In general, formulations have one powdered sugar or sweetener and another one in the syrup form. The importance of using syrups is related to shelf life and resistance to microbial spoilage, as well as the glassy texture that it gives to the final product (Wills, 1998).

2.2. Alternative candies to gummies

The main reason for children to eat candies is due to their high sugar and/or fat contents. It is scientifically proved that the ingestion of fats and sugars, normally induces different effects on physiology, brain, and behaviour (Avena et al., 2009). Some possible alternatives to gummy candies are every foodstuff capable of producing these types of effects in children (see Table 8).

Table 8 Alternative candies to gummies

Food	Types	Main ingredients
Chocolate (National Confectioners Association, 2018)	Dark	Cocoa, cocoa butter, sugar, emulsifier, vanilla or other favouring's
	Milk	Milk solids, cocoa, cocoa butter, sugar, emulsifier, vanilla or other favouring's
	White	Cocoa butter, sugar, milk solids, emulsifier, vanilla or other favouring's
	Organic	Grown without agricultural chemicals
	Bittersweet	Cacao (at least 50%), chocolate liquor and cocoa butter
Chewing gum (Andersen and Wittorff, 2006)	It can be used sugar or sweeteners	Bulk sweeteners/sugars, high intensity sweeteners/sugars, flavouring agents, softeners, emulsifiers, colouring agents, binding agents, acidulants, fillers and antioxidants
Lollipop (Bryk, 2018)		Water, sugar, corn syrup, flavourings (both natural and artificial), and malic or citric acid
Vanilla caramel (Bryk, 2018)		Milk, condensed milk (optional), corn syrup, sugar, oil, whey, calcium carbonate, salt, flavour, butter, vegetable oil, molasses and corn starch
Candy cane (Bryk, 2018)		Water, sugar, corn syrup, cream of tartar, colourants and flavouring agents, salt

2.3. Industrial production

The processing parameters varies according to the binding agents and sweeteners used. As most of the brands use gelatine in their formulations, the following steps are related to a normal gummy candy production and the main source of this description is Judy (2010):

Step 1: Storage of the sweeteners: Prior to production, the sugar and syrup additives may be stored in bulk tanks at room temperature and the syrup in a holding tank where it can be irradiated by ultraviolet light to remove any contaminants;

Step 2: Storage of the sugar slurry after a pre-treatment: The syrup may be dispensed to the mixing vessel manually or automatically. Sugar may be fed through an automated feed system that filters the sugar to remove any sediments, weighs the sugar, and delivers a desired quantity of to the mixing vessel. Prior to reaching the storage buffer tank, the sugar slurry may be heated through a series of heat exchangers to a temperature of approximately 65 ºC to 82 ºC in the mixing vessel; The sugar slurry is processed through a magnetic device, which removes particulates from the slurry, and stored in a storage buffer tank.

Step 3: Blend ingredients: Blend sugars or sweeteners and binding agents in solution (the sugar slurry may contain approximately 70% to 85% sweetener by weight, while the remaining approximately 15% to 30% of the slurry (by weight) may contain the gelling compound and additives) (Grazela and Morrison, 2002). Sodium citrate and other reagents may be added now. In most implementations, the candy slurry may reach a homogeneous mixture in about 5 to 10 minutes (Judy, 2010);

Step 4: Confection and water evaporation: The blend is fed to a high-temperature (104 ºC to 130 ºC), in a static cooker, in which the required solids level (65-75 ºBrix) is achieved for approximately 30 sec. to 60 sec. while the slurry is dehydrated (Judy, 2010).

Step 5: Cooling and drying: The blend leaves the cooker, cooling rapidly in a vacuum chamber. In the vacuum chamber, moisture is drawn from the cooked candy by suction pressure. At this step, the cooked candy may have a brix of approximately 67 to 80 ºBrix, and a pH of approximately 2.8 to 4.0;

Step 6: Filtration, colouring and flavouring: The cooked candy is filtered and consists mainly in a clear sugar solution. To obtain a desired colour and taste, colouring and flavouring may now be added to the cooked candy;

Step 7: Acidification: Finally, to balance the flavour and hardness, food acid solutions are pumped at the required concentrations into the appropriate product line for blending. The solution is filtered to remove possible lumps formed due to the acid addition;

Step 8: Deposition of gummies in the moulds: The final blends are pumped continuously to the moulding lines for deposition. Deposition of the gummies is conducted in large units, called moguls. A mogul is a moulding machine that deposits the cooked candy onto starch covered mould boards that allow the cooked candy to firm and take on the shape of the mould, to produce

a series of shaped gummy candies. Curing process happens in a temperature and humidity controlled curing room, where the candy sits and cools for approximately 24 hours to 48 hours After curing, the target moisture level is from 15 to 22% (Vaclavik and Christian, 2014).

Step 9: Unmoulding: The gummy candies are mechanically separated from the mogul and the starch is returned for recycled. To achieve a clean release from the mould, the moisture content of the starch must be low enough. The starch residues on the gummies must be taken away to provide a clean gummy surface.

Step 10: Application of the glazing agents: The final gummies are either spray-coated with oil tumbling in the drum or is sometimes ladled precisely into the batch. 12 to 15 minutes must be spent for the coating to evenly cover all surfaces.

The current procedure used by DoctorGummy is slightly different from the one described before and is represented in the following scheme (Figure 14).

The main differences reside on the temperature achieved (150 ºC instead of 130 ºC described before in step 4), the fact that they get rid of the bubbles while they filter the final solution (step 7), through a vacuum filter and also the composition of the moulds: as DoctorGummy gummies do not have gluten, they use corn starch moulds, making sure that there is no contamination of gluten in the gummies (step 8).

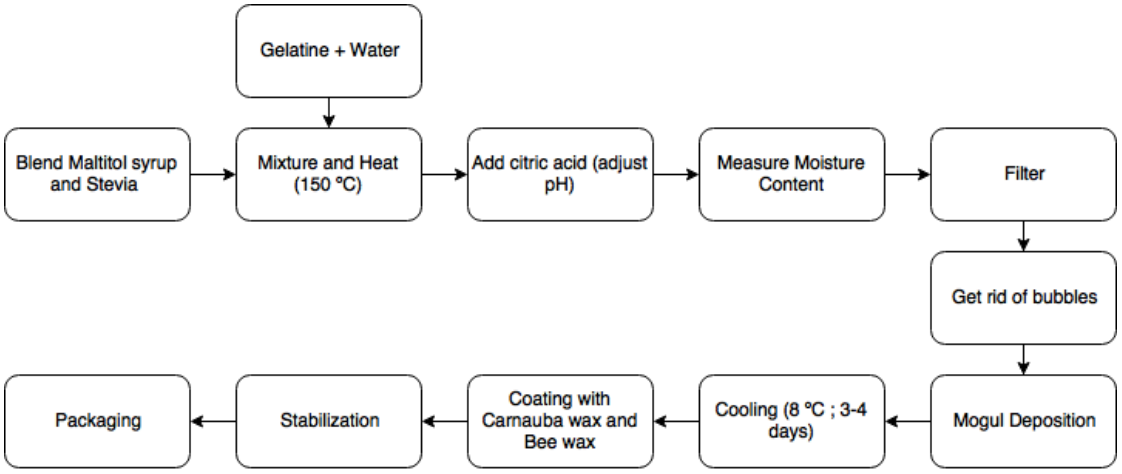


Figure 14 DoctorGummy industrial process

3. Materials and Methods

To start the project, it was created a questionnaire to study the credibility of the topic and whether it is appropriate to the needs and requirements of the current market. The questions made (and the results) are showed in *Appendix* 2.

3.1. Gummy confection

The reagents needed for the gummy confection are presented in Table 9. Maltitol syrup, mannitol and the starches were acquired at Roquette; maltitol, sorbitol, stevia, carrageenan's type I and k, gellan gum, agar-agar and locust bean gum were acquired at Formulab; xanthan gum is from PCA; carrageenan's CF 91-455 and CF 3243 were from CEAMSA and pectin was acquired at Herbstreith and Fox; erythritol was from Naturefoods brand; pineapple flavour was from Firmenich; strawberry flavours were from Firmenich and Takasago; the purple colourant was from CHR HANSEN; trisodium citrate is from Panreac and citric acid from Sigma-Aldrich.

Table 9. Reagents and equipment available for the gummy confection

Sweeteners	Binding agents	Others
Maltitol Syrup E312P	Iota carrageenan PGU 5339	Natural colourants CHR HANSEN GIN 710611
Maltitol P200	Kappa carrageenan: MCH 5308 and MCH 5311	2 natural strawberry flavours: 502473 B and TEG-10312540
Sorbitol E954T	Carrageenan CF 91-455	1 natural pineapple flavour 502434 AP0551
Mannitol E421	Carrageenan CF 3243	Food oil
Stevia E-3721	Modified Starch PG8005	Trisodium citrate di-hydrated PA-ACS 131655.1211
Erythritol	Modified Starch MB70	Citric acid 791725
	Modified Starch LG7015	Paracetamol Sigma-Aldrich, A7085-500G BioXtra>99.0%
	Pectin CF 020-C	
	Locust bean gum E410	
	Gellan gum NP7/18	
	Agar-agar NP8/18	
	Xanthan gum M1607A-G22	

The confection process highly depends on the binding agent used, since some of them need extra care to get dissolved (such as carrageenan) than others. The quantity of water used is very important since it will directly affect the texture of the final product (example of calculation in *Appendix 3).*

3.1.1. Vegan gummies without paracetamol

The binding agent(s) was/were dissolved in water with a magnetic agitation (heat may be needed to promote the dissolution; for example: locust bean gum does not need a pre-heat since

it dissolves in cold water; carrageenan needs the temperature to increase to 70 ºC or higher; xanthan gum needs strong agitation);

The syrup and half of the powdered sweetener were added and mixed; after complete dissolution, the rest of the powdered sweetener was added, making sure everything is solubilized (if need, increase the temperature and the velocity of agitation: carrageenan mixtures needs to be above 90 ºC, otherwise it starts jellifying; for the formulations with trisodium citrate, it is added in this step of the process.

The pH of the formulation was adjusted to the correct value with the addition of citric acid (pH meter Basic 20 from Crison)– see Figure 15 (i) (see Table in *Appendix 4);*

Figure 15 Temperature and pH measurement in a pectin formulation (i) and molding (ii)

After addition of the colourants and flavours, the formulation was transferred to the moulds rapidly to avoid pre-gelation (see Figure 15 (ii)) and the moulds were kept at 8 ºC for at least 24 hours in the refrigerator (Candy CIO 225 EE).

3.1.2. Vegan gummies with paracetamol

For the formulations with paracetamol, the process was the same as described before except the last step, where paracetamol was added at the end of the process, after the colourants and flavours. When fully dissolved, the solution was poured into the moulds and refrigerated.

Thus, it was confectioned 2 separated replicates of a formulation, C40P, with about 100 mg of paracetamol per gummy candy (about 1.6 g of paracetamol per formulation), allowing a wide range of application among children with different ages.

3.2. Moisture content

The moisture content in wet and dry basis were determined, putting the samples (with about 6 g cut into little pieces) in an oven at 120 ºC (WTC binder) until reaching constant weight, allowing the determination of the total water and therefore the final moisture content of the selected formulas, with at least 3 replicates of each.

The actual method used for measuring the water content in DoctorGummy products is Karl-Fischer method (K-F). In order to have faster, simpler and cheaper tests, vacuum and non-vacuum ovens will be used to test the moisture content of the selected formulations.

The evaluation of the drying process in the gummies was measured with a vacuum oven (Vacucell Evo line) at 0.1 bar. Two studies (one at 65 ºC and another one at 55 ºC) were

performed, with frequent measure of the gummy mass until reaching constant value. Between and after measures, the samples were placed in an exccicator until they cool. It was used at east 8 replicates with about 6 g each cut into little pieces.

After measuring the mass of the samples, the equations (2) and (3) can be applied to calculate the moisture content in wet basis (wb) and dry basis (db) in a determined time (for the study of the evaluation of the drying process) or at the end of the process (for the total moisture content).

$$Water\ content\ (wb, \%) = \frac{Mi-Mt}{Mg} \times 100 \qquad (2)$$

Where Mi is the water contained inside the gummy candy at the initial point, Mt the water lost at the determined point and Mg the mass of the gummy before drying.

$$Water\ content\ (db, \%) = \frac{Mi-Mt}{Mg'} \times 100 \qquad (3)$$

Where Mi is the water contained inside the gummy candy at the initial point, Mt the water lost at the determined point and Mg' the mass of the gummy after drying.

3.3. Mechanical properties

The mechanical properties of the films: Hardness (N), Springiness, Cohesiveness, Gumminess (N), Tensile Strength (MPa), Elongation at break (%) and Young's Modulus were measured using a texture analyser (MultiTest-d, mecmesin- see Figure 16) equipped either with a load cell of 19 mm diameter cylindrical probe for compression or with tensile grips for the tensile tests. At least 5 samples of each formulations were tested.

Figure 16 Texture analyser equipment

3.3.1. Compression tests

The samples were cut in circular shapes (about 8 mm of diameter) and the thickness of each sample was measured with a pachymeter three times and an average thickness was used to calculate how much compression corresponded to 50%; the equipment was adjusted to 1 mm/min speed and the test conditions involved two consecutive cycles of 50% compression, according to Periche et al. (2014).

From the compression tests, Hardness, Springiness, Cohesiveness and Gumminess were obtained from the force-time curve, according to Figure 17, where Hardness is the First compression peak value (P1); Springiness is given by the distance associated with B1 divided per distance associated to A1; Cohesiveness: areas (B1+B2)/(A1+A2); Gumminess: Hardness×Cohesiveness.

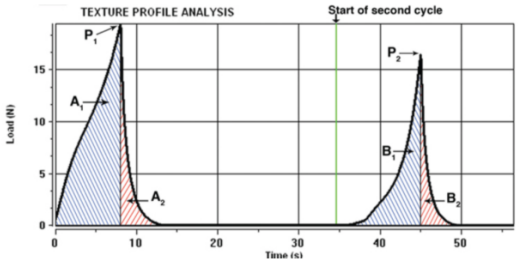


Figure 17 Example of a two compression cycle graph (Adapted from Freeman and Freeman (2015))

3.3.2. Tensile tests

Formulations were confectioned and poured into a flat glass to obtain homogeneous thickness; the samples were cut in rectangular shapes, with around 50 mm length and 15 mm width; the dimensions (width, length and thickness) of the samples were measured in triplicate with a pachymeter for each sample and the distance of the grips (corresponded to elongation= 0%) was defined as 20 mm and the equipment was adjusted to 40 mm/min speed.

From the tension-elongation curve, the Maximum Tensile Strength (MPa), Elongation at break (%) and Young's Modulus were defined.

3.4. Thermal properties

- **Thermal stability of paracetamol**

As paracetamol will be integrated in the gummy formulation during its confection, it will be subject to high temperatures (maximum of 100 ºC). Thus, it will be necessary to evaluate its stability and evolution at high temperatures, through TGA and DSC.

The equipment used for analysing paracetamol is NETZSCH STA 449 F3 Jupiter (see Figure 18), which allows the performance of a TGA and DSC simultaneously. The variables of this procedure are in accordance with the ones used by Oliveira et al., 2017, also for paracetamol.

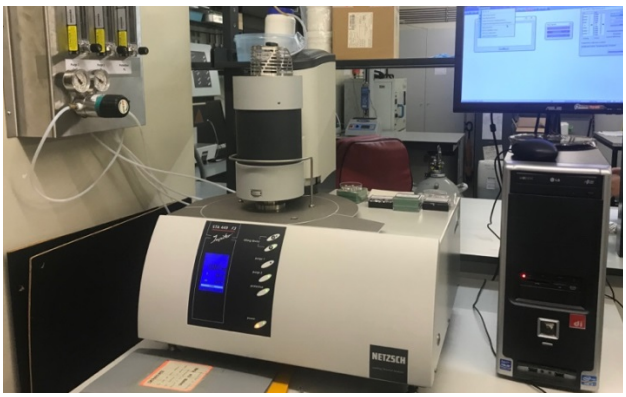


Figure 18 TGA and DSC equipment

The paracetamol sample used for this test is from Sigma-Aldrich, A7085-500G BioXtra>99.0%.

The gas pressure controllers must be: Pair=0.9 bar; Pnitrogen=0.4 bar; Pprotective=0.7 bar and the lab ventilation must be turned off to avoid thermal oscillations; the initial and final temperatures, 30-400 ºC, the velocity of the temperature increase (10 ºC/min) and the masses of

the reference and sample pans (1 mg or less for the blank and 15 mg for paracetamol) were measured.

- **Types of water in the gummy candies**

For the analysation of the water in gummy candies, a DSC with the equipment NETZSCH DSC 214 Polyma was used. Before starting, the equipment must be turned on for 30 minutes to stabilize. The conditions in the "setpoint" window were: 20 ºC, purge 2 and protective purge actives and the temperature segments were selected (in this case the first segment was an increase of the temperature from 20 to 120 ºC, the second segment was an isotherm at 120 ºC for 10 minutes, the third one was a decrease of the temperature to -40 ºC , the fourth was an isotherm for 15 minutes at -40 ºC and finally the fifth segment was a re-increase to 120 ºC);

The reference is an empty pan which mass must be carefully measured, and the sample had about 19 mg of gummy candy. Both pans were made of aluminium and were both covered before being used.

3.5. Quantification of paracetamol

To make sure that the paracetamol included in the formulations did not react chemically with other components, it was necessary the development of a quantification method for paracetamol in the gummy candies. Initially, some tests were performed in order to identify the best way to dissolve both paracetamol and gummy candies in the same solution. A proportion of 50% ethanol/water (v/v) was chosen since paracetamol dissolves better in ethanol and the gummies in water.

3.5.1. UV-VIS spectrum

To evaluate the absorption peaks of paracetamol and gummies, an UV-VIS spectroscopic method was used. A solution with 1 g of gummy (formula C40) was dissolved in 25 mL of ethanol: water (1:1, v/v) and a standard solution 1.25 mg/L of paracetamol diluted in ethanol: water (1:1, v/v) were prepared and a spectrum analysis in the range of 200-400 nm was performed (Jasco V-530) to evaluate the absorption peaks of both solutions;

3.5.2. Calibration curve

Preparation of standard solutions of 25 mL of paracetamol with concentrations of 1.25 mg/L, 5 mg/L, 10 mg/L, 15 mg/L, 20 mg/L, 25 mg/L and 30 mg/L diluted in ethanol: water (1:1, v/v) (solvent) and their absorbances were measured at 247 nm.

3.5.3. Samples preparation and quantification

A sample of the gummy, about 0.5 g, was dissolved in 25 mL of the solvent (ethanol: water (1:1, v/v). Subsequently, 1 mL of this solution was transferred to a 10 mL graduated volumetric flask and the volume was completed with the same solvent. It was measured the absorbance of the samples at 247 nm and the reference used was a solution of a gummy dissolved in the solvent in the same concentration as the samples. It was prepared 4 different samples and each one was

measured 4 times. The content of paracetamol was determined with the use of a calibration curve and expressed in milligrams per gram of gummy.

3.6. Statistical analysis

To determine the existence of significant differences between the mean values obtained for a given parameter, the data were submitted to analysis of variance (ANOVA) Single factor, and the t-Test (Two-Sample Assuming Unequal Variances) was performed, to compare some samples in pairs (less than 3 samples), being considered in all cases a level of significance of 5%. The statistical analysis was performed using Microsoft Excel Supplements.

4. Results and Discussion

To start this project, it has been made a preliminary study that shows that 20% of the people are or are willing to be vegan or vegetarian. This number is growing year by year and there is a need for the development of more vegan products. Even so, the same study says that, having both alternatives, vegan and non-vegan, most of the people (90%) would choose vegetable gummies instead of gelatine ones if the flavour maintains.

Another result considers the number of people (90%) who would choose gummies without sugar (with sweeteners that are non-absorbed by the organism), instead of sugar-gummies. All the results of this study are showed in the *Appendix 2*.

4.1. Development of vegan gummies without paracetamol

Based on some preliminary results (see Figures 19 CP, C0 and P0), the first formulas for this project were determined and tested.

Figure 19 Results from preliminary tests

The formulas that correspond to the results presented in Figure 19 can be seen in Table 10. The problems of these results can be explained due to various factors. First of all, formulation CP has too much solids (~12% wb). Carrageenan is able to form gels in much lower concentrations than gelatine, as well as pectin. They absorb a lot of water, meaning that the quantity of water added for these solids was not enough. Formulation C0, on the other hand, has very low quantities of carrageenan. Still, the adjusted pH was not correct because carrageenan cannot form gels in pH levels below 3.5. Formulation P0 is correct in terms of concentrations; however, the temperature achieved while cooking (40 ºC) did not allow the solids to get fully dissolved and pectin was not able to form a correct firm gel.

Table 10 Formulations from the preliminary results

	Binding agents	Sweeteners	Water	Final pH
CP	5 g carrageenan, 15 g pectin	15 g Erythritol	88 %	3.0
C0	7 g carrageenan	11 g erythritol	93%	3.2
P0	30 g pectin	10 g erythritol, 1 g stevia	83%	3.0

4.1.1. Results of this study

In total, it was tested 69 different vegan formulations, where changes such as type or concentration of some reagents or even pH were studied. All the formulations tested are presented

in *Appendix 5,* Table A5.1. The name of each sample starts with the first letter of the binding agent used (for example, formula CG1 indicates that it has carrageenan and gellan gum).

The first selection phase was based in a sensorial analysis, where all the formulations that solidified were classified in a *9-point Hedonic rating scale* according to some sensorial characteristics. This sensorial analysis studied the hardness (from liquid to rigid), the breakability (from too elastic to breakable), opacity (from translucid to opaque), the bitterness (from sweet to bitter), the aftertaste (from bad to good) and the presence of grumps (from none to a lot). The ideal range of values for each parameter are presented in Table 11.

Table 11 Ideal results from the sensorial test and results of some formulas

	Characteristics					
Ideal	Hardness	Breakability	Opacity	Bitterness	Aftertaste	Presence of grumps
Scale	6-8	3-4	1-4	1-2	7-9	1-2
P1	6	7	9	7	2	1
CG1	9	2	2	2	5	4
CL7	6	2	2	8	2	5
PS2	4	7	9	6	3	2

The results of the sensorial analysis of all formulas are presented in *Appendix 5,* Table A5.2 (it was considered solidified all the formulations that present more than 3 values in the hardness scale of the sensorial test).

In total, 12 formulations did not solidify: for instances, formulations C11 and C12 used kappa-carrageenan as the binding agent and they did not solidify because carrageenan kappa does not form gels by itself but only when mixtured with iota-carrageenan. The rest liquid formulations (PX2, PIS, PG, LX, S1-S4, C33, C34) occurred due to a low evaporation. Several gummies were too hard, such as gummies C31 and C32 due to the excess of carrageenan). On the other side, 11 formulations were too elastic (PISX, G1, CG1, CG2, CL2, CL3, CL7, CL8, C1, C38 and C39). Some of these results are showed in Figure 20 and the sensorial analysis of these solidified gummies are presented in Table 11.

Gummy P1 was a try to improve formula CP from the preliminary results. It was added one more gram of pectin and the temperature achieved while cooking was higher (60 ºC). All the ingredients dissolved well, and pectin formed a hard gel. The disadvantage of this formula was its texture. Although it solidified, the texture was very different from the gelatine gummies.

Formula CG1 was the result of a formulation with gellan gum and a mixture of carrageenan's that went to the oven at 25 ºC, to study the possibility of increasing the solid content after pouring the solution into the moulds. Its texture was a lot sticky and hard.

Formulas CL7 and CL4 are the result of a mixture of carrageenan kappa and iota and locust bean gum. The result of CL7 was a very elastic gummy, without hardness enough while formula CL4, made from the same quantity of solid ingredients, did not solidify because it has more water (75% while formula CL7 has 67%).

Formula PS2 is made of pectin and starch MB70, resulting in a non-elastic gummy as the starch confers both a very breakable texture and a different opaque colour.

Formula C33 represents the fact that carrageenan fibbers hydrolyse at low pH values. This formula had a pH of 3.0. Other formulas with similar ingredients but higher pH values were tried, and the final gummy solidified (for example formulas from C37 to C41). This proves the fact that carrageenan only forms gels at pH levels above 3.5 (Imeson, 2009).

The justification for formula C34, made of carrageenan 91-455 and starch PG8005, did not solidify is probably because it did not evaporate enough water.

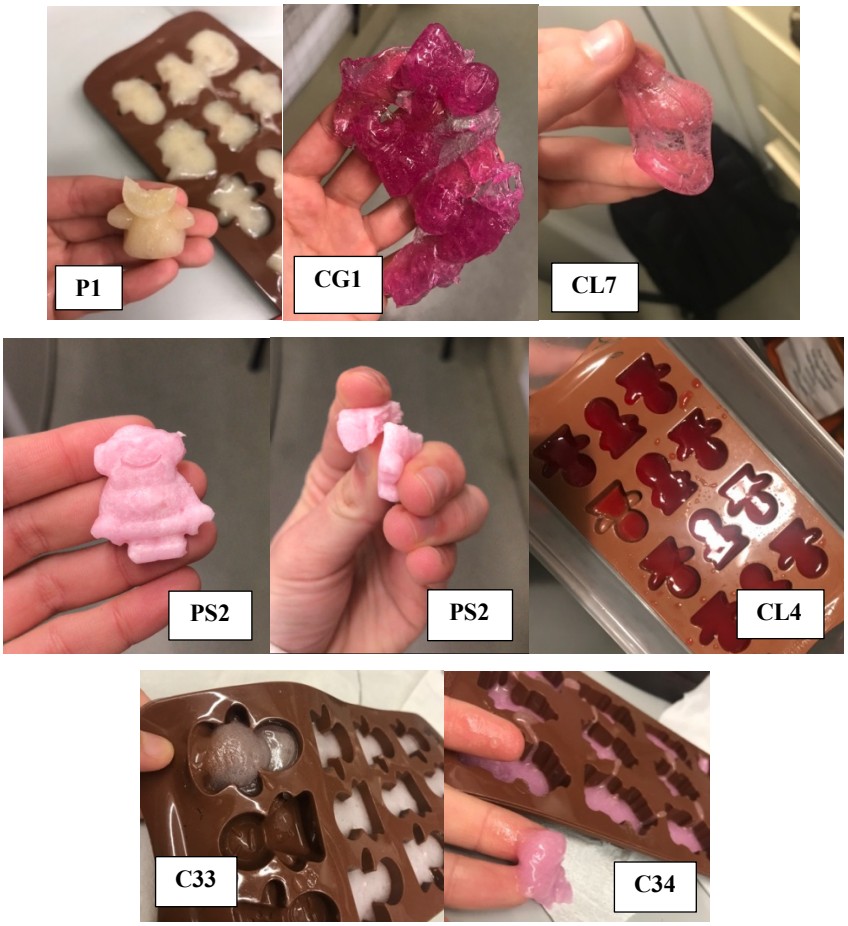


Figure 20 Example of gummies with non-adequate characteristics

4.1.2. Study of the different binding agents

Different binding agents were tested during this project, mainly pectin, agar-agar, locust bean gum, gellan gum, modified starches and several types of carrageenan. It was concluded that neither carrageenan-kappa nor carrageenan-iota are able to form gels by themselves but only when mixed together.

The harder gummies (C31 and C32) were achieved with using 5g (10% db) of CF 91-455 and CF 3243, respectively, being any of each a mixture of special algae derivate carrageenan's both from CEAMSA. Although carrageenan CF 91-455 was designed more specifically to

mixtures with sweeteners instead of sugars, there was no evident difference in terms of texture with both carrageenan's.

The concentration of carrageenan will interfere with the gel rigidity because the increase of carrageenan concentration confers a dense, compact, and hard texture due to evaporation of free water and the formation of temporary bonds between carrageenan molecules followed by cross-link producing nets with strong bonds. When a carrageenan solution is being cooled, the random bonds formed turn into a double helical chain that allows the formation of cross ties, creating a network (gel) (Kaya et al., 2015). An example of this situation is from formula C23 to C32, where a gradual increase of carrageenan concentration from 3g to 5g formed more rigid gummies.

The synergy of carrageenan with locust bean gum was verified between formulation CL7 and C6, producing gummies more elastic and transparent in CL7 than those formed only with carrageenan (C6). However, LBG is very expensive and so this option was discharged.

The easiest vegan gummies in terms of cooking procedures are pectin ones, because its mixture is very easy to dissolve and to mould; they solidify fast and are also very easy to unmould (what was verified in gummies P1-P4). They are also much cheaper than carrageenan gummies. However, they have a characteristic texture in the mouth very different from gelatine. For example, gummy P3 is rigid, however it is breakable, unlike gelatine gummies that are much more elastic.

Although gellan gum produced a very elastic gum, G1, similar to those produced with carrageenan and locust bean gum, this reagent is expensive, and no more tests were proceeded.

Formulations produced only with starch (S1-S4) did not solidify and the addition of pectin allowed the formation of a solid product (PS1 and PS2). However, it was very breakable as mentioned before.

4.1.3. Study of the different sweeteners

Different sweeteners were used along the project, mainly maltitol, sorbitol, mannitol, stevia and erythritol. The final product cannot be sensorially distinguished through the powdered sweetener used, however they differ from each other in terms of confection, sweet index, glycaemic index and price.

In terms of confection, maltitol, mannitol and erythritol behave the same way: they need some time and agitation to get fully dissolved. Maltitol has the higher sweet index, 0.99, compared to mannitol and erythritol, with 0.50 and 0.75, respectively. Among these sweeteners, erythritol is preferable because it does not have laxative effects as the others do. However, it is much more expensive.

Sorbitol differs from the other sweeteners in terms of confection, because when it is added, the solution gets a spongy and soft consistency. Sorbitol dissolves much faster than the other ones and the solution gets a lighter and opaque colour compared to the solution of other sweeteners. A disadvantage of sorbitol is that it has a low sweetener index, only 0.55.

Stevia is the most powerful sweetener in terms of sweetener index, being 200-350 times sweeter than sucrose. Despite being very expensive, its excessive use provides a very bad aftertaste to the solution. Analysing the sensorial results from formulas C15-C32, a decrease of the concentration of stevia from 1 g to 0.3 g had to be done due to the bad aftertaste that it provided. However, its use is very important because the final product get much sweeter when stevia is used.

The addition of maltitol syrup is very important not only because it confers a different glassy texture to the final gummy but also because it is related to shelf life and resistance to microbial spoilage (Wills, 1998). For example, the differences between gummies C13 and C14 is that gummy C13 does not have maltitol syrup and gummy C14 has. In terms of the sensorial analysis, gummy C13 was a little more breakable, rigid and opaque.

4.1.4. Selection of the best formulations

The final selected formulas that proceeded to the characterization tests were C29, C40 and C41 and the ratings of these formulas in the sensorial analysis are presented in Table 12.

Table 12 Results of the sensorial analysis of the selected formula

	Characteristics					
	Hardness	Breakability	Opacity	Bitterness	Aftertaste	Presence of grumps
C29	8	4	3	3	7	4
C40	6	3	4	2	7	3
C41	6	3	4	2	7	3

Although they present some grumps, it is not considered a problem because industrially the temperatures achieved can be higher and the agitation can be stronger, eliminating these grumps. Still, there is a filtering step that removes these grumps. Another alternative would be a more detailed analysis to the quantities of each component, with the attempt to decrease the grumps in the solution. The composition of these formulas is presented in Table 13.

Table 13 Composition of the final selected formulas

	Carrageenan 91-455	Powdered sweetener	Maltitol syrup	Citric acid	Water	Trisodium citrate
C29	4.5 g (9% db)	30 g maltitol, 3 g sorbitol, 0.1 g stevia	25 g (13.75 g solids)	0.07 g	75 g (63% wb)	-
C40	1.8 g (2% db)	50 g sorbitol, 0.1 g stevia	41g (22.55 g solids)	1 g sol. 55%	40 g (44% wb)	0.4 g
C41	1.8 g (2% db)	25 g maltitol, 30 g sorbitol, 0.1 g stevia	41 g (22.55 g solids)	1 g sol. 55%	40 g (42% wb)	0.4 g

These formulas were the most approximate vegan formulations to the known animal origin gummy candies (gelatine-based gummy candies).

Although formula C29 present a much harder texture than C40 and C41, the other ones generate a different feeling in the mouth, seem to be more elastic and are stickier when touching. Formula C29 is brighter than the other ones (see Figure 21-i and ii). Formulas C40 and C41 are

very similar to each other. The fact that gummies C40 and C41 are stickier than C29 can be justified by the less water quantities that they present compared to gummy C29.

Figure 21 Formula C29 (i) and formula C41 (ii)

Trisodium citrate di-hydrated works as a buffer agent and as a chelating and peptizing agent (Cunha, 2007). When trisodium citrate is added to the solution, it will raise the pH before the acid is added, preventing the solution pre-gelation. The higher the buffer salt concentration, the lower the setting temperature and the longer the setting time. When it is added to the solution, as also as citric acid, there is a decrease of the viscosity of the solution, allowing an easier manipulation of the confection (Wankenne, 2012). This is explained because the addition of the buffer/acid will interfere with the hydrocolloid bonds, separating carrageenan molecules from each other (cations from the buffer will bond with the dissociated carboxyl groups of carrageenan). This will delay the formation of the gel network until a new equilibrium is reached (Herbstreith and Fox, 2004). The acid and buffer are added in such an amount that the solution does not set before its complete melting and dissolution (Yasui, 1999).

The advantage of replacing the maltitol powder from formulation C41 to sorbitol in formulation C40 is due to the lowest price of sorbitol compared to maltitol (see *Appendix 6* for more details about their prices) and the objective was to evaluate sensorial differences between these formulas. However, the only difference was that gummy C41 was a bit sweeter than C40.

To simulate the industrial process, some starch was put in the moulds before pouring the gummy solution, to verify if it detaches easily. After unmoulding, gummies were covered in oil to simulate the glazing agents used in industry. Figure 22 presents these situations: (i) is formula C40 (angel moulds) covered in starch and (ii) are formulas C40 and C41 from another moulds (gummy bears) after covered in oil and saved in transparent bags.

Figure 22 Gummy candies with starch (i) and covered in oil (ii) from different moulds

It is relevant to say that none of this laboratory produced formulas is going to be equal as the same formulations produced industrially, due to stabilization/drying processes that cannot be done in the lab, which confer a lower water content to the industrial gummies and therefore they get harder.

4.2. Characterization of gummies without paracetamol

The final selected formulations were characterized through mechanical tests (tensile and compression) and moisture content (MC). This allowed a comparation between the sensorial analysis and these tests results. Table 14 presents all the results obtained for these parameters.

Table 14 Characterization of the final selected formulas: mechanical and moisture content

Tests		Formulations		
		C29	**C40**	**C41**
Tensile tests	Tensile Strength (MPa)	0.13±0.03 [a]	0.009±0.001 [b]	0.010±0.001 [c]
	Elongation at Break (%)	214±13 [a]	190±13 [b]	200±24 [b]
	Young's Modulus (MPa)	$4.6\times10^{-4}\pm0.8\times10^{-4}$ [a]	$8.6\times10^{-5}\pm0.6\times10^{-5}$ [b]	$9.3\times10^{-5}\pm0.7\times10^{-5}$ [c]
Compression tests	Hardness (N)	1.2±0.2 [a]	0.7±0.2 [b]	0.8±0.2 [b]
	Springiness	0.90±0.08 [a]	0.88±0.08 [a]	0.76±0.12 [a]
	Cohesiveness	1.0±0.3 [a]	1.3±0.4 [a]	1.2±0.4 [a]
	Gumminess (N)	1.1 ±0.2 [a]	1.2±0.3 [a]	0.9±0.4 [a]
MC	Wet basis (%)	52±2 [a]	28±2 [b]	27±1 [b]
	Dry basis (%)	111±7 [a]	39±2 [b]	38±2 [b]

a, b and c indicate if there is, by rows, a statistical significance >95%

4.2.1. Evaluation of the mechanical properties

Sensorial characteristics of gummies can be justified by their mechanical properties, through tension and compression tests. The graphs from which these results were taken are presented in *Appendix 7.*

In this study it was obtained values from 0.009 to 0.010 MPa to formulations C40 and C41 and 0.13 MPa for formulation C29 in terms of Tensile Strength. This difference between formulas C40, C41 and C29 is notable when evaluating them sensorially (formula C29 presents 8 points in the hardness scale and C40 and C41 present 6). Still, In et al., 2014 obtained values between 0.12 to 0.18 MPa to 3% (carrageenan: water, g/v) laboratory produced gels, which is in accordance with the values obtained for formulation C29 in this project. However, formulation C29 has more carrageenan (9% db) and is not constituted only by water and carrageenan, what will alter its properties.

Comparing these results with the ones obtained by Mueller and Innerebner (2006) with gelatine and starch-based industrially produced gummy candies, their maximum tensile strength was about 0.95 MPa. They also obtained values below 0.05 MPa for starch-based gummy candies. The fact that the gelatine/starch-based gummies obtained by Mueller and Innerebner (2006) present higher tensile strength indicates that carrageenan produces weaker gels than gelatine. However, as said before, laboratory produced gummies do not have the same texture as the industrial ones and so these values cannot be directly compared. The tensile strength showed to be statistically different between all the 3 formulas, being higher for formula C29 ($p=4.85\times 10^{-4}$ between C29 and C40, $p=5.07\times 10^{-4}$ between C29 and C41 and p=0.04 between C40 and C41). This means that C29 gummies support higher tensile strengths before breaking.

In terms of Elongation at break, gelatine-based gummy candies showed values reaching 330%, while starch-based gummies did not reach 50% of elongation at break, at 58% relative humidity (Mueller and Innerebner, 2006). The formulations of this study reached elongations from 190% (formula C40) to 214% (formula C29). The statistical analysis showed that there is a significant difference between formulas C29 and C40/C41 in terms of elongation at break. Contrary to the sensorial analysis results (says that C40 and C41 are more elastic than C29), statistics indicates that formula C29 presents higher elongation values and therefore is more elastic than the other ones ($p=3.18\times 10^{-6}$ between C29 and C40; $p=4.60\times 10^{-5}$ between C29 and C41 and p=0.11 between C40 and C41). The presence of more water in formulation C29 (63% wb) makes it more swelled and therefore it seems to be less elastic than the other formulations. However, when the products are subject to a traction force is notable that formulation C29 handles more strength and elongates more than the other ones.

The Young's Modulus obtained by Mueller and Innerebner (2006) is about 1.000 MPa for gelatine gummies and 0.800 MPa to starch-based gummy candies. In et al. (2014) found values between 0.45 MPa and 0.50 MPa for 3% carrageenan gels. Values obtained in this project vary from $8.6\times10^{-5}\pm0.6\times10^{-5}$ MPa to $4.6\times10^{-4}\pm0.8\times10^{-4}$ MPa. This value is obtained through the slope of the initial deformation-stress curves, meaning that the gummy candies of this project deform more before starting to stress compared to the other ones, resulting in a lower slope value. There are two main reasons for the difference of these values between studies: values obtained by Mueller and Innerebner (2006) and In et al. (2014) were obtained through a compressive test with a 500 N load cell and the ones from this project were obtained through a tensile test. Still, their solutions were made of the gelling agent and water and the one from this project have much more constituents that influence the mechanical properties of the final product. All the formulas were statistically different, and C29 was, one more time, the one with higher values, meaning that it is stiffer than the other ones, having lower elastic deformations for the same load applied compared to gummies from formulas C40 and C41 (p=0.002 between C29 and C40, p=.003 between C29 and C41 and p=0.004 between C40 and C41). As gummy C29 has more carrageenan (9%

compared to gummies C40 and C41 that have 2%, in dry basis), the intermolecular forces in gummy C29 are stronger and therefore the product is stronger.

The results of the compressive tests are presented in Table 14. Comparing the hardness results with the ones obtained by Periche et al. (2014), these gummy candies are much less hard (with values between 0.7 to 1.2 N) than the ones made of gelatine and sugar (between 21.2-62.1 N). The difference between gummy C29 and the other ones is sensorially notable, since gummy C29 presented 8 points of rigidity and C40/C41 presented 6, in the hardness parameter.

The Springiness do not vary that much, with values between 0.760 and 0.90 in this project and 0.95 and 0.98 in the comparative study. It is associated with the rate at which the gummy goes back to its undeformed condition after deforming force is removed and this means that these gummies recover easily its shape after being deformed.

The fact that it was obtained Cohesiveness values above 1 indicates that these gummies support very well a second deformation, not having any type of permanent deformation after the first compression cycle. Gelatine gummy candies present cohesiveness values varying between 0.900 to 0.961, according to (Periche et al., 2014).

Gumminess is associated with the energy needed to disintegrate a semi-solid product to be able to be swallowed (Ecslab, 2015). Gumminess values obtained vary from 0.9 to 1.2 N in these vegan gummies, compared to 19.3 N to a maximum of 56.3 N in gelatine formulations. This difference can be felt when ingesting both types of gummies, since the laboratory produced vegan ones are much easier to swallow and bite (deform faster) than the gelatine ones (industrially produced).

Statistical analysis (ANOVA and t-student) showed that there is no significant difference comparing values between formulas C29, C40 and C41, for most of the parameters (springiness, cohesiveness and gumminess), with p-values above 0.05 for all the situations. Only hardness showed statistically different results between formula C29 (1.2 N) and formulas C40 and C41 (0.7 and 0.8 N, respectively). This is in accordance with the sensorial analysis, since gummy C29 presented higher hardness than the other ones. The p-values obtained were p=0.003 between C29 and C40, p=0.002 between C29 and C41 and p=0.61 between C40 and C41. This can actually be confirmed by touching since formula C29 creates harder gummy candies than the other formulas.

4.2.2. Characterization of the type of water in the gummies (DSC)

In order to define the drying temperature for moisture content test, the types of water that exist in gels were studied and a DSC was performed. According to Watase et al., 1988, there are three forms of water in polymer gels: non-freezing water, freezable disordered water and free water. Non-freezable water is associated with a polymer matrix and does not show a phase transition by calorimetric analysis; freezable water is also bound to the polymer matrix but shows a melting and crystallization temperatures different from bulk water. Free water shows similar melting/crystallization temperatures as bulk water (Watase et al., 1988). The results of the DSC

(see *Appendix 8*) showed that there is one absorption peak divided in three from 20 to 120 ºC for formulation C29 and one main peak at about 120 ºC for formulations C40 and C41. Formula C29 also presents an absorption peak in the 120 ºC isotherm. This means that the peaks observed at 120 ºC may be due to freezable water, since its evaporation temperature is different from 100 ºC.

The differences between formula C29 and the other ones can be visually explained by the fact that as gummy C29 is much more swelled and as it does not stick in the hand as the other ones do, it means that it may have free water molecules in the outside molecular layer of the gummy. So, the first absorption peaks that appear below 100 ºC in formulation C29 may be due to the evaporation of free water molecules that the other formulations do not have.

As formulas C40 and C41 have trisodium citrate in their compositions, it can trap water molecules, diminishing the free water molecules that would evaporate first. Still, the percentage of water that is initially added to formulation C29 is around 63% and in formulations C40 and C41 is around 44% and 42%, respectively.

Mineo and Katsuyoshi (1987) studied the effect of the temperature in carrageenan gels through DSC and they found an endothermic peak at about 60 ºC, corresponding to the gel-sol transition. This peak is observable in gummy C29 but not in the other ones. The explanation may residue in the fact that formulations C40 and C41 have a higher melting temperature due to the difference in the ingredients of the formulations. The same study detected that lower concentrations of carrageenan gels show smaller endothermic peaks, which also explains why formulas C40 and C41, that have less carrageenan than C29, do not show that peak.

4.2.3. Determination of the water content

The determination of the total water content in the gummies was performed placing them in an oven and promoting its drying through water evaporation. According to the results obtained from the DSC to the gummy candies, a measurement of the total water content in gummies was performed at 120 ºC in an oven. The results obtained for the water content of gummies is showed in Table 14, presented above in the beginning of this chapter.

Formula C29 has evidently more water than the other ones, presenting an average water content value (52%, wb) higher than formulas C40 and C41 (28% and 27%, respectively, in wb). This was expected since it can be verified with the sensorial tests: the fact that gummy C29 seems to be harder and less elastic than the other ones, is actually because it is more swelled with water.

A statistical analysis (ANOVA and t-test) also stated this difference, where formulation C29 is statistically different from the others ($p=1.11\times 10^{-12}$ wb and $p=1.47\times 10^{-5}$ db compared to formula C41 and $p=1.36\times 10^{-13}$ wb and $p=1.5510\times^{-5}$ db compared to formula C40), while formulations C40 and C41 were statistically equal (p=0.78 wb and 0.29 db).

The normal value for the water content of the actual gummy candies sold is between 15-22% (Delgado and Bañón, 2015). However, those gummies are subject to a drying/stabilization process

during the industrial confection that removes the extra water, what was not done in lab. This is the main reason for the difference in water content values between lab and industrial products.

Delgado and Bañón (2015) produced laboratory gummy candies and studied their moisture content, measuring values between 20-25%. However, their gummies were made of gelatine and just after cooking, the moisture content was adjusted by applying −0.6 kg cm^{-2} vacuum pressure to increase the solid soluble content of the hot liquor at 78 °Brix. This explains the different values among the projects. Despite that, the fact that the formulations have different ingredients also interfere with the properties of the final product: gelatine and carrageenan have very different structures and therefore will absorb different quantities of water, what means that there is a possibility that a gummy candy made of gelatine have different water content than one made of vegetable binding agents, even industrially.

It was industrially produced at pilot scale vegan gummies with the same ingredients as these and they acquired a texture much more similar to gelatine gummy candies than from those produced in laboratory and a moisture content below 20% (technical specifications in *Appendix 9*), which indicates that these formulations will probably have an acceptable moisture content value after an industrial production. In terms of sensorial analysis, this gummy is much chewier and harder than C29, C40 and C41. It does not have any grump and is very elastic.

4.2.4. Evaluation of the drying process in the gummies

As laboratory produced gummies have higher water contents than gummies produced industrially, it was studied the evolution of the drying process of the final selected formulas using a vacuum oven in two different temperatures with frequent measurements until constant weight.

A graph that represent the evaluation of moisture content (in wet basis) with time (at 65 ºC and 55 ºC) are presented in Figure 23. The same graphic in dry basis is presented in *Appendix 10.*

All the samples stabilize with some water that is not removed with the vacuum drying (equilibrium humidity) (Celestino, 2010) and the difference between drying at 55 or 65 ºC is verified specially for the first part of the curves, since samples heated at 55 ºC present lower slope values than samples heated at 65 ºC. For example, in 2h at wet basis graph, formulation C29 at 65 ºC shows 15% of water content while at 55 ºC it still shows about 26%. However, after about 10h all the formulations at both temperatures are stabilized.

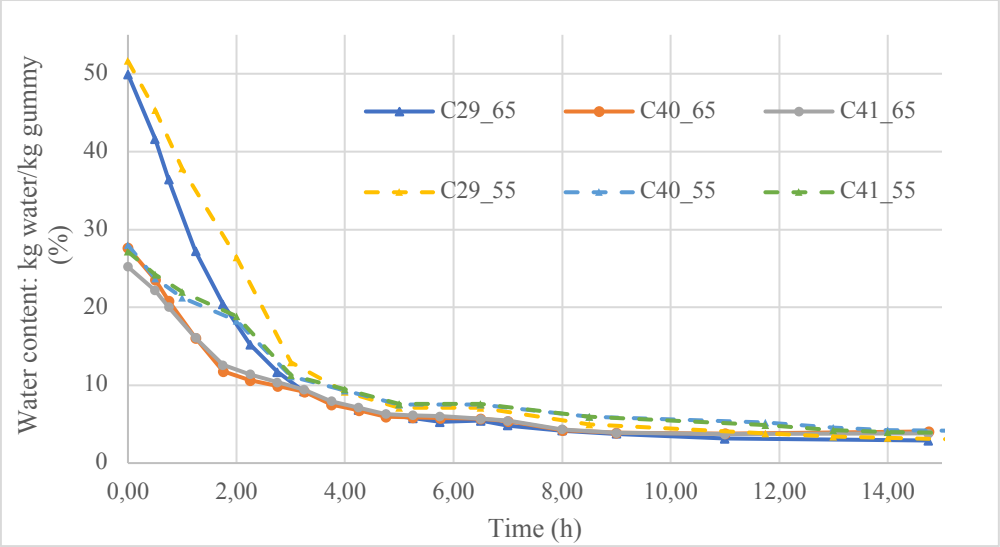


Figure 23 Water content evolution (wet basis) for 65 and 55 ºC

The drying rate of all the samples are presented in Figure A10.2 in *Appendix 10*. Formula C29 starts with a higher rate, diminishing very fast with the time. After 4 hours, this value only fluctuates a little bit and is already close to 0. Formulas C40 and C41 behave in a similar way with each other: in the first 30 minutes the rate of drying increases and then starts decreasing to 0. The fact that gummy C29 does not show an increase in the drying rate in the graph is because its increase occurs from 0 to 30 minutes and was not measured.

Despite that gummy C29 starts with a much higher moisture content, its drying rate is faster than the other ones, making them all stabilize at about 4h, achieving 0 speed after 10h in the vacuum oven.

Through graph from Figure 23 and knowing that the final moisture content of industrial gummies is between 15-22% wet basis, it is possible to verify how much time these formulas need to be inside the vacuum oven in order to achieve the desired humidity.

Gummy C29 achieves 20% water content after 1h45 inside the vacuum oven at 65 ºC; gummy C40 only needs about 40 minutes and gummy C41, 45 minutes.

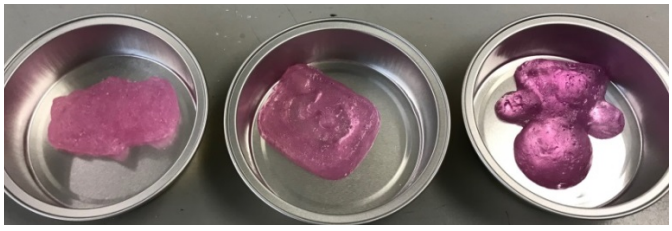


Figure 24 Gummies C29, C40 and C41 with approx. 20% humidity

These formulations with about 20% of water content are presented in Figure 24. However, they cannot be directly compared to the industrial gummies because this drying process, unlike the industrial one, dried more the outside layers of the gummies, making them more humid inside than in the outside; this was verified by touching (heterogeneous drying). However, it was

possible to verify that the consistency of the gummies with 20% of water were much closer to the industrial ones because they got harder, less opaque and less breakable.

4.2.5. Estimation of the costs of the final selected formulas

A succinct study of the costs associated to the selected formulas revealed that formula C41 is the most expensive one and formula C29 is the cheaper one. The results are presented in Table 15. The prices considered for these calculations are presented in *Appendix 6*. As the cost of carrageenan varies, it was considered €18.45/kg because the carrageenan type used in these formulations is one of the most expensive ones, since it is obtained from a specific selection of algae with determined characteristics. The cost of the water considered was €2.1482/m^3, obtained from (Águas do Porto, 2017), specially for industries.

Table 15 Estimated costs for each formulation

Formulation	Cost/ kg
C29	€1.76
C41	€2.79
C42	€2.82

Although formulation C29 has more concentration of carrageenan, the other ones have trisodium citrate, that increases the final price.

It is also relevant to say that this estimation is only based on the price of the reagents and other expenses such as fabric renting, energy, water for cleansing, labour pays, etc. were not considered.

4.3. Development of vegan gummies with paracetamol

4.3.1. Evaluation of the thermal stability of paracetamol

In order to evaluate the possible thermal decomposition of paracetamol during the production of vegan gummies, the loss of paracetamol mass and the energy absorption were evaluated with a heat increase of 10 ºC/min (Thermogravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC)).

The TGA shows that paracetamol starts losing its mass at around 230 ºC, with a total mass loss of 99.2% at about 350 ºC (see Figure 25 (i)), which are in accordance to the results obtained by Oliveira et al., 2017. The fact that paracetamol disintegrates by a complete thermal composition is demonstrated by the non-formation of residues during the heating (Oliveira et al., 2017). If there was formation of residues, the mass would not decrease until almost 0 %.

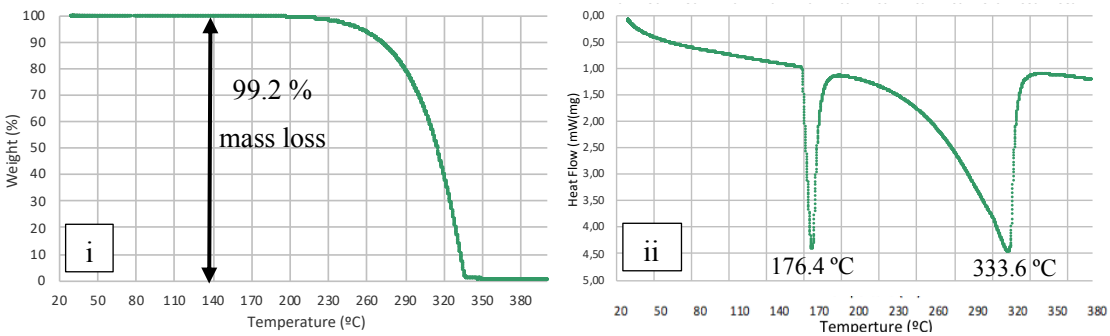


Figure 25 TGA (i) and DSC (ii) obtained for paracetamol

The peak showed in the DSC graph (Figure 25 (ii)) at 176.4 ºC corresponds to the melting temperature of paracetamol. Tomassetti et al., 2005 obtained a similar graph with a melting temperature of 175.5 ºC. The second peak, at 333.6 ºC, corresponds to vaporization and is also in accordance to the one obtained by Tomassetti et al. (2005), 327.9 ºC. This means that the mass changes presented in the TGA, that starts at 230 ºC and ends at about 350 ºC are due to the evaporation of paracetamol and not due to structural changes.

Tomassetti et al. (2005) studied the possible interactions between paracetamol and the tableting excipients (polyvinylpyrrolidone, magnesium stearate, citric acid, aspartame, mannitol, cellulose and starch) through TGA and DSC. They concluded that the temperature of vaporization of paracetamol is not substantially affected due to the presence of excipients in the pharmaceutical formulations. However, little deviations in the melting temperature occurred.

As the confection of the gummies require temperatures below 176.4 ºC, paracetamol will not decompose thermally (individually) or produce other toxic/unpleasant substances. The introduced quantity of paracetamol will not decrease due to temperature increases.

4.3.2. Development of a quantification method for paracetamol in the gummies

In order to quantify paracetamol in gummies, it was necessary to develop a quantification method that allowed the analysis of paracetamol in the gummy C40P. For that, a UV-VIS spectrum of a paracetamol solution and a gummy C40 solution were made, in order to select the most adequate wavelength to measure paracetamol after incorporation in the gummies formulations. It was chosen formula C40 since it is the most likely to be industrialized.

Through the λ=200-400 nm spectrum (see Figure 26), a peak at λ=247 nm was chosen to measure the absorbances both of the standard solutions and the samples. It is possible to see that there is another peak for paracetamol at 200 nm wavelength. However, the gummy spectrum also presents a peak at the same wavelength. In this context, 247 nm was chosen and the reference sample for the measurements was the gummy solution with the same concentration as the samples, in order to subtract the absorbances and obtain a more accurate result.

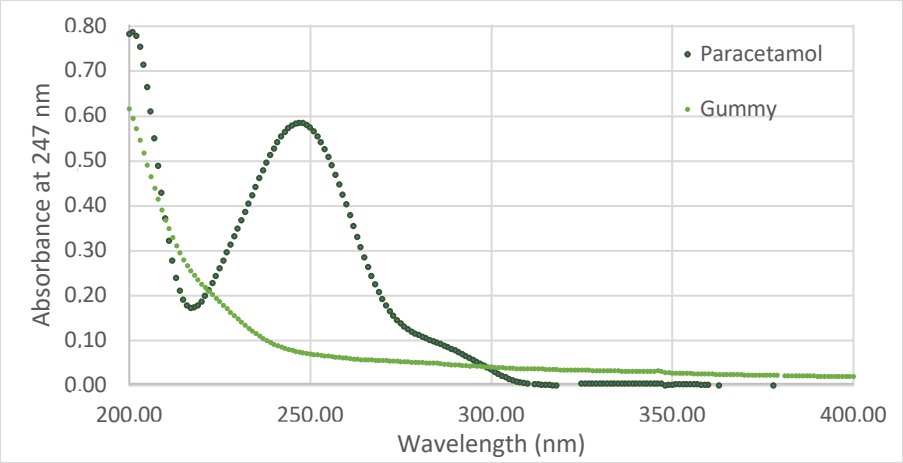


Figure 26 Paracetamol and gummy C40 spectrums in the 200-400 nm region

The calibration curve obtained is presented in Figure 27.

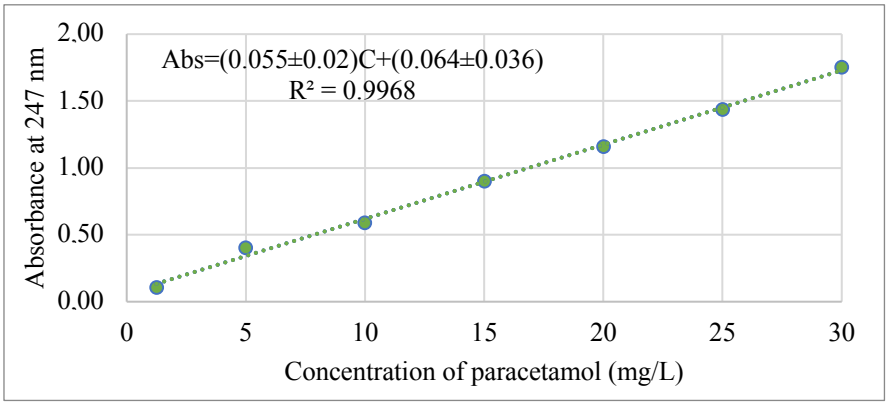


Figure 27 Calibration curve obtained at 247 nm for paracetamol in an ethanol: water (1:1, v/v) solution

In order to make sure that the calibration curve was suitable for using, the validation parameters were studied and are showed in Table 16.

Table 16 Validation parameters of the calibration curve for paracetamol quantification

Parameter	Experimentally	Criterion	Verified?
Number of points (n)	7	≥5	Yes
Linearity range	Factor of 24	Minimum factor: 10	Yes
S$\boldsymbol{a}$/a × **100**	1.800	<5%	Yes
$\boldsymbol{b-sb}$	0.047	$b-sb<0<b+sb$	No
$\boldsymbol{b+sb}$	0.082		
Correlation coefficient (R)	0.998	>0.995	Yes

The only parameter that was not verified was $b-sb<0<b+sb$. This may mean that the deviation of the ordinate from the origin is not due only to noise but to an error in the method of analysis such as the presence of an impurity in the solutions. All the other parameters from the calibration curve were verified. The lower absorbance value measured from the samples was 0.96,

which is higher than the minimum quantification limit (yLQ, 0.24) and detection values (yLD, 0.12); the lower concentration obtained from the samples measurements (16.25 mg/L) was also higher than the minimum required (xLD, 0.99 mg/L and xLQ, 3.18 mg/L).

The results obtained for the quantification of paracetamol are presented in *Appendix 11*). All the absorbances of the samples presented values within the range of the calibration curve.

It was estimated the quantity of paracetamol that should be added to the formulations in order to have about 100 mg of paracetamol per gummy candy. As each gummy has a mass of about 6 g (average) and the quantity of paracetamol obtained was 16.6±0.8 mg/g of gummy, this result demonstrates that all the paracetamol included in the formulations are measured through this method, then meaning that probably there are no chemical reactions between paracetamol and the other constituents of the gummy candies (unless it had formed a similar chemical species that absorb at the same wavelength).

Despite these good results, it was verified that paracetamol confers a bitter taste to the gummies. There are several solutions that can be tested to avoid this situation. Some of them include:

- Try adding more sweetener quantities to the formulation;
- Try adding more flavour to the formulation;
- Encapsulate paracetamol with a compound that is not degraded by salivary amylase;

It was tested the effect of the addition of more sorbitol (+ 20%), stevia (2x more) and both to formula C40P, resulting in three new formulations:

	Carr. 91-455	Powdered sweetener	Maltitol syrup	Citric acid	Water	Trisodium citrate	Paracetamol
C40P1	1.8 g (2% db)	60 g sorbitol, 0.1 g stevia	41g (22.55 g solids)	1 g sol. 55%	40 g (44% wb)	0.4 g	1.6 g
C40P2	1.8 g (2% db)	50 g sorbitol, 0.2 g stevia	41g (22.55 g solids)	1 g sol. 55%	40 g (44% wb)	0.4 g	1.6 g
C40P3	1.8 g (2% db)	60 g sorbitol, 0.2 g stevia	41g (22.55 g solids)	1 g sol. 55%	40 g (44% wb)	0.4 g	1.6 g

All the formulations showed a decrease in the bitter taste of paracetamol, meaning that there is the possibility of improving the taste of the formulations that have paracetamol included.

5. Conclusion

Through this project it was concluded that it is possible to create vegan, no sugared gummy candies with a similar texture as the gelatine ones. Unlike most of the vegan gummies that are already sold which use pectin as the binding agent, these ones use carrageenan to solidify. It was also concluded that there is the possibility of adding paracetamol to those gummies, allowing children to swallow paracetamol in a funny and easy way.

Between the 69 formulations tested, 3 of them (C29, C40 and C41) were available to proceed to the tests (moisture content and mechanical tests). The biggest differences between these formulas reside not only in the fact that they have different concentrations of the ingredients but also because formulations C40 and C41 have trisodium citrate and C29 does not.

Formula C29 has a moisture content of around 52±2% in wet basis and 111±7% in dry basis; formula C40 has 28±2% of water in wet basis and 39±2% in dry basis; finally, formula C41 has a moisture content of 27±2% in wet basis and 38±2% in dry basis. These values are higher than the required ones at industrial level: 15-22%. This is justified by the fact that there are industrially stabilization and drying processes which increase the solid content of gummies that were not performed in the lab.

In terms of the compression tests, formula C29 present higher hardness values, 1.2±0.2 N, compared to the other ones, 0.7±0.2 N for C40 and 0.8±0.2 N for C41. The Springiness, Cohesiveness and Gumminess were similar among the formulations, with values ranging from 0.76±0.12 to 0.90±0.08 for Springiness, 1.0±0.3 to 1.3±0.4 for Cohesiveness and 0.9±0.4 N to 1.2±0.3 N for Gumminess.

The tensile tests showed that formulation C29 elongates more before breaking (214±13%) than the other ones (190±13% for C40 and 200±24% for C41) and also presents a higher Tensile Strength of 0.13±0.03MPa, unlike formula C40 with a maximum of 0.009±0.001MPa and C41 with 0.010±0.001MPa. The Young's Modulus varied between $4.6\times10^{-4}\pm0.8\times10^{-4}$MPa for formula C29 and $9.3\times10^{-5}\pm0.7\times10^{-5}$ MPa for formula C41.

The cost estimated for the production of 1 kg of these gummies was €1.76 for C29, €2.79 for C40 and €2.82 for C41, considering only the reagents prices of each formulation.

The inclusion of paracetamol in the gummies was possible and it did not decompose during the production, being obtained an average value of 16.6±0.8 milligrams of paracetamol per gram of gummy candy. Producing gummy candies with about 100 mg of paracetamol each, the quantity of gummies taken will vary according to the age of the child.

6. Limitations and Future Work

Despite the good results obtained and the main goals of this work have been achieved, there were some things that could have gone better.

As this work requires a confidential term, the practical experiments could only start after everything was dealt with and legalized, which took some time.

The ideal method for measuring the moisture content of the gummies is the Karl-Fischer method. Although FEUP has a K-F equipment, it is broken and therefore could not be used in this project.

When the DSC was performed to the gummies, the ideal was to use it accomplished with a TGA analysis to understand better some of the DSC results, but the equipment broke during the semester and cannot be used.

The measurement of the water activity of the final selected gummies was going to be done. However, the equipment needed moved to another research centre where we could not use.

As future work, the study of the water sorption and water activity of the gummies would be very interesting since it is related to the water behaviour and availability in the gummies.

A study of the macromolecules of the gummies (protein, carbohydrate and lipids) is important and obligatory if the gummies are sold in the future.

A deeper study of the gummies with paracetamol is important to be sure that the compound measured with the UV-VIS equipment is actually paracetamol and no other similar chemical compound. It would also be very interesting to simulate the delivery rate of paracetamol from carrageenan gels either *in vivo* or *in vitro* with a simulation of the human body environment. Some extra tests should be done, trying to improve the flavour of the gummies with paracetamol, since they acquired a bitter taste after adding the active ingredient. The addition of more sweetener helps improving its taste, however more studies should be done.

At this moment, a sensorial probe is being prepared to be handled in July and the confidential terms are being signed to make a pilot scale production of one of the chosen formulas, industrially.

7. References

Abdel-Daim, M., Abushouk, A. I., Reggi, R., Yarla, N. S., Palmery, M., Peluso, I. (2017). Association of antioxidant nutraceuticals and acetaminophen (paracetamol): Friend or foe?. Journal of Food and Drug Analysis, 26(2), S78-S87. doi:10.1016/j.jfda.2017.11.004

Abdo, K. M., Huff, J. E., Haseman, J. K., Alden, C. J. (1986). No evidence of carcinogenicity of d-mannitol and propyl gallate in F344 rats or B6C3F1 mice. Food and Chemical Toxicology, 24(10), 1091-1097. doi:10.1016/0278-6915(86)90293-0

Águas do Porto. (2017). Tarifário. Retrieved from https://www.aguasdoporto.pt/assets/misc/PDF%27S/Contrato/Tarifário_2017.pdf (last accessed on 16-06-18)

Allingham, R. P. (1982). Polydextrose - A new Food Ingredient. In Chemistry of Foods and Beverages: Recent Developments, 293-303: Academic Press. Groton.

Andersen, L. and Wittorff, H. (2006). Chewing gum having improved release of chewing gum ingredients: Google Patents (US20090226383A1).

Anderson, D. M., Brydon, W. G., Eastwood, M. A. (1988). The dietary effects of gellan gum in humans. Food Additives & Contaminants, 5(3), 237-249. doi:10.1080/02652038809373701

Armisén, R. and Gaiatas, F. (2009). Agar. In Handbook of Hydrocolloids, 82-107: Woodhead Publishing. Burgos

Armisén, R. and Galatas, F. (1987). Production, Properties and uses of agar. In Production and utilization of products from commercial seaweeds: FAO Fisheries & Aquaculture. Burgos

Avena, N. M., Rada, P., Hoebel, B. G. (2009). Sugar and Fat Bingeing Have Notable Differences in Addictive-like Behavior. The Journal of Nutrition, 139(3), 623-628. doi:10.3945/jn.108.097584

Babiker, R., Merghani, T. H., Elmusharaf, K., Badi, R. M., Lang, F., Saeed, A. M. (2012). Effects of gum Arabic ingestion on body mass index and body fat percentage in healthy adult females: two-arm randomized, placebo controlled, double-blind trial. Nutrition Journal, 11(111), 1475-2891. doi:10.1186/1475-2891-11-111

Baines, D. and Seal, R. (2012). Natural Food Additives, Ingredients and Flavourings, 488: Woodhead Publishing. United Kingdom

Bar-Shalom, D., Slot, L., Fischer, G., Hemmingsen, P. H. (2006). Swellable dosage form comprising gellan gum: Google Patents (EP2258349A3)

Bera, D., Lahiri, D., Leonardis, A. D., De, K. B. (2006). A Novel Azeotropic Mixture for Solvent Extraction of Edible Oils. Agricultural Engineering International: the CIGR Ejournal, 06(005), VIII. Italy.

Bian, X., Chi, L., Gao, B., Tu, P., Ru, H., Lu, K. (2017). The artificial sweetener acesulfame potassium affects the gut microbiome and body weight gain in CD-1 mice. Public Library of Science One, 12(6), 178-426. doi:10.1371/journal.pone.0178426

Biester, A., Wood, M. W., Wahlin, C. S. (1925). Carbohydrate Studies. American Journal of Physiology-Legacy Content, 73(2), 387-396. doi:10.1152/ajplegacy.

Boesten, D. M. P. H. J., Hartog, G. J. M., Cock, P., Bosscher, D., Bonnema, A., Bast, A. (2015). Health effects of erythritol. International Journal on Nutraceuticals, Functional Foods and Novel Foods from Research to Industrial Applications, 14(1), 3-9. doi:10.1007/s13749-014-0067-5

Bornet, F. R. J. (1994). Undigestible sugars in food products. The American Journal of Clinical Nutrition, 59(3), 763-769. doi:10.1093/ajcn/59.3.763S

Bowen, W. H., Young, D. A., Pearson, S. K. (1990). The Effects of Sucralose on Coronal and Root-surface Caries. Journal of Dental Research, 69(8), 1485-1487. doi:10.1177/00220345900690080701

Bryk, N. E. V. (2018). How Products are made. Advameg, Inc. Retrieved from http://www.madehow.com/Volume-6/Lollipop.html (last accessed on 05-06-18)

Burg, J. C. (1998). Generating Yummy gummies. Natural Products Insider. Retrieved from https://www.naturalproductsinsider.com/articles/1998/05/generating-yummy-gummies.aspx (last accessed on 22-11-17)

Burt, J. (2014). A Brief History of Gummy Bears. Retrieved from https://www.bonappetit.com/entertaining-style/pop-culture/article/history-gummy-bears (last accessed on 21-05-18)

Bush, S. (2017). Erythritol vs. Stevia vs. Xylitol. Retrieved from https://www.livestrong.com/article/497773-erythritol-vs-stevia-vs-xylitol/ (last accessed on 02-03-18)

Cairns, P., Miles, M. J., Morris, V. J. (1986). Intermolecular binding of xanthan gum and carob gum. Nature, 322, 89-90. doi:10.1038/322089a0

Carlson, T. and Woo, A. (2004). Use of low glycemic index sweeteners in food and beverage compositions. Google Patents (US20040052915A1)

Castro, A. G., Diogo, A. C., Covas, J. A. (2001). Reologia e Suas Aplicações Industriais. Instituto Piaget, 464 (last accessed on 07-06-18)

Castro, C. (2018). DoctorGummy. Gomas saudáveis chegam aos supermercados. Dinheiro Vivo. (last accessed on 20-05-18)

Celestino, S. M. C. (2010). Princípios de Secagem de Alimentos. Embrapa. Retrieved from https://www.infoteca.cnptia.embrapa.br/bitstream/doc/883845/1/doc276.pdf (last accessed on 04-06-2018)

Chattopadhyay, S., Raychaudhuri, U., Chakraborty, R. (2014). Artificial sweeteners – a review. Journal of Food Science and Technology, 51(4), 611-621. doi:10.1007/s13197-011-0571-1

Cheney, P. A., Stares, J., Vernon, A. J. (1979). Food product thickened or gelled with carrageenan and glucomannan: Google Patents (US4427704A).

Clarke, M. A. (2003). Syrups. In Encyclopedia of Food Sciences and Nutrition. 5711-5717: Academic Press. Oxford.

Clifford, J., Maloney, K., Anderson, J., Young, L. (2016). Sugar and sweeteners. Food and nutrition series. Health. Retrieved from http://extension.colostate.edu/docs/pubs/foodnut/09301.pdf. (last accessed on 16-05-18)

Company, D. C. (2002). The use of DOWEX ion exchange resins and adsorbents in corn sweetener processing. Retrieved from http://msdssearch.dow.com/PublishedLiteratureDOWCOM/dh_0037/0901b80380037728.pdf?filepath=liquidseps/pdfs/noreg/177-01563.pdf%26fromPage=GetDoc (last accessed on 16-06-18)

Cpkelco. (2001). Carrageenan book. Retrieved from http://www.bisi.cz/cmsres.axd/get/cms$7CVwRhc3USVqgzxkKF96gI$2BChNrXcTq$2BOUdiEtz5TfYA$2Fg1ADRHMfXfdEjUsYQagqUs9N6byPOkok$3D (last accessed on 14-06-18)

Cunha, C. R. (2007). Papel da Gordura e do Sal Emulsificante em Análogos de Requeijão Cremoso. Universidade Estadual de Campinas. Retrieved from http://repositorio.unicamp.br/bitstream/REPOSIP/255794/1/Cunha_ClarissaReschkeda_D.pdf (last accessed on 10-06-18)

Curry, L. L., Roberts, A. (2008). Subchronic toxicity of rebaudioside A. Food Chemical Toxicology, 46(7), 11-20. doi:10.1016/j.fct.2008.04.042

Silva B. V., Fernandes, F. L. B., Fidalgo, T. K., Martins, C., Mattos, C. T., de Souza, I. P., Maia, L. C. (2015). Mother-to-child transmission of Streptococcus mutans: a systematic review and meta-analysis. Journal of Dentistry, 43(2), 181-191. doi:10.1016/j.jdent.2014.12.001

Dakia, P. A. (2011). Carob (*Ceratonia siliqua L.*) Seeds, Endosperm and Germ Composition and Application to Health. In Nuts and Seeds in Health and Disease Prevention. 293-299: Academic Press. San Diego.

Daly, J., Tomlin, J., Read, N. W. (1993). The effect of feeding xanthan gum on colonic function in man: correlation with in vitro determinants of bacterial breakdown. British Journal of Nutrition, 69(3), 897-902. doi:10.1079/BJN19930089

Daneshvari, D. (2013). Wet granulation process and granulate material comprising arabic gum: Google Patents (EP2836083B1).

Deis, R. C. (2000). Polyols in Confectionery the Manufacturing Confectioner. Retrieved from http://www.aactcandy.org/firstpage/a2000200010053.pdf (last accessed on 08-02-18)

Delgado, P.; Bañón, S. (2015). Determining the minimum drying time of gummy confections based on their mechanical properties. Journal of Food, 13(3), 329-335. doi:10.1080/19476337.2014.974676

Demodaran, S., Parkin, K. L., Fennema, O. R. (2008). Fennema's Food Chemistry. 1158: CRC Press. Florida

Deosen Biochemical Ltda. (2015). Os tipos de gomas e as suas aplicações na indústria. Retrieved from http://aditivosingredientes.com.br/upload_arquivos/201601/20160100303330014534883327.pdf (last accessed on 14-06-18)

Zia-ud D., Xiong, H., Fei, P. (2015). Physical and Chemical Modification of Starches - A Review. Critical Reviews in Food Science and nutrition, 57(12), 2691-2705. Doi: 10.1080/10408398.2015.1087379

Dionísio, M. and Grenha, A. (2012). Locust bean gum: Exploring its potential for biopharmaceutical applications. Journal of Pharmacy and Bioallied Sciences, 4(3), 175-185. doi:10.4103/0975-7406.99013

Dobbing, J. (2012). Dietary Starches and Sugars in Man: A Comparison. In Human Nutrition Reviews: Springer London, 256. Manchester

Dodi, G., Hritcu, D., Popa, I. M. (2011). Carboxymethylation of guar gum: Synthesis and characterization Cellulose Chemistry and Technology 45(3), 171-176. doi:10.15761/AMS.1000125

DrugBank. (2018). Acetaminophen. Retrieved from https://www.drugbank.ca/drugs/DB00316 (last accessed on 14-06-18)

Ecslab. (2015). What is texture analysis? Retrieved from http://www.ecslabonline.com/pdf/brookfield-ct3-tekstur-analizoru.pdf (last accessed on 03-06-18)

Embrapa. (1998). Manual para produção de geléias de frutas em Escala Industrial. Retrieved from https://www.agencia.cnptia.embrapa.br/Repositorio/doc29-1998_000gc3pmnuc02wx5ok01dx9lcy4av4k9.pdf (last accessed on 06-03-18)

Endreß, H. U. and Christensen, S. H. (2009). Pectins. In Handbook of Hydrocolloids, 274-297: Woodhead Publishing. doi:10.1533/9781845695873.274. Germany

Ervin, G. and Ervin, E. (2011). Health characteristic chewy or gummy candy confection: Google Patents (US20110313055A1).

European Parliament and Council Directive on Sweeteners in Foodstuffs, S. D. (2003). Directive 2003/115/EC. Retrieved from http://www.ajinomoto-aspartame.com/products/pdf/Consolidated.pdf (last accessed on 02-03-18)

Farmer, S. and Reusch, W. (2013). Disaccharides. Retrieved from https://chem.libretexts.org/Core/Organic_Chemistry/Carbohydrates/Disaccharides (last accessed on 13-06-18)

Farmer, S. and Reusch, W. (2016). Polysaccharides. Retrieved from https://chem.libretexts.org/Under_Construction/Textmaps_and_Wikitexts/MVC%3A_Chem_1406/Chapters/14%3A_Carbohydrates/14.7%3A_Polysaccharides (last accessed on 15-06-18)

FDA- Food and Drug Administration. (2013). Everything Added to Food in the United States (EAFUS). Retrieved from: https://www.accessdata.fda.gov/scripts/fcn/fcnnavigation.cfm?rpt=eafuslistinganddisplayall=falseandpage=6 (last accessed on 22-11-17)

Figdor, S. K. and Rennhard, H. H. (1981). Caloric Utilization and Disposition of [14C] Polydextrose in the Rat. Journal of Agricultural and Food Chemistry, 9(6), 1181–1189. doi:10.1021/jf00108a022

Food Science. (2016). Chemical solvent extraction in food. Retrived from: http://www.foodscience-avenue.com/2016/06/chemical-solvent-extraction-in-food.html (last access on 10-06-18)

Freeman, C. and Freeman, C. (2015). The Gold Standard for Texture Analysis. Retrieved from https://www.laboratoryequipment.com/article/2015/09/gold-standard-texture-analysis (last accessed on 07-06-18)

Gómez, L. P. (2013). A Novel Carrageenan for Vegetable Gummy Candy Production. Marketing & Technology. Retrieved from https://www.harnisch.com/uploads/tx_harnisch/food_5_Novel_Carrageenan.pdf (last accessed on 06-02-18)

Gonze, J. G. M. (2000). Erythritol. The Manufacturing Confectioner. Retrieved from http://www.gomc.com/firstpage/200001071.pdf (last accessed on 22-11-17)

Gornicka, B. and Gorecki, L. (2010). TGA/DTG/DSC investigation of thermal ageing effects on polyamide–imide enamel. Journal of Thermal Analysis and Calorimetry, 101(2), 647-650. doi:10.1007/s10973-010-0883-9

Gostner, A., Blaut, M., Schäffer, V., Kozianowski, G., Theis, S., Klingeberg, M., Scheppach, W. (2007). Effect of isomalt consumption on faecal microflora and colonic metabolism in healthy volunteers. British Journal of Nutrition, 95(01), 40-50. doi:10.1079/bjn20051589

Grazela, A. J. and Morrison, N. (2002). Gelatin-free gummy confection using gellan gum and carrageenan: Google Patents (US20020076478A1).

Grotz, V. L. and Munro, I. C. (2009). An overview of the safety of sucralose. Regulatory Toxicology and Pharmacology, 55(1), 1-5. doi:10.1016/j.yrtph.2009.05.011

Gupta, R. C. (2015). Medication Safety. Retrieved from http://kidshealth.org/en/parents/medication-safety.html (last accessed on 02-03-18)

Hardy, G. (2000). Nutraceuticals and functional foods: introduction and meaning. Nutrition, 16(7-8), 688-689. doi:10.1016/S0899-9007(00)00332-4

Hartel, R. W., Elbe, J. H., Hofberger, R. (2018). Jellies, Gummies and Licorices. In Confectionery Science and Technology, 329-359: Springer International Publishing. Burlington

Hayashibara, K. and Sugimoto, K. (1976). Containing lactitol as a sweetener: Google Patents (US3973050A)

Herbstreith & Fox. (2004). Confectionery Gum and Jelly Products. Retrieved from http://www.herbstreith-fox.de/fileadmin/tmpl/pdf/broschueren/Suesswaren_englisch.pdf (last accessed on 10-06-18)

Hiele, M., Ghoos, Y., Rutgeerts, P., Vantrappen, G. (1993). Metabolism of erythritol in humans: comparison with glucose and lactitol. British Journal of Nutrition, 69(1), 169-176. doi:10.1079/BJN19930019

Hispaganar. (2013). Products: Agar. Retrieved from http://www.hispanagar.com/agar.html (last accessed on 14-06-18)

Hobby Takt Ltd. (2005). The dried exudate (gum) from Acacia Senegal and related species (Acacia Seyal). Retrieved from http://www.hobbytakt.com/products/3856466109.pdf (last accessed on 15-06-18)

Hoppu, P., Jouppila, K., Rantanen, J., Schantz, S., Juppo, A. M. (2007). Characterisation of blends of paracetamol and citric acid. Journal of Pharmacy and Pharmacology, 59(3), 373-381. doi:10.1211/jpp.59.3.0006

Hsieh, M. H., Chan, P., Sue, Y. M., Liu, J. C., Liang, T. H., Huang, T. Y., Chen, Y. J. (2003). Efficacy and tolerability of oral stevioside in patients with mild essential hypertension: a two-year, randomized, placebo-controlled study. Clinical Therapeutics, 25(11), 2797-2808. Doi:10.1016/S0149-2918(03)80334-X

Hui, Y. H. (2006). Handbook of Food Science, Technology and Engineering. CRC Press: Vol. 3, 712. United States of America

Ibarra-Reynoso, L. D. R., Lopez-Lemus, H. L., Garay-Sevilla, M. E.; Malacara, J. M. (2017). Effect of Restriction of Foods with High Fructose Corn Syrup Content on Metabolic Indices and Fatty Liver in Obese Children. Obesity Facts, 10(4), 332-340. doi:10.1159/000476069

Iglauer, S., Wu, Y., Shuler, P., Tang, Y.; Goddard, W. A. (2011). Dilute iota- and kappa-Carrageenan solutions with high viscosities in high salinity brines. Journal of Petroleum Science and Engineering, 75(3), 304-311. doi:10.1016/j.petrol.2010.11.025

Imeson, A. P. (2009). Carrageenan and furcellaran. In Handbook of Hydrocolloids, 164-185: Woodhead Publishing. United Kingdom

In, E., Naguib, H., Haider, M. (2014). Mechanical stability analysis of carrageenan-based polymer gel for magnetic resonance imaging liver phantom with lesion particles. Journal of Medical Imaging, 1(3), 35-50. doi:10.1117/1.JMI.1.3.035502

Infarmed. (2014). Paracetamol Generis 40 mg/ml Xarope Paracetamol. Retrieved from http://app7.infarmed.pt/infomed/download_ficheiro.php?med_id=57034andtipo_doc=fi (last accessed on 4-03-18)

Ionescu, C. M., Sehnal, D., Falginella, F. L., Pant, P., Pravda, L., Bouchal, T. Koča, J. (2015). AtomicChargeCalculator: interactive web-based calculation of atomic charges in large biomolecular

complexes and drug-like molecules. Journal of Cheminformatics, 7(50). doi:10.1186/s13321-015-0099-x

Isokangas, P., Soderling, E., Pienihakkinen, K., Alanen, P. (2000). Occurrence of dental decay in children after maternal consumption of xylitol chewing gum, a follow-up from 0 to 5 years of age. Journal of Dental Research, 79(11), 1885-1889. doi:10.1177/00220345000790111201

Jackson, R. and Lees, E. B. (1973). Sugar Confectionery and Chocolate Manufacture. 1-379: Leonard Hill Books. New York

Jarret, T. N. (2012). Acids in Confections. In The Manufacturing Confectioner. Retrieved from https://pdfs.semanticscholar.org/d43a/1e9273d851703f6f42cc253329355e21d45a.pdf (last accessed on 27-02-18)

Jie, Z., Bango-yao, L., Ming-jie, X., Zu-kang, Z., Ting-song, W., Craig, S. A. (2000). Studies on the effects of polydextrose intake on physiologic functions in Chinese people. The American Journal of Clinic Nutrition, 72(6), 1503–9. doi:10.1093/ajcn/72.6.1503

Johnson, L., Mander, A. P., Jones, L. R., Emmett, P. M., Jebb, S. A. (2007). Is sugar-sweetened beverage consumption associated with increased fatness in children? Nutrition Journal, 23(7), 557-563. doi:10.1016/j.nut.2007.05.005

Jones, T. W., Borg, W. P., Boulware, S. D., McCarthy, G., Sherwin, R. S., Tamborlane, W. V. (1995). Enhanced adrenomedullary response and increased susceptibility to neuroglycopenia: Mechanisms underlying the adverse effects of sugar ingestion in healthy children. The Journal of Pediatrics, 126(2), 171-177. doi:10.1016/S0022-3476(95)70541-4

Judy, D. (2010). Chewable drug: Google Patents (EP2445485A1).

Klingeberg, M., Kozianowski, G., Kunz, M., Theis, S. (2004). Use of isomalt as prebiotic: Google Patents (DE10328180A1).

Kulkarni, V. S. and Shaw, C. (2016). Use of Polymers and Thickeners in Semisolid and Liquid Formulations. In Essential Chemistry for Formulators of Semisolid and Liquid Dosages, 43-69: Academic Press. Boston

Kuusisto, J., Tokarev, A. V., Murzina, E. V., Roslund, M. U., Mikkola, J.-P., Murzin, D. Y., Salmi, T. (2007). From renewable raw materials to high value-added fine chemicals—Catalytic hydrogenation and oxidation of d-lactose. Catalysis Today, 121(1), 92-99. doi:10.1016/j.cattod.2006.11.020

Landin, K., Holm, G., Tengborn, L., Smith, U. (1992). Guar gum improves insulin sensitivity, blood lipids, blood pressure and fibrinolysis in healthy men. The American Journal of Clinical Nutrition, 56(6), 1061-1065. doi:10.1093/ajcn/56.6.1061

Lee, J. H., Hong, S. K., Hong, S. J., Lee, M. H. (2014). Agar-derived neoagarooligosaccharide composite composition prepared by daga enzyme reaction and having anti-obesity and anti-diabetes effects: Google Patents (WO2014077575A1).

Lefkowitz, A. R. (2010). Probiotic confection and lipid compositions: Ganeden Biotech Inc. (US9597286B2)

Livesey, G. (2003). Health potential of polyols as sugar replacers, with emphasis on low glycaemic properties. Nutrition Research Reviews. 6(2), 163-91. doi:10·1079/NRR200371

Lund, A. E. (2008). Xylitol gummy bears reduce plaque in young children. Journal of the American Dental Association, 139(10), 312.

Lustig, R. H., Schmidt, L. A., Brindis, C. D. (2012). The toxic truth about sugar. Nature, 27, 482. doi:10.1038/482027a

Malik, V. S., Pan, A., Willett, W. C., Hu, F. B. (2013). Sugar-sweetened beverages and weight gain in children and adults: a systematic review and meta-analysis. The American Journal of Clinical Nutrition, 98(4), 1084-1102. doi:10.3945/ajcn.113.058362

Marcinkowski, E. d. A. (2006). Estudo da cinética de secagem, curvas de sorção e predição de propriedades termodinâmicas da proteína texturizada de soja. Universidade Federal do Rio Grande do Sul. Retrieved from http://www.lume.ufrgs.br/bitstream/handle/10183/13433/000638112.pdf (last accessed on 11-06-18)

Martino, M. and Chiarugi, A. (2015). Recent Advances in Pediatric Use of Oral Paracetamol in Fever and Pain Management. Pain and Therapy, 4(2), 149-168. doi:10.1007/s40122-015-0040-z

Maydeo, A. (2010). Lactitol or lactulose in the treatment of chronic constipation: result of a systematic. Journal of the Indian Medical Association, 108(11), 789-792.

McClements, D. J. (2003). Analysis of Food Products. Retrieved from http://people.umass.edu/~mcclemen/581Toppage.html (last accessed on 22-11-17)

McCooey, A. (2016). Sugar and Sweetener Guide. Retrieved from http://www.sugar-and-sweetener-guide.com/sweetener-values.html (last accessed on 13-06-18)

Medeiros, A. F. D., Santos, A. F. O., de Souza, F. S., Procópio, J. V. V., Pinto, M. F., Macêdo, R. O. (2007). Thermal stability of paracetamol and its pre-formulates obtained by spray drying. Journal of Thermal Analysis and Calorimetry, 88(2), 377-382. doi:10.1007/s10973-006-8006-3

Melnick, R. L., Huff, J., Haseman, J. K., Dieter, M. P., Grieshaber, C. K., Wyand, D. S., Lilja, H. S. (1983). Chronic effects of agar, guar gum, gum arabic, locust-bean gum, or tara gum in F344 rats and B6C3F1 mice. Food and Chemical Toxicology, 21(3), 305-311. doi:10.1016/0278-6915(83)90065-0

Miles, M. J., Morris, V. J., Carroll, V. (1984). Carob gum-κ-carrageenan mixed gels: mechanical properties and x-ray fiber diffraction studies. Macromolecules, 17(11), 2443-2445. doi:10.1021/ma00141a041

Mineo, W. and Katsuyoshi, N. (1987). Rheological and thermal properties of carrageenan gels. Effect of sulfate content. Die Makromolekulare Chemie, 188(9), 2213-2221. doi:10.1002/macp.1987.021880918

Minnesota Dental Association (2017). The Effects of Sour Candy on Oral Health. Retrieved from https://www.mndental.org/files/Pucker-Up-5.pdf (last accessed on 14-06-18)

Mitsubishi- Chemical Foods Corporation. (1998). What is Erythritol? Retrieved from http://www.mfc.co.jp/en/erythritol/about.html (last accessed on 23-06-18)

Miyazaki, S., Ishitani, M., Takahashi, A., Shimoyama, T., Itoh, K., Attwood, D. (2011). Carrageenan gels for oral sustained delivery of acetaminophen to dysphagic patients. Biological and Pharmaceutical Bulletin, 34(1), 164-166. Doi:10.1248/bpb.34.164

Mohammed, K. (2014). Thermogravimetric Analysis. Retrieved from https://pt.slideshare.net/KalsoomMohammed/thermogravimetry-analysis-tga (last accessed on 19-06-18)

Mudgil, D., Barak, S., Khatkar, B. S. (2014). Guar gum: processing, properties and food applications: A Review. Journal of Food Science and Technology, 51(3), 409-418. doi:10.1007/s13197-011-0522-x

Mueller, R. and Innerebner, F. (2006). Starch-based rubber-elastic confectionery: Google Patents (US206/013431A1).

Muzzarelli, R. A. A., Muzzarelli, C. (2009). Chitin and chitosan hydrogels. In Handbook of Hydrocolloids, 849-888: Woodhead Publishing.

Nasri, H., Baradaran, A., Shirzad, H., Rafieian-Kopaei, M. (2014). New Concepts in Nutraceuticals as Alternative for Pharmaceuticals. International Journal of Preventive Medicine, 5(12), 1487-1499. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/25709784 (last access on 21-07-18)

National Confectioners Association. (2018). Kinds of chocolate. Retrieved from https://www.candyusa.com/story-of-chocolate/what-is-chocolate/kinds-of-chocolate/ (last accessed on 04-06-18)

National Center for Biotechnology Information. (2018). D-Glucose. Retrieved from https://pubchem.ncbi.nlm.nih.gov/compound/5793 (last accessed on 13-06-18)

National Center for Biotechnology Information. (2018). D-sorbitol. Retrieved from https://pubchem.ncbi.nlm.nih.gov/compound/D-Sorbitol#section=Top (last accessed on 13-06-18)

National Center for Biotechnology Information. (2018). Xylitol. Retrieved from https://pubchem.ncbi.nlm.nih.gov/compound/6912 (last accessed on 23-06-18)

Network, T. T. D. (2015). HSDB: Acetaminophen. U.S. National Library of Medicine. Retrieved from https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+hsdb:@term+@rn+103-90-2:@odhsdb@/cgi-bin/sis/search2/f?./temp/~Hr1Tsf:1@ (last accessed on 14-06-18)

NETZSCH. (2016). STA 449 F3 Jupiter ®. Retrieved from https://www.netzsch-thermal-analysis.com/pt/produtos-solucoes/termogravimetria-calorimetria-exploratoria-diferencial-simultaneas/sta-449-f3-jupiter/ (last accessed on 14-06-18)

Nguyen, S., Alund, S. J., Hiorth, M., Kjøniksen, A. L., Smistad, G. (2011). Studies on pectin coating of liposomes for drug delivery. Colloids and Surfaces B: Biointerfaces, 88(2), 664-673. doi:10.1016/j.colsurfb.2011.07.058

NTP. (1993). NTP Toxicology and Carcinogenesis Studies of Acetaminophen (CAS No. 103-90-2) in F344 Rats and B6C3F1 Mice (Feed Studies). National Toxicology Program Technical Report Series, 394, 1-274. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/12637965 (last access on 04-05-18)

Nunes, D. F. (2016). DoctorGummy: Dona dos chocolates Regina investe em gomas saudáveis. Dinheiro Vivo. (last accessed on 13-06-18)

Nussinovitch, A. (2012). Gum technology in the food and other industries. In Hydrocolloid Applications: Springer US. London

Nwachukwu and Ahunanya, G. (1985). Studies on Cassava Syrup as a possible brewing adjunct. Thesis: University of Nigeria.

O'Neil, M. J. (2001). The Merck Index. In An Encyclopedia of Chemicals, Drugs and Biologicals. Whitehouse Station, N.J Merck.

Oliveira, G. G. G., Feitosa, A., Loureiro, K., Fernandes, A. R., Souto, E. B., Severino, P. (2017). Compatibility study of paracetamol, chlorpheniramine maleate and phenylephrine hydrochloride in physical mixtures. Saudi Pharmaceutical Journal: Saudi Pharmaceutical Journal, 25(1), 99-103. doi:10.1016/j.jsps.2016.05.001

Oliveira, J. T., Martins, L., Picciochi, R., Malafaya, P. B., Sousa, R. A., Neves, N. M., Reis, R. L. (2010). Gellan gum: A new biomaterial for cartilage tissue engineering applications. Journal of Biomedical Materials Research Part A, 93A(3), 852-863. doi:10.1002/jbm.a.32574

Osilesi, O., Trout, D. L., Glover, E. E., Harper, S. M., Koh, E. T., Behall, K. M., Tartt, J. (1985). Use of xanthan gum in dietary management of diabetes mellitus. American Journal of Clinical Nutrition, 42(4), 597-603. doi:10.1093/ajcn/42.4.597

Periche, A., Heredia, A., Escriche, I., Andrés, A., Castelló, M. L. (2014). Optical, mechanical and sensory properties of based-isomaltulose gummy confections. Food Bioscience, 7, 37-44. doi:10.1016/j.fbio.2014.05.006

Peroni, F. H. G. (2003). Características estruturais e físico-químicas de amidos obtidos de diferentes fontes botânicas. Retrieved from https://repositorio.unesp.br/handle/11449/88414 (last accessed on 06-03-18)

Phillips, G. O. (1996). The chemical identification of PNG-carrageenan. Gums and Stabilisers for the food industry. 403-421: Newtech Innovation Centre. United Kingdom

Polaha, J., Dalton, W. T. 3rd., Lancaster, B. M. (2008). Parental report of medication acceptance among youth: implications for everyday practice. South Medicine Journal, 101(11), 1106-1112. doi:10.1097/SMJ.0b013e318180645d

Professional, C. (2018). Drawing of molecules (Version 16.0.1.4).

National Tecnology Program. (2005). NTP report on the toxicology studies of aspartame (CAS No. 22839-47-0) in genetically modified (FVB Tg.AC hemizygous) and B6.129-Cdkn2atm1Rdp (N2) deficient mice and carcinogenicity studies of aspartame in genetically modified [B6.129-Trp53tm1Brd (N5) haploinsufficient] mice (feed studies). National Toxicology Program Genetically Modified Model Report, (1), 1-222. Retrived from https://www.ncbi.nlm.nih.gov/pubmed/18685711 (last access 20-07-18)

Protection, A. C. (2002). Food energy – Methods of analysis and conversion factors. In Food and Nutrition Paper. Report of a technical workshop. Rome

Rizkalla, S. W. (2010). Health implications of fructose consumption: A review of recent data. Nutrition and Metabolism, 7, 82-82. doi:10.1186/1743-7075-7-82

Roberfroid, M. B. (2000). Prebiotics and probiotics: are they functional foods?. American Society for Clinical Nutrition, 71(6), 1682–1687, doi:10.1093/ajcn/71.6.1682S

Rocha, F. (2016). Amido Modificado e Gomas Em Alimentos. Retrieved from https://pt.scribd.com/presentation/360900387/Amido-Modificado-e-Gomas-Em-Alimentos (last accessed on 16-03-18)

Ruiz-Ruiz, J. C., Moguel-Ordoñez, Y. B., Segura-Campos, M. R. (2017). Biological activity of Stevia rebaudiana Bertoni and their relationship to health. Critical Reviews in Food Science and Nutrition, 57(12), 2680-2690. doi:10.1080/10408398.2015.1072083

Samuel, P., Ayoob, K., Ashwell, M., Magnuson, B., Lin, K. G., Wölwer-Rieck, U. (2017). Stevia Facts. Retrieved from https://www.purecirclesteviainstitute.com/resources/infographics/stevia-facts (last accessed on 13-06-18)

Kayaa, A. O. W., Suryanib, A. Santoso, J., RusliSanderson, M.A, (1990). The Effect of Gelling Agent Concentration on the Characteristic of Gel Produced From the Mixture of Semi-refined Carrageenan and Glukomannan. 201-232: International Journal of Sciences. Indonesia

Savino, F., Muratore, M. C., Silvestro, L., Oggero, R., Mostert, M. (1999). Allergy to Carob Gum in an Infant. Journal of Pediatric Gastroenterology and Nutrition, 29(4), 475-476. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/10512412 (last access 21-07-18)

Scariotto, M. C. (2013). Estudo da utilização da goma Xantana como auxiliar no processo de floculação em tratamento de água para abastecimento. Universidade Tecnológica Federal Do Paraná. Retrieved from http://repositorio.roca.utfpr.edu.br/jspui/bitstream/1/1583/1/CM_COEAM_2013_1_20.pdf (last accessed on 13-03-18)

Schiele, J. T., Quinzler, R., Klimm, H.-D., Pruszydlo, M. G., Haefeli, W. E. (2013). Difficulties swallowing solid oral dosage forms in a general practice population: prevalence, causes and relationship to dosage forms. European Journal of Clinical Pharmacology, 69(4), 937-948. doi:10.1007/s00228-012-1417-0

Schiffman, S. S. and Rother, K. I. (2013). Sucralose, A Synthetic Organochlorine Sweetener: Overview of Biological Issues. Journal of Toxicology and Environmental Health. Part B, Critical Reviews, 16(7), 399-451. doi:10.1080/10937404.2013.842523

Schiweck, H., Bär, A., Vogel, R., Schwarz, E., Kunz, M. (2012). Sugar Alcohols. In Ullmann's Encyclopedia of Industrial Chemistry: Wiley-VCH Verlag GmbH & Co. KGaA. doi:10.1002/14356007.a25_413.pub3

Shafer, R. B., Levine, A. S., Marlette, J. M., Morley, J. E. (1987). Effects of xylitol on gastric emptying and food intake. American Journal of Clinical Nutrition, 45(4), 744-747. Doi: 10.1093/ajcn/45.4.744

Sousa, J. and Rufato, S. S. (2018). Determinação da Umidade do Café- Secagem e Armazenagem de Café – Tecnologias e Custos. Retrived from https://www.researchgate.net/publication/266595202_DETERMINACAO_DA_UMIDADE_DO_CAFE (last access on 05-05-18)

Standards, A. (2002). S 882: Standard Test Method for Tensile Properties of Thin Plastic Sheeting. Reviewed from https://www.astm.org/DATABASE.CART/HISTORICAL/D882-02.htm (last access on 18-06-18)

Stroka, J., Dossi, N., Anklam, E. (2003). Determination of the artificial sweetener Sucralose by capillary electrophoresis. Food Additives and Contaminants, 20(6), 524-527. doi:10.1080/0265203031000070803

Sundar Raj, A. A., Rubila, S., Jayabalan, R., Ranganathan, T. V. (2012). A review on pectin: Chemistry due to general properties of pectin and its pharmaceutical uses. Scientific Reports, 1(12), 1-3. Doi: 10.4172/scientificreports.553

Sworn, G. (2009). Xanthan gum. In Handbook of Hydrocolloids, 186-203: Woodhead Publishing. Burgos

Sworn, G. (2009). Gellan gum. In Handbook of Hydrocolloids, 204-227: Woodhead Publishing. Burgos

Tavčar, E., Turk, E., Kreft, S. (2012). Simple Modification of Karl-Fischer Titration Method for Determination of Water Content in Colored Samples. Journal of Analytical Methods in Chemistry, 2012, 379-724. doi:10.1155/2012/379724

Thabuis, C., Y Cheng, C., Wang, X., Pochat, M., Han, A., Miller, L., Guerin-Deremaux, L. (2013). Effects of maltitol and xylitol chewing-gums on parameters involved in dental caries development, 14(4), 303-8. Doi:10.1080/ 20002297.2017.1374152

Thiébaud, D., Jacot, E., Schmitz, H., Spengler, M., Felber, J. P. (1984). Comparative study of isomalt and sucrose by means of continuous indirect calorimetry. Metabolism, 33(9), 808-813. doi: 10.1016/0026-0495(84)90106-9

Thomson, S. A., Tuleu, C., Wong, I. C. K., Keady, S., Pitt, K. G., Sutcliffe, A. G. (2009). Minitablets: New Modality to Deliver Medicines to Preschool-Aged Children. Pediatrics, 123(2), 235-238. doi:10.1542/peds.2008-2059

Tobacman, J. K. (2001). Review of harmful gastrointestinal effects of carrageenan in animal experiments. Environmental Health Perspectives, 109(10), 983-994. doi:10.1289/ehp.01109983

Toledo, M. (2012). Karl Fischer - The method at a Glance. Retrieved from https://www.mt.com/dam/MT-NA/KF/KarlFischer-Guide4.pdf (last accessed on 05-04-18)

Tomassetti, M., Catalani, A., Rossi, V., Vecchio, S. (2005). Thermal analysis study of the interactions between acetaminophen and excipients in solid dosage forms and in some binary mixtures. Journal of Pharmaceutical and Biomedical Analysis, 37(5), 949-955. doi:10.1016/j.jpba.2004.10.008

Toolbox, T. E. (2010). Water - boiling points at Vacuum Pressure. Retrieved from https://www.engineeringtoolbox.com/water-evacuation-pressure-temperature-d_1686.html (last accessed on 18-04-18)

TOXNET. Acetaminophen Retrieved from https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+hsdb:@term+@rn+@rel+103-90-2 (last accessed on 16-05-18)

Trivedi, J., D. Thaker, M., C. Trivedi, H. (2014). Synthesis and characterization of poly(butyl methacrylate) grafted sodium salt of partially carboxymethylated guar gum. In Cellulose Chemistry and Technology, Vol. 32. India

TutorVista. (2018). Pectin. Retrieved from https://chemistry.tutorvista.com/organic-chemistry/pectin.html (last accessed on 14-06-18)

Vaclavik, V. and Christian, E. W. (2014). Essentials of food science. Springer-Verlag New York: 572. New York

Vervloet, D., Senft, M., Dugue, P., Arnaud, A., Charpin, J. (1983). Anaphylactic reactions to modified fluid gelatins. Journal of Allergy and Clinical Immunology, 71(6), 535-540. doi:10.1016/0091-6749(83)90433-5

Wahl, R. and Kleinhans, D. (1989). IgE-mediated allergic reactions to fruit gums and investigation of cross-reactivity between gelatine and modified gelatine-containing products. Clinical and Experimental Allergy, 19(1), 77-80. doi:10.1111/j.1365-2222.1989.tb02348.x

Wankenne, M. A. (2008). Agar ou Agar-Agar, o mais antigo Ficocolóide. In Aditivos & Ingredientes. Editora Insumos Ltda. Retrieved from http://insumos.com.br/aditivos_e_ingredientes/materias/87.pdf (last access on 10-04-18)

Wankenne, M. A. (2012). Carragenas: Kappa, Iota, Lambda, Mu, Nu e Theta!. In Aditivos and Ingredientes. Editora Insumos Ltda. Retrieved from http://insumos.com.br/aditivos_e_ingredientes/materias/87.pdf (last access on 10-04-18)

Watase, M., Nishinari, K., Hatakeyama, T. (1988). DSC study on properties of water in concentrated agarose gels. In Food Hydrocolloids, 2(6), 427-438. doi:10.1016/S0268-005X(88)80043-2

Weiner, M. L. (2014). Food additive carrageenan: Part II: A critical review of carrageenan in vivo safety studies. Critical Review of Toxicology, 44(3), 244-269. doi:10.3109/10408444.2013.861798

Whistler, R. L. and Belfort, A. M. (1961). Nutritional value of chemically modified corn starches. Science, 133(3464), 1599-1600

Williams, P. A. and Phillips, G. O. (2009). Introduction to food hydrocolloids. In Handbook of Hydrocolloids), 1-22: Woodhead Publishing. Burgos

Williams, P. A. and Phillips, G. O. (2009). Gum arabic. In Handbook of Hydrocolloids, 252-273: Woodhead Publishing. Burgos

Wills, D. (1998). Water Activity and its Importance in Making Candy. In The Manufacturing Confectioner, 71-74. LMC International. London

Yalkowsky, S. H., He, Y., Jain, P. (2010). Acetaminophen. In Handbook of Aqueous Solubility Data. Retrived from https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+hsdb:@term+@rn+@rel+103-90-2 (last access on 14-05-18)

Yamabe, F., Masumoto, K., Suzuki, K., Osada, K., Yamada, Y. and Yamada, I. (2017). High-moisture-content gummi candy: Google Patents (EP3187053A1)

Yamada, H., Kiyohara, H., Matsumoto, T. (2003). Recent Studies on Possible Functions of Bioactive Pectins and Pectic Polysaccharides from Medicinal Herbs on Health Care. In Advances in Pectin and Pectinase Research, 481-490: Springer Dordrecht. Netherlands

Yamamoto, Y., Sogawa, I., Nishina, A., Saeki, S., Ichikawa, N., Iibata, S. (2000). Improved Hypolipidemic Effects of Xanthan Gum-Galactomannan Mixtures in Rats. Bioscience, Biotechnology and Biochemistry, 64(10), 2165-2171. doi:10.1271/bbb.64.2165

Yang, R. K. and Oh, V. (1998). Consumable gummy delivery system: Google Patents (US5928664A)

Yaranossian, N. and Lynch, M. G. (2014). Confectionery Containing Low Viscosity Iota Carrageenan: PMC Corporation (US20170251692A1)

Yasui, H. (1999). Gumi candies and a process for making the same (US5932273A).

8. Appendix

Appendix 1. Karl-Fischer method

The method used for determining the moisture content in DoctorGummy products is K-F method. Some information about this method is presented below.

For **coulometric** titrations, a smaller compartment with a catholyte solution is immersed in the main anode compartment of the titration cell, that has the sample dissolved in anolyte (iodide and a sulphur dioxide/base buffer). These solutions must first be pre-titrated. Iodine is generated electrochemically and consumed for water titration. Constant current is detected potentiometrically by two detector electrodes. At the equivalence point, excess iodine abrupt voltage drop. The current necessary to generate the iodine is measured (Tavčar et al., 2012).

The sample is injected, via a septum, into the reagent. Modern titrators calculate the amount of water automatically after injection of the sample.

The **volumetric** titration is based on the same principles except that the anode solution is used as the titrant solution (Tavčar et al., 2012). In a two-component reagent the reactive components are separated. The titrant contains a pre-set amount of iodine in a solvent, which can be either methanol or ethanol. The burette of the titrator is then filled with this solution. A base and sulphur dioxide are dissolved in the ‘‘K-F solvent’’, which is added to the titration cell and pretitrated to dryness. The sample is then added, and its water content determined. It is important that the pH of the system is maintained between 5-7 to assure that the method is effective.

K-F method applied to solids

Solids may have water bounds in two different states: It can be adsorbed on the surface of the solid or in the form of crystallized water or trapped water inside the solid. In order to determine the whole water content, the sample must be fully dissolved. If the material does not dissolve in methanol, additional solvents are often added to aid solubility. For lipophilic substances, a working medium is used. Normally it consists of 50% methanol or K-F solvent and the remainder is chloroform or a suitable alcohol (sometimes it may inhibit the dissolution of inorganic salts and some sugars). To dissolve polar substances the working medium is made up of 50% formamide (it is also suggested when analysing carbohydrates, proteins and inorganic salts). It is recommended to reduce the size of large pieces for better solubility by crushing the sample using a pestle and mortar, ground in a laboratory mill, or cutting into small pieces.

If there is no working medium that dissolves the sample, it should be tried an extraction titration. The sample is ground as fine as possible and suspended in the working medium. The water is then titrated. The hygroscopic solvent extracts the water from the solid. After the End

Point has been reached, it is recommended that a subsequent standard addition is made, to ensure that the water is completely extracted. To improve the distribution of the sample, a high-speed stirrer or homogenizer can also be installed inside the titration cell. The sample is dispersed during the titration, which optimizes the conditions for such samples (Utilities, 2017).

Another possibility for determining the water content of insoluble samples is external extraction: a known amount of a hygroscopic solvent, typically dried methanol, is added to the sample in a volumetric flask. After a period of stirring, an aliquot is taken, and the water content titrated. The result must take into account the blank value of the methanol. When there is a low water content, it should be determined the blank value of the solvent and the blank of the procedure. For the blank of the procedure a volumetric flask is taken, filled with the same volume of methanol, stirred under the same conditions for the same length of time and an aliquot taken and titrated to the same volume. Already titrated K-F solvent can also be used to extract water from samples.

For gummy candies, Toledo (2012) recommend a direct titration with added formamide and heat to 50 ºC. The titrant is a two-component reagent 5 mg H_2O/mL, the solvent 40 mL K-F solvent /formamide 1:1 and the stir time 10-15 minutes.

Appendix 2. Results from the questionnaire

Table A2.1 shows the results obtained with the questionnaire about gummy candies:

Table A2.1 Results from the questionnaire

Are you vegan or vegetarian?	Yes	4%
	No	79%
	Not yet, but I want to	16%
Do you eat gummy candies?	Yes	33%
	No	67%
Have you ever eaten gummies without sugar?	Yes	73%
	No	27%
Have you ever eaten vegan gummies?	Yes	11%
	No	89%
Would you choose vegan gummies in spite of those with animal origin, if the flavour maintains?	Yes	90%
	No	10%
Do you know any brand of gummies without sugar?	Yes	15%
	No	85%
Sugar or natural sweeteners?	Sugar	10%
	Sweetener	90%
Do you know any brand of vegan gummies?	Yes	7%
	No	93%
Which vegan brand do you know?	DoctorGummy	30%
	NaturSoy	30%
	Fini	10%
	DreamPills	10%
	Trolli	10%

	Beauty Sweeties	10%
Which gummy brands do you eat?	Pingo Doce	18%
	Continente	22%
	Biobon	1%
	DoctorGummy	7%
	Fini	12%
	Habribo	31%
	Natursoy	1%
	Trident	1%
	Vidal	4%
	Other	1%
	I don't eat gummies	1%
What is your favourite candy?	Gummies	15%
	Chocolate	69%
	Marshmallow's	4%
	Chwing gum	10%
	Lollipops	2%
	Hard candies	1%

Appendix 3. Moisture content calculation

The final product must have about 75% solids and during the process there is a lot of dehydration, that should be considered when the quantity of water is defined.

Example:

Formula C29:

- 4.5 g carrageenan CEABLOOM 91-455
- 30 g maltitol powder
- 25 g maltitol syrup
- 0.1 g stevia
- 3 g sorbitol
- Citric acid pH 3.7

Having in consideration that maltitol syrup has about 55% solids and the rest of the ingredients may be considered about 100% solids:

$$4.5 + 30 + 25 \times 0.55 + 0.1 + 3 = 51.35\ g \text{ of solids} \qquad \text{(i)}$$

$$\frac{75}{100} = \frac{51.35}{x} <=> x = 40.47\ g \text{ (ii)}$$

To have 75% solids, according to Eq. ii, the water added should be:

$$40.47 - 0.45 \times 25 = 54.72\ g \qquad \text{(iii)}$$

However, after some labour tests, it was verified that carrageenan absorbs much more water than gelatine. Besides that, there are some evaporation during the procedure. Thus, after some tests and considering that about 37% of the water added will be evaporated (in gelatine gummies there is usually an evaporation of about 30%):

$$54.72 + 54.72 \times 0.37 \approx 75\ g \qquad \text{(iv)}$$

Appendix 4. Correct pH for each binding agent

The following table (Table A4.1) presents the pH range that should be used when confectioning gummy candies.

Table 4.1 Correct pH for each binding agent

Pectin	Carrageenan	Gellan Gum	Carob gum	Gelatine
3.0-3.4 (Endreß and Christensen, 2009)	**>3.5** (Imeson, 2009)	**3.5-8.0** (Nussinovitch, 2012)	**5.4-7.0** (Kulkarni and Shaw, 2016)	**2.8-4.0** (Judy, 2010)

Although carrageenan stability is at a pH above 3.5, it is recommended that in acidic foods, the acid is added at the end of the formulation. Further breakdown does not occur after jellification (Baines and Seal, 2012). Starches and agar gelation do not depend on the pH but also on the temperature and concentration (Armisén and Gaiatas, 2009).

Appendix 5. Formulas tested and sensorial analysis to the gummies

The following table (Table A5.1) resumes all the formulations tested for this study. They are grouped according to the binding agent used. Variations in the type and concentration of the reagents and pH were tested.

The results of the sensorial analysis of the gummies are presented in Table A5.2.

Table A5.1 List of formulas tested in this project, in grams (g)

	Binding Agent													Sweetener						Acid/buffer		Water
	Carrageenan					Starches								Syrup	Powder							
	K	K+	I	91-455	CF3243	P	G	LBG	PG8005	LG7015	MB70	A	X	Mt	Mt	Sb	Mn	S	E	pH	TC	%
P1						31												1	10	3		83
P2						28												1	10	3		84
P3						25													10	3		85
P4						7.5								15		20				3.5		61
PX1						10							10					1	10	3		87
PX2						5							5					0.5	10	3		91
PA						20						15		10	2			1		3		80
PISX			17			4			7.5				1.96	45						3		80
PIS			1.5			0.5			2.5					30				1	10	3.6		70
PS1						5			15					30	35					3.6		43
PS2						3					10			30			35			3.5		45
PG						1	1.3							30	40					4	1.25	50
LX								2					1.5	25	30					3.5		56
S1									15					30	35					3.4		45
S2									11					45		27				3.4		47
S3											15			30			35			3.6		45
S4										4	8			45	12	25		1		3.8		40
G1							1.2							39	39					4.0	1.25	43
CG1		0.5	2				0.5							45		20		2		3.7		45
CG2			1				1.5							30	40					3.6		52
CL1		7.2						1.8											11	3.5		93
CL2		0.5	2					1.5							30					3.5		67
CL3		1	1.5					1.5							15	15				3.5		67
CL4		1	1.5					1.5							15	15				3.4		75
CL5	7.2							1.8						15	20				11	3.7		81
CL6	7.2							1.8						15	20				11	4.3		76
CL7	0.5		2					1.5							30					3.5		67
CL8	1		1.5					1.5							15	15				3.5		75
C1			1.5											45		46				3.6	0.45	45
C2	1		2											20	30					3.7		61
C3	1		2											20	30					3.7		83
C4	1.5		1.5											25		30				3.8		63
C5	1.5		1.5											25	30					3.2		63
C6	0.5		2											25	30					3.8		64
C7	0.5		2											25	30					3.7		64
C8	0.5		2											25	30					3.5		64
C9	1.5		1.5											25	30					3.8		63
C10	1.5		1.5											25	30					3.8		63
C11		2.5												25		15				2.5		66
C12		2																	11	3.3		95
C13					3									0	43					3.7		63
C14					3									25	30					3.7		63
C15				3										25	30			1		3.7		63
C16					3									25	30			1		3.4		63
C17					3									25	30			1		3.7		63
C18				3										25	30			1		3.4		63
C19				3										25	30			0.8		3.7		63
C20					3									25	30			0.8		3.7		63
C21				3										25	30			0.5		3.7		63
C22					3									25	30			0.5		3.7		63
C23				3										25	30			0.3		3.7		63
C24					3									25	30			0.3		3.7		63
C25				3.5										25	30	3		0.3		3.7		62
C26					3.5									25	30	3		0.3		3.7		62
C27				4										25	30	3		0.3		3.7		61
C28					4									25	30	3		0.3		3.7		61
C29				4.5										25	30	3		0.1		3.7		63
C30					4.5									25	30	3		0.1		3.7		63
C31				5										25	30	3		0.1		3.7		62
C32					5									25	30	3		0.1		3.7		62
C33				1.7										45		34		0.03		3.0		43
C34				1.6					0.5					45		50		0.04		3.8		35
C35					3.5									25		30		0.3		3.7		63
C36					3.5									50				0.3		3.7		71
C37				1.5										41	51					3.7	0.45	40
C38				1.5										41		50		0.1		3.7	0.4	44
C39				1.5										41	25	30		0.1		3.7	0.4	42
C40				1.8										41		50		0.1		3.7	0.4	44
C41				1.8										41	25	30		0.1		3.7	0.4	42

Being K, kappa; K+, kappa + elastic; I, iota; P, pectin; G, gellan gum; C, carob gum; A, agar-agar; X, xanthan gum; Mt, maltitol; Sb, sorbitol; Mn, mannitol; S, Stevia; E, erythritol; TC, Trisodium citrate

Table A5.2. Results from the sensorial analysis

Appendix 6. Reagent Prices

The following Table, A6.1, presents the costs of each reagent used during the project. Paracetamol was acquired in Sigma-Aldrich (A7085).

Table A6.1 Costs of the most important reagents used in this project

Reagent	Price
Carrageenan	€11.00-18.45/kg
Carob Gum	€23.40/kg
Pectin	€7.80/kg
Gellan Gum	€30/kg
Agar-agar	€25/kg
Modified Starch	€1.01/kg
Maltitol powder	€2.75/kg
Maltitol Syrup	€1.90/kg
Sorbitol powder	€2.50/kg
Erythritol	€7.59/kg
Strawberry flavour 1	€205.00/kg
Strawberry flavour 2	€24.35/kg
Strawberry flavour 3	28.60 €/kg
Pineapple flavour	€30/kg
Paracetamol	€437/kg
Natural purple colour	€40/kg
Stevia (97%)	€210/kg
Citric acid	€69/ kg
Trisodium citrate	€195/kg

Appendix 7. Mechanical Properties

The following graphs (Figures A7.1, A7.2 and A7.3) present the curves obtained from the tensile tests for each gummy formulation. Each formulation has several replicants. Figures A7.4, A7.5 and A7.6 represent the curves obtained from the compressive tests for each gummy.

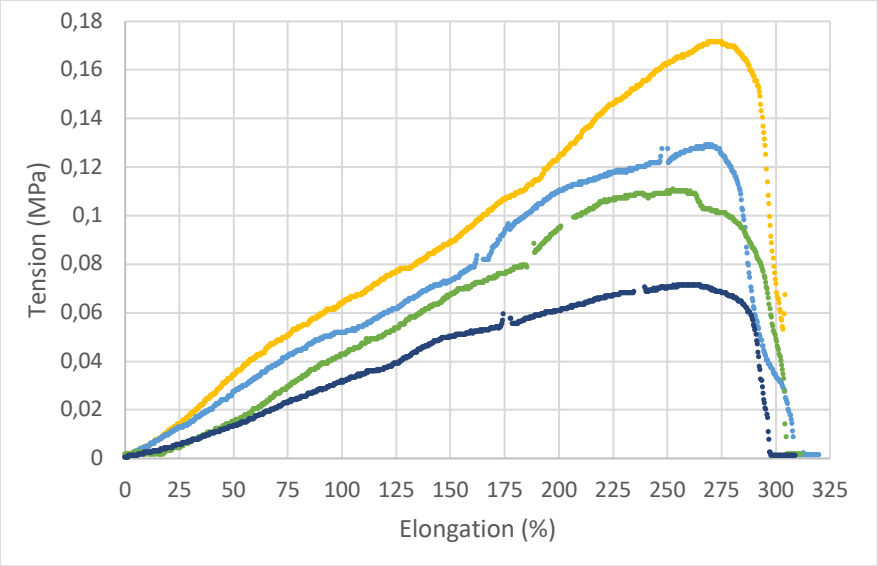


Figure A7.1 Replications of the tensile tests of formulation C29

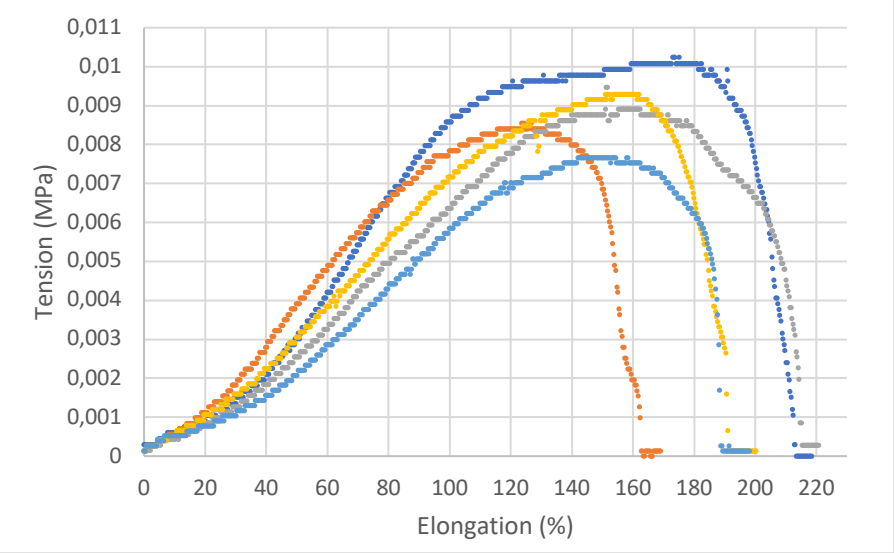


Figure A7.2 Replications of the tensile tests of formulation C40

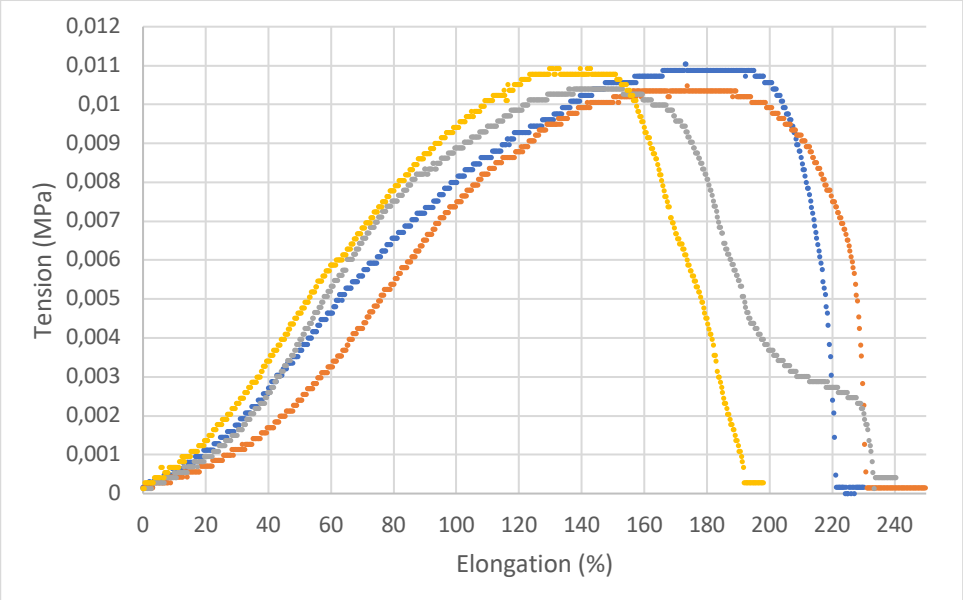


Figure A7.3 Replications of the tensile tests of formulation C41

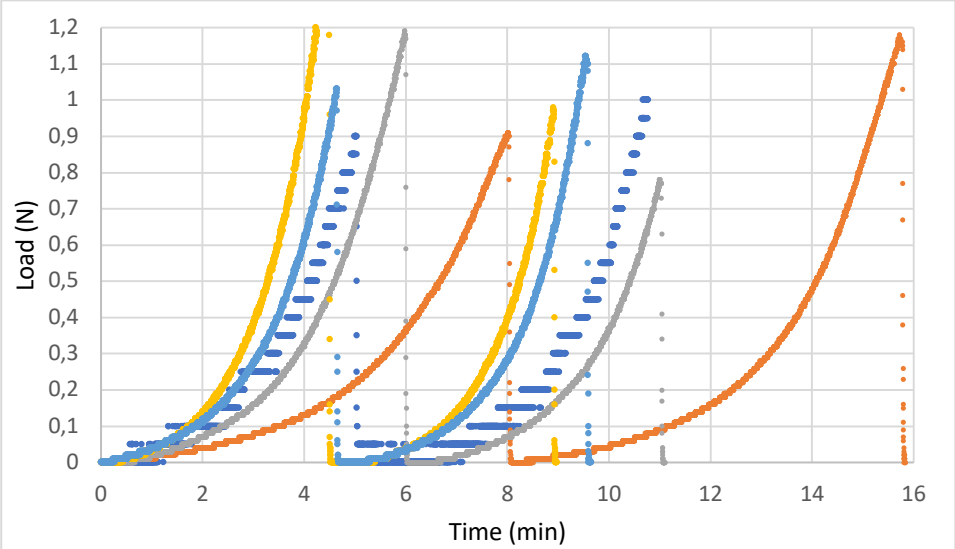


Figure A7.4 Replications of the compressive tests of formulation C29

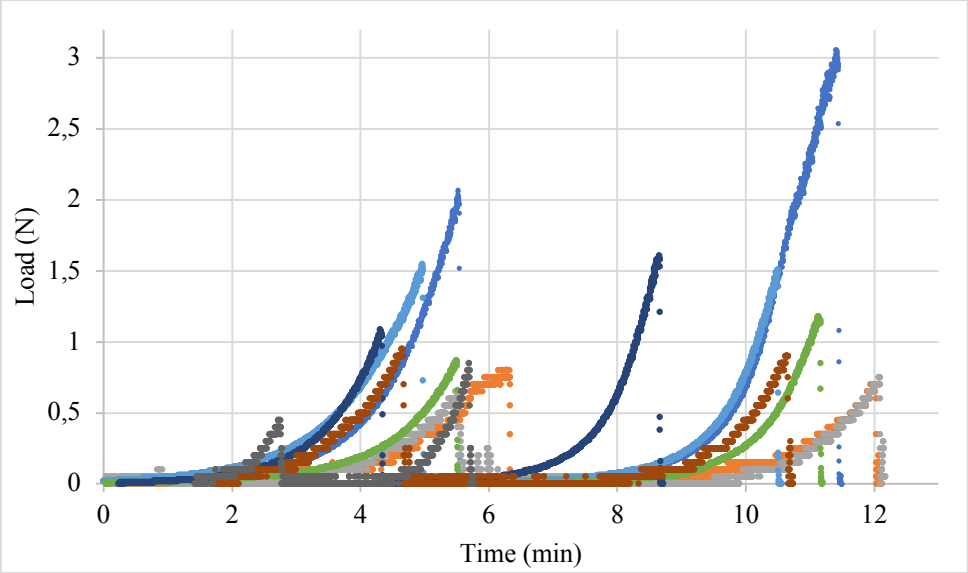


Figure A7.5 Replications of the compressive tests of formulation C40

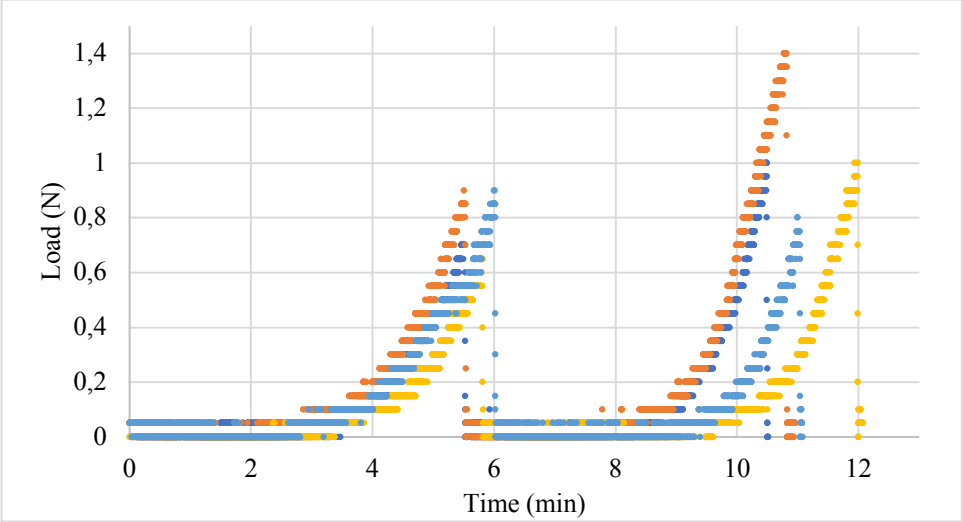
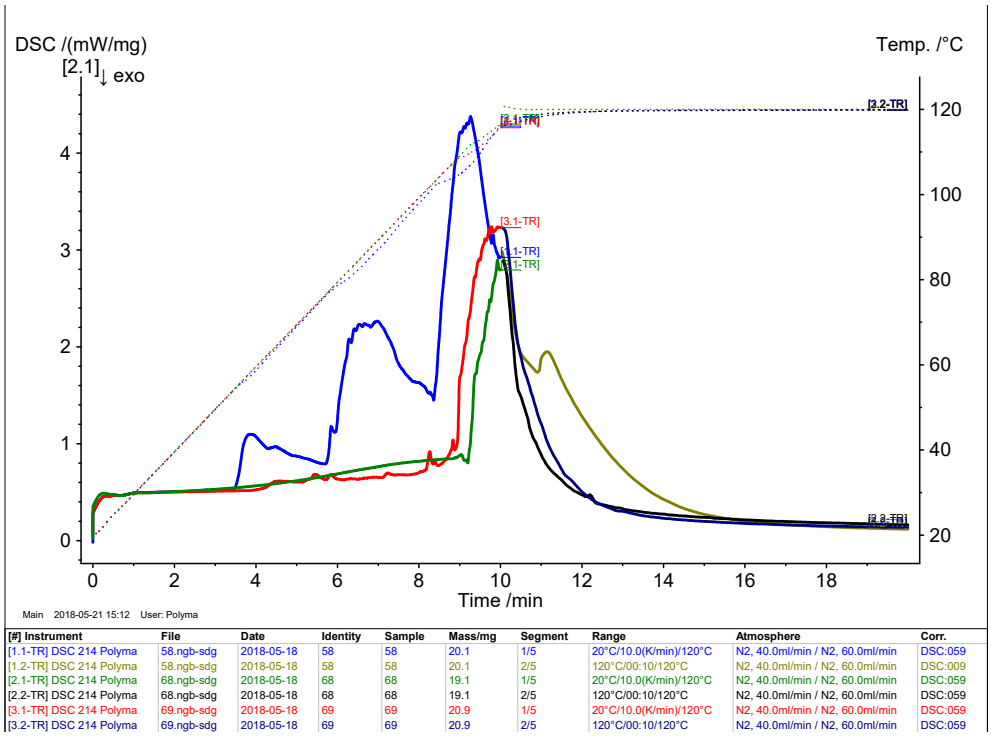


Figure A7.6 Replications of the compressive tests of formulation C41

Appendix 8. Graphs of the DSC to the gummies

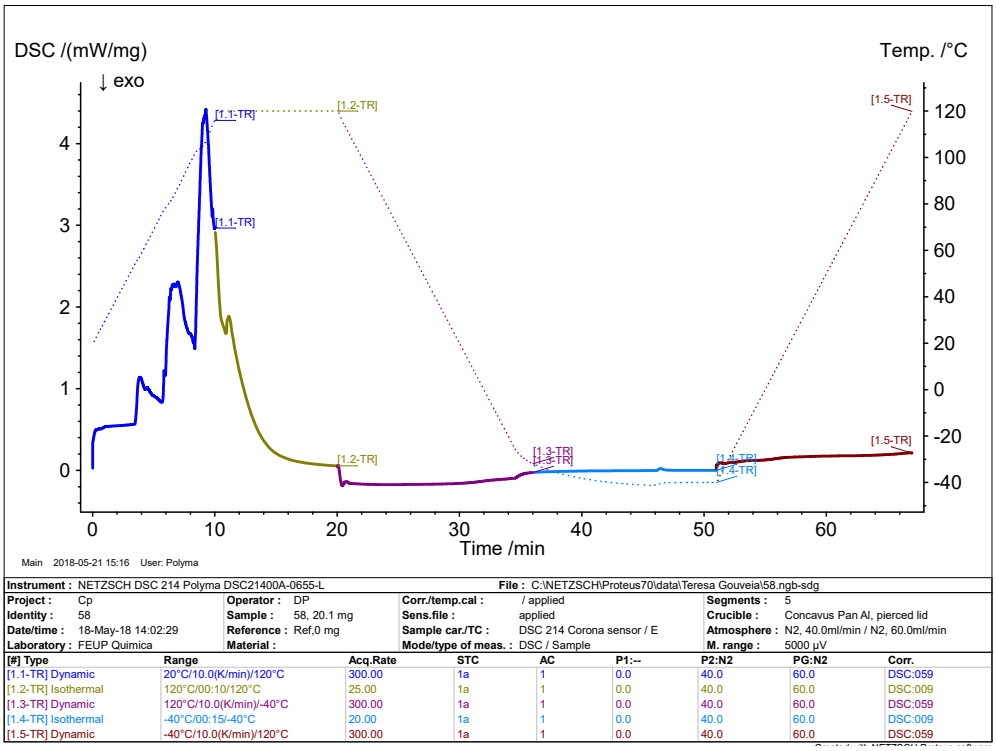
The graphs presented in Figures A8.1, A8.2, A8.3 and A8.4 were extracted from the DSC equipment program. Figure i presents the heating phase (1st segment). It is possible to see that formula C29 (light blue and olive-green colours) shows a different behave than the other ones. There is one main absorption peak with 3 divisions starting at 30 ºC with a maximum at 120 ºC, where the other 2 formulas (C40 and C41) only show one absorption peak at 120 ºC. Formula C29 also shows a peak when the temperature is maintained at 120 ºC (10 minute' isotherm- 2nd segment). The other formulas (red black for gummy C40 and green and dark blue for gummy C41) only present one peak at 120 ºC.



[#] Instrument	File	Date	Identity	Sample	Mass/mg	Segment	Range	Atmosphere	Corr.
[1.1-TR] DSC 214 Polyma	58.ngb-sdg	2018-05-18	58	58	20.1	1/5	20°C/10.0(K/min)/120°C	N2, 40.0ml/min / N2, 60.0ml/min	DSC:059
[1.2-TR] DSC 214 Polyma	58.ngb-sdg	2018-05-18	58	58	20.1	2/5	120°C/00:10/120°C	N2, 40.0ml/min / N2, 60.0ml/min	DSC:009
[2.1-TR] DSC 214 Polyma	68.ngb-sdg	2018-05-18	68	68	19.1	1/5	20°C/10.0(K/min)/120°C	N2, 40.0ml/min / N2, 60.0ml/min	DSC:059
[2.2-TR] DSC 214 Polyma	68.ngb-sdg	2018-05-18	68	68	19.1	2/5	120°C/00:10/120°C	N2, 40.0ml/min / N2, 60.0ml/min	DSC:059
[3.1-TR] DSC 214 Polyma	69.ngb-sdg	2018-05-18	69	69	20.9	1/5	20°C/10.0(K/min)/120°C	N2, 40.0ml/min / N2, 60.0ml/min	DSC:059
[3.2-TR] DSC 214 Polyma	69.ngb-sdg	2018-05-18	69	69	20.9	2/5	120°C/00:10/120°C	N2, 40.0ml/min / N2, 60.0ml/min	DSC:059

Figure A8.1 Heating segment of gummies C29, 40 and C41

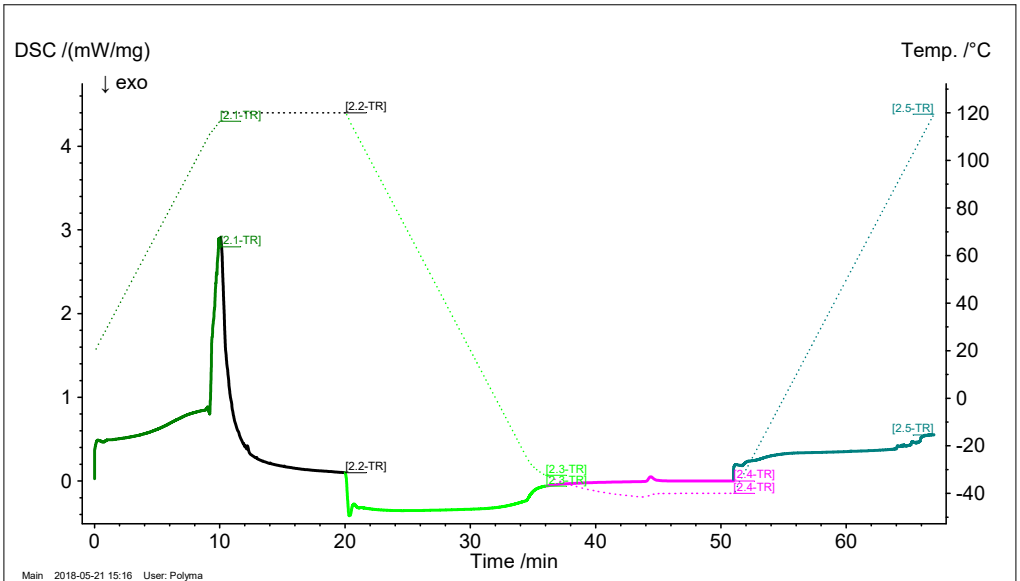
Analysing Figures ii, iii and iv it can be seen that the decrease of the temperature to -40 ºC (3rd segment) and the second re-heat cycle to 120 ºC (5th segment) did not show any change in the energy of none of the systems.



Instrument :	NETZSCH DSC 214 Polyma DSC21400A-0655-L				**File :**	C:\NETZSCH\Proteus70\data\Teresa Gouveia\58.ngb-sdg		
Project :	Cp	**Operator :**	DP	**Corr./temp.cal :**	/ applied	**Segments :**	5	
Identity :	58	**Sample :**	58, 20.1 mg	**Sens.file :**	applied	**Crucible :**	Concavus Pan Al, pierced lid	
Date/time :	18-May-18 14:02:29	**Reference :**	Ref,0 mg	**Sample car./TC :**	DSC 214 Corona sensor / E	**Atmosphere :**	N2, 40.0ml/min / N2, 60.0ml/min	
Laboratory :	FEUP Quimica	**Material :**		**Mode/type of meas. :**	DSC / Sample	**M. range :**	5000 µV	

[#] Type	Range	Acq.Rate	STC	AC	P1:--	P2:N2	PG:N2	Corr.
[1.1-TR] Dynamic	20°C/10.0(K/min)/120°C	300.00	1a	1	0.0	40.0	60.0	DSC:059
[1.2-TR] Isothermal	120°C/00:10/120°C	25.00	1a	1	0.0	40.0	60.0	DSC:009
[1.3-TR] Dynamic	120°C/10.0(K/min)/-40°C	300.00	1a	1	0.0	40.0	60.0	DSC:059
[1.4-TR] Isothermal	-40°C/00:15/-40°C	20.00	1a	1	0.0	40.0	60.0	DSC:009
[1.5-TR] Dynamic	-40°C/10.0(K/min)/120°C	300.00	1a	1	0.0	40.0	60.0	DSC:059

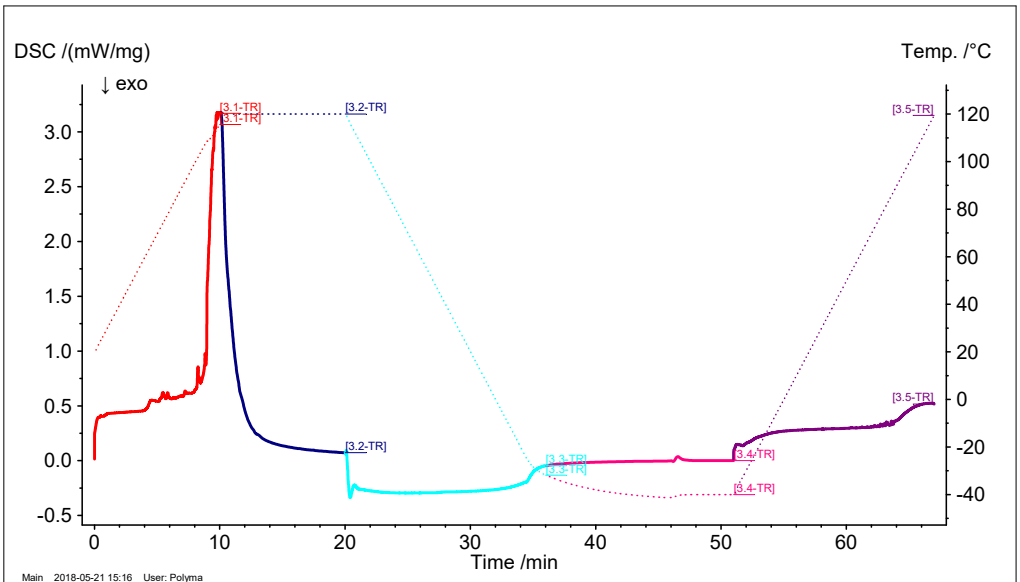
Figure A8.2 DSC of gummy C29



Instrument :	NETZSCH DSC 214 Polyma DSC21400A-0655-L			**File :**	C:\NETZSCH\Proteus70\data\Teresa Gouveia\68.ngb-sdg			
Project :	Cp	**Operator :**	DP	**Corr./temp.cal :**	/ applied	**Segments :**	5	
Identity :	68	**Sample :**	68, 19.1 mg	**Sens.file :**	applied	**Crucible :**	Concavus Pan Al, pierced lid	
Date/time :	18-May-18 11:05:12	**Reference :**	Ref,0 mg	**Sample car./TC :**	DSC 214 Corona sensor / E	**Atmosphere :**	N2, 40.0ml/min / N2, 60.0ml/min	
Laboratory :	FEUP Quimica	**Material :**		**Mode/type of meas. :**	DSC / Sample	**M. range :**	5000 µV	

[#] Type	Range	Acq.Rate	STC	AC	P1:--	P2:N2	PG:N2	Corr.
[2.1-TR] Dynamic	20°C/10.0(K/min)/120°C	300.00	1a	1	0.0	40.0	60.0	DSC:059
[2.2-TR] Isothermal	120°C/00:10/120°C	25.00	1a	1	0.0	40.0	60.0	DSC:009
[2.3-TR] Dynamic	120°C/10.0(K/min)/-40°C	300.00	1a	1	0.0	40.0	60.0	DSC:059
[2.4-TR] Isothermal	-40°C/00:15/-40°C	20.00	1a	1	0.0	40.0	60.0	DSC:009
[2.5-TR] Dynamic	-40°C/10.0(K/min)/120°C	300.00	1a	1	0.0	40.0	60.0	DSC:059

Figure A8.3 DSC of gummy 40



Instrument :	NETZSCH DSC 214 Polyma DSC21400A-0655-L			**File :**	C:\NETZSCH\Proteus70\data\Teresa Gouveia\69.ngb-sdg			
Project :	Cp	**Operator :**	DP	**Corr./temp.cal :**	/ applied	**Segments :**	5	
Identity :	69	**Sample :**	69, 20.9 mg	**Sens.file :**	applied	**Crucible :**	Concavus Pan Al, pierced lid	
Date/time :	18-May-18 12:43:59	**Reference :**	Ref,0 mg	**Sample car./TC :**	DSC 214 Corona sensor / E	**Atmosphere :**	N2, 40.0ml/min / N2, 60.0ml/min	
Laboratory :	FEUP Quimica	**Material :**		**Mode/type of meas. :**	DSC / Sample	**M. range :**	5000 µV	

[#] Type	Range	Acq.Rate	STC	AC	P1:--	P2:N2	PG:N2	Corr.
[3.1-TR] Dynamic	20°C/10.0(K/min)/120°C	300.00	1a	1	0.0	40.0	60.0	DSC:059
[3.2-TR] Isothermal	120°C/00:10/120°C	25.00	1a	1	0.0	40.0	60.0	DSC:009
[3.3-TR] Dynamic	120°C/10.0(K/min)/-40°C	300.00	1a	1	0.0	40.0	60.0	DSC:059
[3.4-TR] Isothermal	-40°C/00:15/-40°C	20.00	1a	1	0.0	40.0	60.0	DSC:009
[3.5-TR] Dynamic	-40°C/10.0(K/min)/120°C	300.00	1a	1	0.0	40.0	60.0	DSC:059

Figure A8.4 DSC of gummy C41

Appendix 9. Technical specifications of an industrial vegan, sugar-free gummy

DoctorGummy asked a fabric to produce gummies with the same ingredients as the ones from this project. The technical specification of a sample is presented in Figure A9.1.

ANDROS CONFISERIE
PROVISIONAL

SPECIFICATION

GEA-072-08

PRODUCT DENOMINATION	fruit
SALES DENOMINATION :	Flavoured confectionery
INGREDIENTS :	Maltitols, modifiad starch, gelling agent: carrageenan, acidity regulator: sodium citrate, acid: citric acid, natural flavouring, colour: E160c, coconut and rapeseed oil, galzing agent: carnauba wax.WHEAT starch.
ALLERGENS TO BE LABELLED	Wheat starch
SPECIFIC MENTION	
STORAGE CONDITIONS	store in a cool (max 27°C) dry place

PHYSICAL CHARACTERISTICS

Shape :	Peach
Average net weight : (indicative value)	6,0 g

ORGANOLEPTIC CHARACTERISTICS

Colours:	Orange
Flavours:	peach

PHYSICO-CHEMICAL CRITERIA

Criterion	Values	Tolerances	Method of analysis
HUMIDITY	≤ 20%	-	KARL FISCHER

NUTRITIONAL VALUES

Average energy values per 100g	**kcal**	213
	kJ	888
Tolerances are not governed by any regulation but we recommend applying those defined by the Guidelines of the European Commission of December 2012 ("tolerances for nutrient values declared on a label")	**Fat**	0,21 ou Traces ou < 0,5
	Of which fatty saturated acids	0,14
	Carbohydrate	82
	Of which sugars	0 donc Traces ou < 0,5
	Of which polyols	77
	Dietary fibre	0,9
	Protein	0,1 ou Traces ou < 0,5
	Salt	0,7

This is a computer printout and has therefore not be signed by hand

This product and its components are permitted in food according to European Regulation. It is the user responsibility to ensure that the usage and labelling of the product are in compliance with the relevant legislation governing the application for which you intend to use it.

Figure A9.1 Technical Specification of a vegan industrially produced gummy

Appendix 10. Moisture content

The following graphs (Figures A10.1 and A10.2) present the drying process of the selected gummies at two different temperatures (55 and 65ºC) in dry basis and the drying rate of the same formulas, respectively.

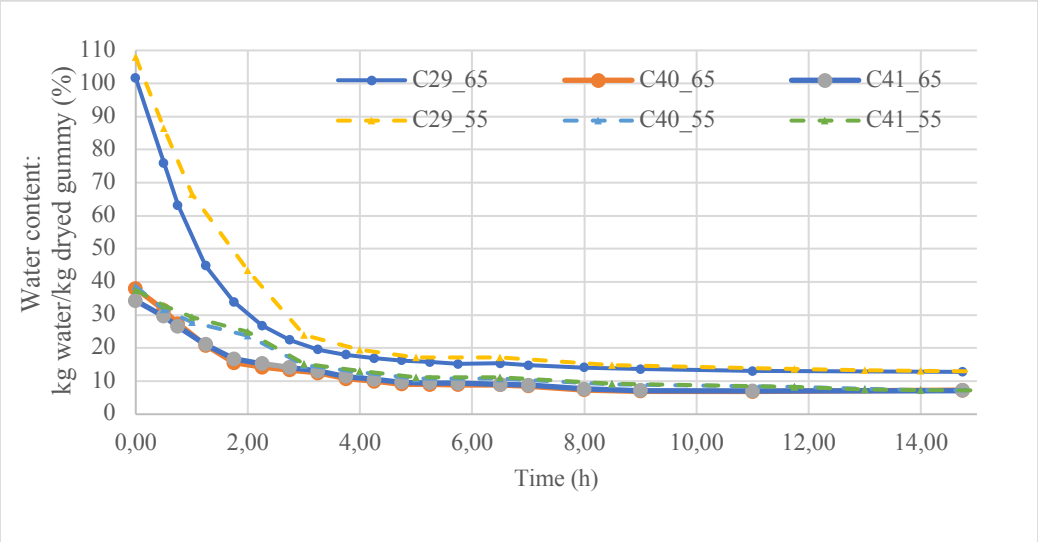


Figure A10.1 Water content evolution (dry basis) for 65 and 55 ºC

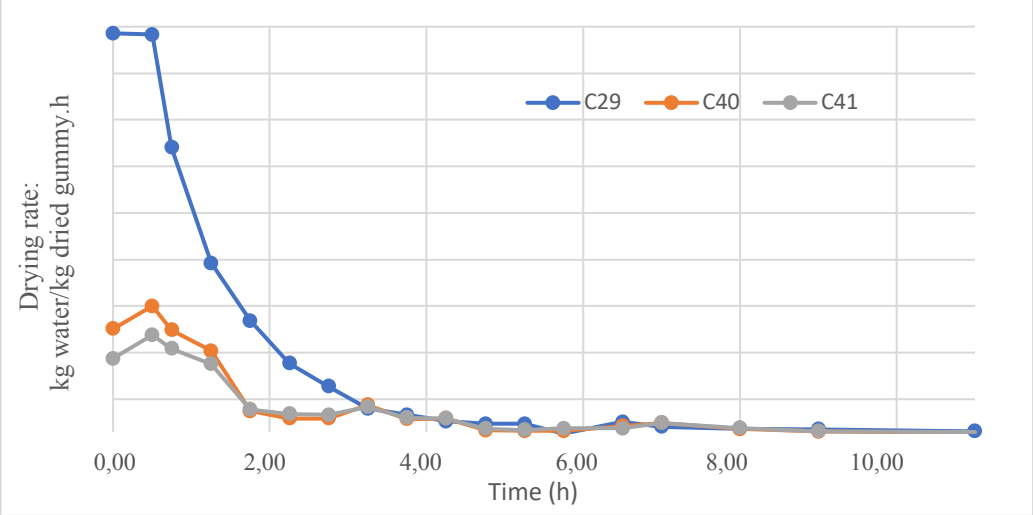


Figure A10.2 Drying rate of the selected formulations

Appendix 11. Quantification of paracetamol

The quantification of paracetamol was made through an UV-VIS measurement at 247 nm. Table A11.1 presents the results obtained. X1 and X2 are diferente gummies from the same formulation, as well as Y1 and Y2; X and Y represente diferente formulations of the same formula (C40P).

Table A11.1 Results from the quantification of paracetamol

	X1	X2	Y1	Y2
Absorbance at 247 nm	0.971±0.005	1.115±0.003	0.940±0.009	1.09±0.02
Concentration (mg/L)	16.35±0.09	18.93±0.05	16.3±0.2	18.41±0.37
Paracetamol/ gram of gummy	**16.6±0.8 mg/g**			