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EVALUATION OF THE PRINTABILITY AND FUNCTIONAL PROPERTIES OF *PROSOPIS LAEVIGATA* SEEDS

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Evaluation of the Printability and Functional Properties of Prosopis laevigata Seeds

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INTERNATIONAL IBERIAN NANOTECHNOLOGY LABORATORY

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Abstract

Mesquite, as an underutilized legume in Europe, merits a better understanding of its nutritional properties. Additionally, within all the emergent technologies in the food sector, 3D food printing is a rising tendency and its inclusion in this project aimed to evaluate the printability of mesquite flour edible inks.

A partial nutritional characterization was performed, including the content of soluble protein, antioxidant capacity and ash content. Moisture content was also assessed. The preliminary printability tests served as a base for the 3D printing of cookies with raw and extruded mesquite flour inks. Shape fidelity, colour and texture of the cookies were evaluated.

Finally, this work concluded that mesquite flour, either raw or extruded, presents favourable characteristics in terms of nutrition, as well as good printability faculties that might be valuable in the future for creation of foods containing this lesser-known ingredient.

Keywords (theme): 3D printing, nutrition, mesquite

Resumo

O mesquite, como uma leguminosa subutilizada na Europa, merece uma melhor compreensão das suas propriedades nutricionais. Adicionalmente, entre todas as tecnologias emergentes no setor alimentar, a impressão 3D de alimentos é uma tendência crescente e a sua inclusão neste projeto teve como objetivo avaliar a capacidade de impressão de tintas comestíveis de farinha de mesquite.

Foi realizada uma caracterização nutricional parcial, incluindo o teor de proteína solúvel, capacidade antioxidante e teor de cinzas. O teor de humidade também foi avaliado. Os testes preliminares de impressão serviram de base para a impressão 3D de bolachas com tintas de farinha de mesquite crua e extrudida. Foram avaliadas a fidelidade da forma, cor e textura das bolachas.

Finalmente, este trabalho concluiu que a farinha de mesquite, crua ou extrudida, apresenta características favoráveis em termos de nutrição, bem como boas propriedades de impressão que podem ser valiosas no futuro para a criação de alimentos que contenham este ingrediente menos conhecido.

Declaration

I hereby declare, under word of honour, that this work is original and that all non-original contributions are indicated and due reference is given to the author and source.

Assinado por: **Mónica Isabel Amorim Marques**
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Notation and Glossary

List of Acronyms

AAE	Ascorbic Acid Equivalent
AOAC	Association of Official Analytical Chemists
BCA	Bicinchoninic Acid Assay
BJ	Binder Jetting
CAD	Computer-Aided Design
CIELAB	Commission Internationale de l´Eclairage L*a*b*
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
DPPH	2,2-diphenyl-1-picrylhydrazyl
FDM	Fused Deposition Modelling
GAE	Gallic Acid Equivalent
INL	International Iberian Nanotechnology Laboratory
LOM	Laminated Object Manufacturing
RGB	Red Green Blue
SLA	Stereolithography
SLS	Selective Laser Sintering
TEAC	Trolox Equivalent Antioxidant Capacity
TPA	Texture Profile Analysis

1 Introduction

1.1 Framing and presentation of the work

The food industry, in recent years, has faced a revolution in what concerns methods, objectives and obstacles. For example, meat products are becoming less and less consumed due to environmental awareness and moral concerns that alter consumer choices, which gives place to alternative “meats”. The personalisation of foods creates a new way to consume what is needed and/or desired, and it comes in various forms. Some of the characteristics that might be personalised are the texture, nutritional values, colour, flavour and organoleptic properties of foods such as cakes or cookies (Sun et al., 2015). Texture may be customised by altering the proportions of ingredients or the ingredients themselves and/or by changing the structure of the food, namely with the resource to 3D printing, for example (Dankar et al., 2018). The progression of that technology prompted some adaptations to several materials, some of them being food products.

The work developed at INL within the scope of food personalisation is intended to innovate and create solutions for various challenges. The challenge assessed in the present work is the creation of a 3D-printed nutritional snack that aims to resolve some consumption difficulties experienced by a great share of the population, such as celiac disease. This snack also may be personalized to respond to other necessities in the scope of alimentary difficulties and needs namely diabetes, obesity, and allergies. The laboratorial work assessed the nutritional values of an underutilised legume flour, mesquite (P*rosopis laevigata*), and aimed to create a sweet snack that is nutritionally balanced and physically compatible with consumption by all individuals. To achieve this objective, printability studies were carried out, as well as different food ink formulations, cooking, and respective textural analyses.

1.2 Presentation of the company

The International Iberian Nanotechnology Laboratory (INL) is a research organisation that was established in 2005 by the governments of Portugal and Spain. It is located in Braga, Portugal, and operates as an autonomous international research organisation, governed by a joint board of representatives from Portugal and Spain.

INL's mission is to promote scientific research and technological development in the field of nanoscience and nanotechnology, with a focus on addressing some of the most pressing global challenges in areas such as health, energy, and the environment. To achieve this mission, INL conducts cutting-edge research and development in nanotechnology (nanoelectronics, nanobiotechnology, nanomedicine, nanomaterials, and nanophotonics), provides advanced training and education programs, and collaborates with academic institutions, industry partners, and other organisations to advance the understanding and application of nanotechnology.

The Food Processing and Nutrition research group at INL focuses on three main areas of research. Firstly, it aims to develop innovative food packaging solutions that are smart, active, and even edible, in order to extend the shelf life of perishable foods and reduce food losses. Secondly, the group investigates micro and nanoencapsulation of bioactive food ingredients to improve their bioavailability and enhance their bioactivity, by increasing their stability and targeted delivery. Lastly, the group also works on designing and modifying the micro and nanostructure of food polymers in order to create new and improved rheological properties in food.

1.3 Contribution of the author to the work

The work presented in this dissertation was initiated by the author within the framework of a new project at INL. Although this was very challenging, it delayed the initiation of the laboratory work, since the materials and methods were not completely defined within the research group. All the laboratory work was performed by the author, namely the nutritional properties assays, the preparation and analysis of food inks, its printing and baking, colour analysis and texture analysis, with resource to the knowledge and experience of supervisors and colleagues to assist in some activities.

1.4 Organisation of the dissertation

The present work is organized in five sections, starting with a brief context in Chapter 2 regarding food personalisation techniques, alimentary necessities, food 3D printing and mesquite properties and history. The succeeding chapter presents the methods performed to achieve the results of each assay or procedure, and afterwards, in Chapter 4, the corresponding results are presented and discussed. Finally, in Chapter 5, the conclusion summarises the results and in Chapter 6 a final work assessment is completed.

2 Context and state of the art

2.1 Food personalisation

The market of food personalisation has been a rising tendency in the last decades for numerous motives. Medical diagnoses allow every individual to assess their personal allergenic data, as well as possible nutritional deficiencies and particular attributes that condition one's alimentary choices. Preferences and ease of access to personalised food services have also entered into the equation with the creation of customisable meals in fast food restaurants and delivery services (e.g. gluten-free meals and exclusion of allergens). With these recent innovations and information, individuals begin to progressively choose a more tailored alimentary plan to favour all the necessities and preferences.

At the industrial level, there are some technologies that allow the complete or partial personalisation of foods, e.g., vacuum impregnation, encapsulation and 3D printing (Derossi et al., 2019; McClements, 2020).

Vacuum impregnation is a food processing method in which food is placed in a vacuum-sealed chamber and subjected to a high-pressure differential. This procedure allows liquid or gas to enter the food item swiftly and evenly, thereby improving the taste, texture, and nutritional content of the product (Radziejewska-Kubzdela et al., 2014). This method is commonly used to infuse foods with marinades, brines, and other flavourings, and it can also be employed to introduce nutrients into food products.

Encapsulation is the technique of encapsulating microscopic particles within a protective exterior, such as taste compounds or nutrients. This technology increases the stability of food items and permits the controlled release of active substances (Đorđević et al., 2014). It is commonly used in functional foods, such as sports drinks and nutritional supplements.

3D food printing is a technology that allows the creation of three-dimensional food products using a customised printer. This process involves creating a digital model of the desired food product and afterwards using a printer to layer edible materials, such as dough or pureed foods, to create the final product (Sun et al., 2015).

All of the previous technologies allow the personalisation of foods at various levels, such as shape in the case of 3D printing, flavour in the case of vacuum impregnation and medical properties within the encapsulation method. Nevertheless, there are numerous additional factors that may be considered when creating a food product, which are schematically presented in Figure 1, divided into three main categories: organoleptic properties, sustainability and health conditions.

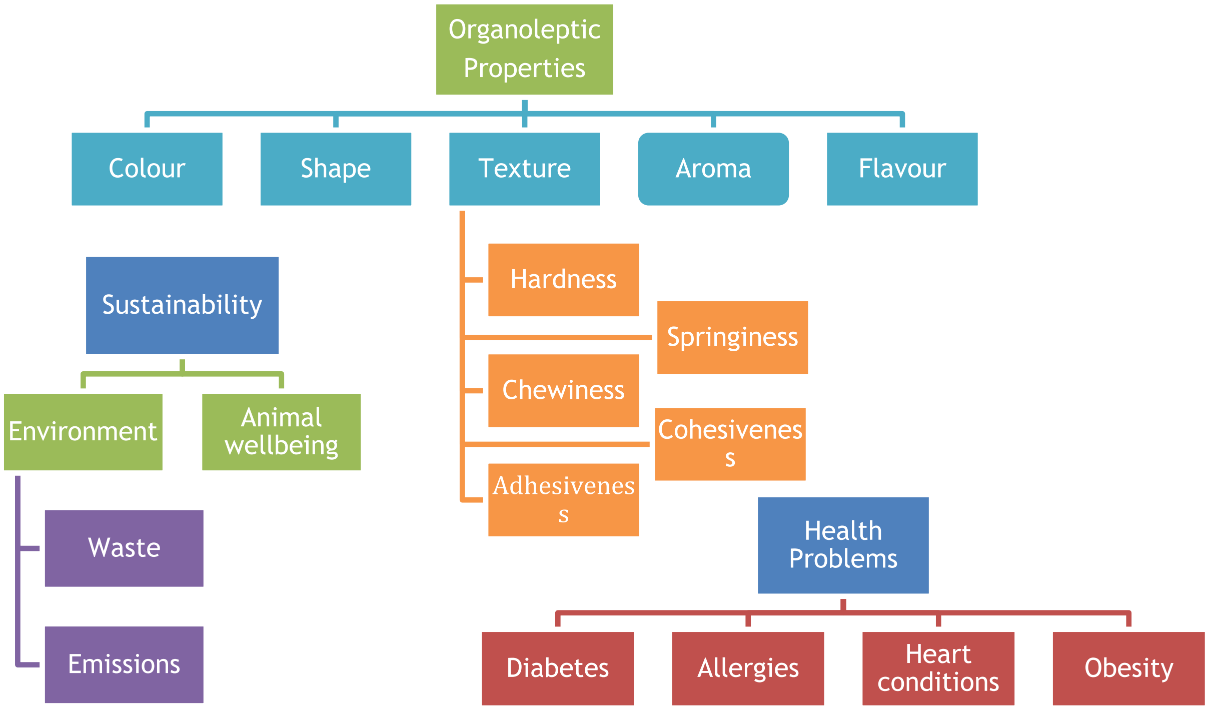

Figure 1 - Food personalisation variables.

2.2 Nutritional/Alimentary necessities

Some individuals, within the scope of health problems presented in Figure 1, suffer from one or various conditions which may alter their eating patterns.

Food allergies, in contrast to the common misconception of the general public, are not food intolerances. An allergy is an excessive reaction of the immune system to a compound, which is caused by an exaggerated cascade of molecular mechanisms in reaction to the said compound. Food intolerances do not affect the immune system and are usually caused by the deficiency or absence of an enzyme (Turnbull et al., 2015). The most common allergens in adults are shellfish, nuts and fish, while the most prevalent intolerances are to milk and milk products.

Despite the vast incidence of food allergies and intolerances, other alimentary necessities still take place in a broad share of the population. Diabetes, for example, affects roughly 500 million people around the world (Cho et al., 2018). This disease demands a strict diet in terms of sugars, which can be hampered by the availability of suitable products in developing countries. Celiac disease, on the other hand, is not a food allergy or intolerance. It has been studied for several years to conclude that it is indeed an autoimmune response to the ingestion of gluten (Caio et al., 2019).

These restrictions require strict care with alimentation routines and habits, for what new products and solutions are being constantly developed and becoming progressively more accessible. One of the recently proposed resolutions for celiac disease is based on food gene editing. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is an innovative technology that allows for the editing of DNA in food and humans, offering the potential to develop new food products where allergenic DNA has been modified, thus avoiding harmful allergic reactions. This advancement can broaden the food options available to consumers with allergies and autoimmune diseases. However, the technology still carries a high cost, and while some allergens can be eliminated, certain complex crops, such as wheat, pose challenges in completely removing allergenic genes through genetic manipulation (D'Auria et al., 2019). Additionally, the long-term effects of consuming gene-edited foods are not yet fully understood, highlighting the need for further research in this field.

While medical therapies are being developed, most of the celiac disease carriers choose to avoid gluten in order to eliminate the possibility of an immunologic response (De Martinis et al., 2020), while also striving to discover food products with similar organoleptic properties. Mesquite seed flour presents pleasant characteristics in terms of nutrition for those with alimentary constraints, since it is a gluten-free alternative to wheat flour, as well as presenting low levels of sugar and a high percentage of protein.

2.3 Mesquite history

Mesquite, also known as *algarrobo* or *huisache*, is a common tree found throughout Mexico, and also in the United States and African continent, particularly in the arid and semi-arid regions (Jacoby & Ansley, 2021). This versatile plant has a long history of use in Mexican culture and has played an important role in the economy and daily life of many communities for centuries.

Mesquite has long been a valuable resource for fuel and construction materials in Mexico. It is a popular fuel source, particularly in rural areas where other types of fuel may be difficult to obtain (Pérez-Serrano et al., 2021). The wood is dense and hard, which allows it to burn slowly and produce a great amount of heat. It emanates a pleasant aroma when burned, which can be an attractive alternative to the smell of other types of fuels, such as propane or kerosene. Additionally, its physical properties also create strong and durable structures, being ideal for building houses, barns, and fences (Beresford-Jones & Whaley, 2022). Furthermore, mesquite wood has a unique and attractive grain pattern that gives it a distinctive appearance highly valued in decorative settings.

Mesquite has also played an important role in Mexican folklore and tradition. The tree is often featured in local myths and legends, and its wood is used to make intricate carvings and sculptures.

Nevertheless, one of the most well-known uses of mesquite in Mexico is as a food source. The tree produces a sweet and flavourful pod (composed of endocarp, mesocarp, and seed – see Figure 2), which can be ground into flour and used in a variety of meals. Mesquite flour has a rich, nutty flavour and is often used in baked goods from Latin-America, such as bread, cakes, and cookies (González-Montemayor et al., 2019). It is also used as a thickener in soups and stews (Nwokocha & Williams, 2016) and as a seasoning for meats and vegetables, and even its wood is used in cooking methods such as barbecuing and smoking. In fact, mesquite wood is considered by many to be the best wood for smoking meat, as it imparts a distinct and delicious flavour (Del Toro-Gipson et al., 2020; Jaffe et al., 2017).

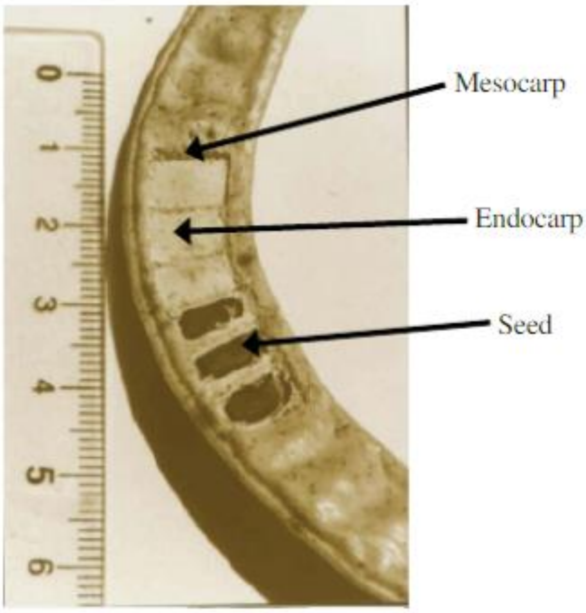

Figure 2 - Mesquite pod constituents. Reproduced with permission. (Felker et al., 2013) *Copyright 2013, Taylor & Francis.*

In addition to its culinary uses, mesquite has also been used in traditional medicine for centuries. The plant has a long list of medicinal properties, including its cardioprotective and anti-hypertensive, anti-inflammatory, antioxidant, antibacterial, and antidiabetic activities (relevant studies presented in Table 1). There are even some studies related to the usage of mesquite in Alzheimer's disease therapy (Henciya et al., 2017). Mesquite pods are also a good source of vitamins and minerals, including calcium, magnesium, and potassium (Maciej Serda et al., 2021).

Table 1 - Mesquite medicinal properties studies.

Health benefit	Species	Tree Section	Reference
Cardioprotection	*Prosopis laevigata*	Leaves	(García-Andrade et al., 2013)
	Prosopis laevigata	Seeds	(Díaz-Batalla et al., 2022)
Antioxidant	*Prosopis nigra, Prosopis alba*	Pods	(Pérez et al., 2014)
	Prosopis africana	Leaves	(Yanda et al., 2022)
Antidiabetic	*Prosopis cineraria*	Bark	(Soni et al., 2018)
	Prosopis juliflora	Leaves	(Jangid et al., 2022)
	Prosopis cineraria	Bark	
	Prosopis farcta	Roots	(Feyzmand et al., 2017)
Antibacterial	*Prosopis juliflora*	Leaves	(Thakur et al., 2014)
	Prosopis juliflora	Pods	(dos Santos et al., 2013)
	Prosopis juliflora	Pods	(Tajbakhsh et al., 2015)
Antihyperlipidemic	*Prosopis cineraria*	Leaves	(Sharma et al., 2013)
	Prosopis farcta	Roots	(Saidi et al., 2016)

2.3.1 Mesquite nutritional characterisation

Mesquite trees belong to the *Prosopis* genus, which englobes more than forty species. The majority of the species are native to the Americas, however, as its characteristics are appealing, it has been introduced to the African continent in the beginning of the 20th century (Felker, 1979). The most employed and know species of Mesquite is *Prosopis juliflora*, but several others are used and studied as well: *Prosopis glandulosa*, *Prosopis alba*, *Prosopis pubescens*, *Prosopis laevigata*, *Prosopis africana,* among others.

There are various studies assessing the nutritional values of the *Prosopis genus*, some of them presented in Table 2.

Table 2 - Prosopis genus nutritional values (presented in %).

Species	Ash	Moisture	Protein	Fat	Carbohydrates	Fibre	Reference
Prosopis Juliflora (seed)	3.09 ± 0.12	9.38 ± 0.20	17.55 ± 0.44	1.18 ± 0.02	66.89 ± 0.86	0.91 ± 0.02	(Cavalcante et al., 2022
Prosopis alba (pod)	4.22 ± 0.01	3.46 ± 0.10	62.09 ± 6.21	12.20 ± 0.05	8.97 ± 0.05	9.06 ± 1.00	Cattaneo et al., 2016
Prosopis cineraria (pod)	9.712 ± 1.034	8.55 ± 0.341	28.42 ± 0.56	2.30 ± 0.328	51.01 ± 1.179	5.00 ± 2.541	Malik et al., 2013
Prosopis africana (seed)	6.91 ± 0.53	4.98 ± 0.11	11.77 ± 0.26	9.91 ± 0.26	53.36 ± 0.83	11.35 ± 0.44	(Aremu et al., 2015)
Prosopis alpataco (pod)	3.33 ± 0.05	n.a.	10.2 ± 0.02	3.23 ± 0.05	62.0 ± 3	25.5 ± 2	(Piñuel et al., 2017)
Prosopis laevigata (seed)	4.14 ± 0.03	8.28 ± 0.15	36.51 ± 0.36	4.83 ± 0.04	38.45 ± 0.66	7.73 ± 0.46	(Díaz-Batalla et al., 2018)

The results of the above presented studies show that the nutritional values of mesquite depend on the species and the fraction of the pod. However, on average, mesquite presents a high content of protein and carbohydrates and a low percentage of lipids, which is a fairly good composition for human consumption.

Besides being a great source of protein and carbohydrates, mesquite is also a plausible substitute for wheat flour, since gluten is not part of its composition, and a suitable product for diabetic individuals for its low levels of sugar in seed flour (Díaz-Batalla, Hernández-Uribe, Román-Gutiérrez, et al., 2018).

2.4 3D printing

3D printing, also known as additive manufacturing, is the process of creating three-dimensional objects by layering a material according to a pattern (Matias & Rao, 2015). This process goes back to the 1970s, when Johannes Gottwald patented the Liquid Metal Recorder (F Gottwald, 1971), a continuous inkjet metal material device to form a metal fabrication for immediate use or for printing again by remelting. Since then, many other techniques were developed and perfected, some of them presented in Table 3.

Table 3 - 3D Printing patents.

3D Printing Technology	Inventor(s)	Date of Invention	Reference
Stereolithography (SLA)	Chuck Hull	1984	(Hull, 1984)
Fused Deposition Modelling (FDM)	Scott Crump	1989	(Crump, 1989)
Selective Laser Sintering (SLS)	Carl Deckard	1989	(Deckard & Beaman, 1989)
Laminated Object Manufacturing (LOM)	Michael Feygin	1991	(Feygin & Hsieh, 1991)
Binder Jetting (BJ)	MIT	1993	(Sachs et al., 1993)

The most known and accessible type of 3D printing is the FDM. It has become an extraordinary tool for the industry but also in individual's homes, since it is now reasonably affordable and allows the production of both simple and intricate 3D shapes for various applications. The FDM technology consists in a filament that is extruded by the printer, after being heated to melting point (Figure 3). After deposition in a base or on top of another layer, the thermoplastic filament resolidifies and fuses to the previous layer to create a solid object (Mwema & Akinlabi, 2020).

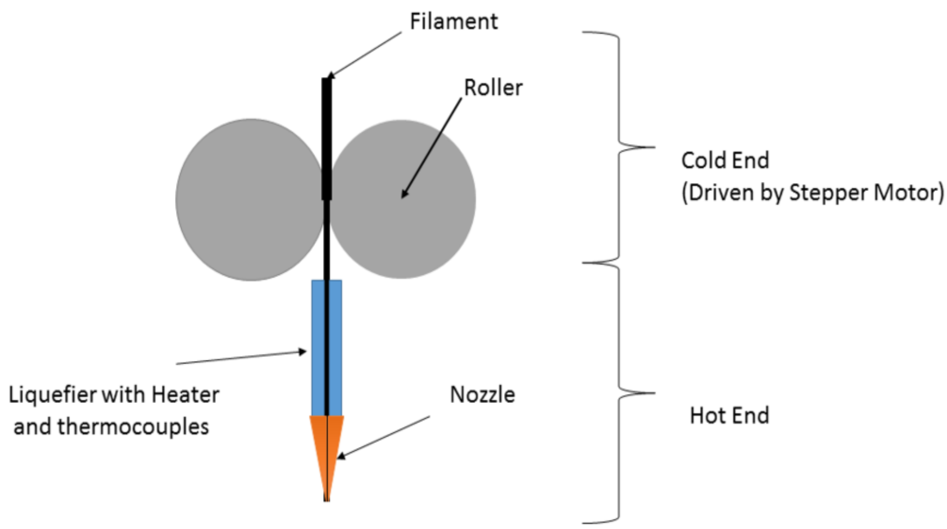

Figure 3 - FDM scheme. Reproduced with permission. (3D Printer Extruder.Png - Wikimedia Commons, n.d.) *Copyright, Wikimedia Commons*

More recently, the same technology has been applied to alimentary products, allowing the customisation of shapes, density, composition, among others.

2.4.1 3D Food Printing

The application of 3D printing technologies in the realm of gastronomy has been a rising tendency since the first invention in this area in 2006. Cornwell University students created an open-source, multi-material 3D printer, in which materials such as chocolate, dough or melted cheese could be loaded and posteriorly printed in a base, similarly to an FDM printer (Diogo et al., 2014). This machine was the first of its kind, since all the additive manufacturing technologies available until then were voluminous, expensive and unavailable to the general public.

A typical extrusion-based 3D food printer works by depositing a filament of dough or paste in a base, resorting to a nozzle of the desired width and a syringe, as seen in Figure 4. These cartesian machines pushes the piston to create a constant rate of extrusion and move it in 3 axes (x, y, z) to create the desired form.

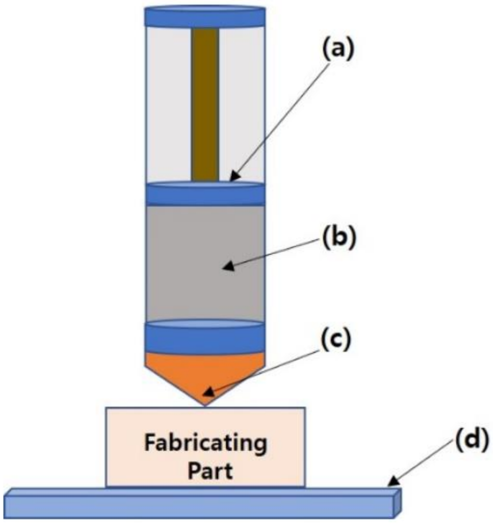

Figure 4 – Scheme of extrusion-based 3D food printing. Reproduced with permission. (Lee & Babi´cbabi´c, 2021) Copyright 2021, MDPI. (a) Piston; (b) Formulated "food ink"; (c) Nozzle; (d) Printer bed

The process of 3D printing a food product involves the development of a three-dimensional model in computer-aided design (CAD) programs such as AutoCAD or Tinkercad. The model is created on the CAD software and exported as an STL file (created for stereolithography), for example. Afterwards, the STL file is uploaded to a slicer, which slices the shape in horizontal segments, defines the printing parameters such as nozzle diameter and printing speed, and saves the commands in G-code for the printer to read and execute (Guo et al., 2019). The post-processing, when necessary, is usually a baking or cooking process, followed or preceded by trimming the unwanted fragments of the printed product. Figure 5 represents the essential steps for a food 3D printing process.

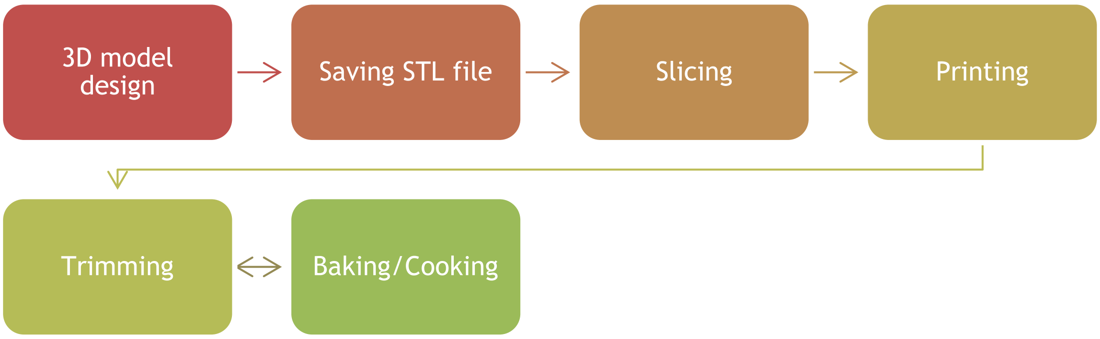

Figure 5 - 3D printing steps.

Turning an ingredient into a printable dough or paste relies on the physical properties of the latter. The viscosity should be ideally low to allow the extrusion, but also high enough to support the structure of the final product. This requirement is defined as shear-thinning behaviour. In some cases, a binding agent, such as xanthan gum, is added to the paste or dough to aid in the obtention of the ideal viscosity (always complying with food safety standards) (Godoi et al., 2016).

Currently, many food creations are being 3D printed in laboratories and even some restaurants. The most researched products are chocolate, doughs, alternative meats, vegetable purées and even soft cheese. Table 4 represents the number of studies concerning the mentioned subjects in Scopus database between 2013 and 2023. The search was performed by writing the term “3D print*” followed by the designation of the desired food product. The words were searched within the title, abstract and keywords, and restriction to document type was set to present only research articles (in this way, review articles, conference papers and book chapters are excluded in order to display only novel scientific advancements).

Table 4 - Number of studies regarding the printability of food products.

Food Product	Nº of Studies
Chocolate	54
Dough	59
Meat	88
Vegetables	59
Cheese	19

It should be highlighted that within the spectrum of doughs, there are no studies published about mesquite-based 3D printed foods.

3 Materials and methods

3.1 Flour characterisation

Two types of mesquite (*Prosopis laevigata*) flour were received from the researcher Luis Díaz-Batalla, Mexico: raw seed flour and extruded seed flour.

3.1.1 Moisture

Moisture assay was performed by weighing 2 grams of flour sample in a crucible, followed by a period of dehydration in an oven at 105 ºC for 24 hours (based on AOAC method (Association Of Official Analytical Chemists, 1998)) 925.10. Total moisture values were calculated following Equation 1.

$$\%\ \text{Moisture} = \frac{\text{Total weight loss}}{\text{Total weight}} \times 100 \quad (1)$$

3.1.2 Ash content

Ash content was calculated by the method AOAC 923.03 (Association of Official Analytical Chemists, 1998), as follows: approximately 2 grams of each sample were put in a kiln at 550 ºC for 2 hours. Samples were taken out of the kiln and cooled to room temperature in a desiccator. Ash content values were calculated according to Equation 2.

$$\%\ \text{Ash} = \frac{\text{Final weight}}{\text{Total weight}} \times 100 \quad (2)$$

3.1.3 Antioxidant activity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

This protocol was provided by the coordinators at INL.

Preparation of the DPPH solution
An 8.87 mM DPPH solution was prepared by dissolving 35 mg of DPPH in 10 ml of methanol.

Preparation of the sample solutions
The sample solutions were prepared by mixing 0.5 g of raw or extruded mesquite flour and 10 ml of methanol/H_2O.

Preparation of the Trolox stock solution and standard curve

A 5 mM Trolox stock solution was prepared by dissolving 12.5 mg of Trolox in 10 ml of methanol/H_2O (50:50, V/V).From the 5mM Trolox solution, ½ serial dilutions were performed with methanol/H_2O (50:50, V/V) to obtain the standard curve concentrations: 2.500, 1.250, 0.625, 0.313, and 0.156 mM.

Determination of Antioxidant Capacity

The antioxidant capacity was determined as follows. A 0.5 ml aliquot of the concentrated DPPH stock solution was diluted in 25 ml methanol/H_2O (50:50, V/V) to achieve an absorbance of DPPH near 1.000 at 520 nm. In a 96-well microplate, 10 µl of sample (or solvent for the blank) were added, followed by 190 µl of diluted DPPH solution to complete a total of 200 µl in each well. The reaction was incubated at room temperature (c.a. 20 °C, protected from light, for 30 minutes. Then, the absorbance of each sample was read at a wavelength of 520 nm. Finally, the TEAC (Trolox Equivalent Antioxidant Capacity) of the samples was calculated following Equations 3 and 4:

$$\text{TEAC}_{\text{sample}}\,(\text{mM}) = \frac{ABS_{sample} - b}{a} \tag{3}$$

$$\text{TEAC}_{\text{sample}}\,(\text{mM} \cdot \text{g}_{\text{sample}}{}^{-1}) = \frac{TEAC}{m_{sample}} \tag{4}$$

where *a* is the slope and *b* is the independent term of the Trolox standard curve ($y = ax+b$); m is the mass of flour sample used in the assay in grams; and ABS is the absorbance read at 520nm.

Determination of total phenolic content by Folin-Ciocalteu assay

This protocol was provided by the coordinators at INL.

Preparation of the Sodium Carbonate (Na2CO3)

A 75 $g \cdot l^{-1}$ solution of sodium carbonate was prepared by dissolving 0.75 grams in 10 ml of distilled water.

Preparation of Gallic Acid stock solution and standard curve

A 250 $mg \cdot l^{-1}$ solution of gallic acid was prepared by adding 13.8 mg of gallic acid monohydrate to 50 ml of methanol/water (50:50, V/V).

The standard curve ranging from (200 $mg \cdot l^{-1}$ – 5 $mg \cdot l^{-1}$) was made by serial dilutions

Determination of the content of total phenols by Folin-Ciocalteau method

In a 96-well microplate, 20 µl of sample (or solvent for the blank) were added, followed by 80 µl of sodium carbonate solution and 100 µl of Folin Ciocalteau reagent to complete a total of 200 µl in each well. The reaction was incubated at 43°C, protected from light, for 30 min in an oven. Then, the absorbance of each sample was read at 750 nm. The total phenolic content of the samples under study was calculated using Equations 5 and 6:

$$\text{Total Phenols } (\text{mg GAE} \cdot \text{l}_{\text{sample}}{}^{-1}) = \frac{ABS_{sample} - b}{a} \quad (5)$$

$$\text{Total Phenols } (\text{mg GAE} \cdot \text{g}_{\text{sample}}{}^{-1}) = \frac{[\text{GAE}] \times 10^{-3}}{[\text{sample}]} \quad (6)$$

where a is the slope and b is the independent term of the standard curve ($y = ax + b$); [sample] is the load of flour in the sample used for the assay in grams per milliliter; [GAE] is the concentration of gallic acid in milligrams per liter and ABS is the absorbance read at 750 nm.

3.1.4 Determination of protein content

The bicinchoninic acid assay (BCA) was performed for protein content according to the Thermo Scientific™ "Pierce™ BCA Protein Assay Kit" protocol. However, the albumin standards were prepared by dissolving 20 mg of bovine serum albumin in 10 ml of water and posteriorly performing serial dilutions according to the protocol.

3.2 Food inks

3.2.1 Preliminary food inks preparation

Food inks were prepared by hand mixing raw or extruded mesquite flour and liquid beverages or oil in a 40/60 solid to liquid ratio, in weight.

The flours were mixed with water, a commercial (Shoyce) or a homemade oat milk, and sunflower oil (Fula).

Additionally, heated food inks were prepared by using the same formulations followed by thermally treating the inks at 86 ºC in a Thermomixer (Eppendorf® ThermoMixer® C) for 30 minutes.

3.2.2 Preliminary printability tests

Preliminary printability tests were performed by extruding the ink with a 1 ml syringe and a 1.60 mm nozzle attached. Filament adequacy was assessed by a series of four tests: freestanding filament, line on surface, square on surface, and stacked dots (Oliveira et al., 2020).

3.2.3 Syneresis assay

Syneresis was assessed by performing centrifugation at 8060 g for ten minutes. The supernatant was weighed, and its volume was determined. Syneresis values were calculated as follows in Equation 6:

$$\% \text{ Syneresis} = \frac{m_{supernatant}\ (\text{g})}{m_{sample}\ (\text{g})} \times 100 \quad (6)$$

3.2.4 Food inks preparation and cooking

<u>**Oat milk**</u>

Oat milk was prepared by blending 100 ml of oat flakes (Salutem) and 300 ml of water. The mixture was then strained with a dishcloth and posteriorly stored in a sealed and refrigerated glass bottle.

<u>**Food inks**</u>

Food inks were prepared with homemade oat milk in three concentrations for each flour (raw and extruded): 40/60, 35/65, and 30/70 ratio of flour to milk. A commercial stevia sweetener (Continente) and salt (NaCl – Sigma Aldrich) were added for flavour purposes in the proportions of 0.5 % and 0.01 % of mesquite flour weight, respectively (based on Martinez-Saez et al., 2017).

<u>**Cooking**</u>

The 3D printed cookies were baked in a ventilated oven at 185 ºC for 8 minutes and cooled for 10 minutes (based on Naseer et al., 2021).

3.3 3D Printing

A cookie model was made using an online CAD software (Tinkercad). The model consists of a cylinder with a diameter of 2.5 cm and a 4.8 mm height (Figure 6).

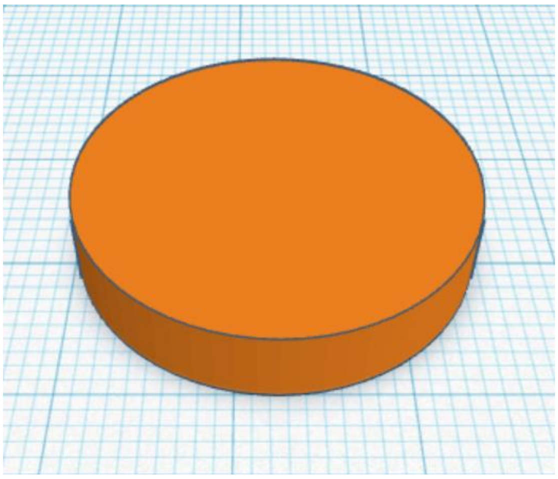

Figure 6 - Cookie CAD model.

The model was exported as an STL file and uploaded to a slicing software (Slic3r), where the print, printer and filament settings were defined. The filament and nozzle diameter were set to 1.6 mm, according to the nozzle diameter chosen for extrusion when performing printability tests. Print settings were defined as follows in Figure 7:

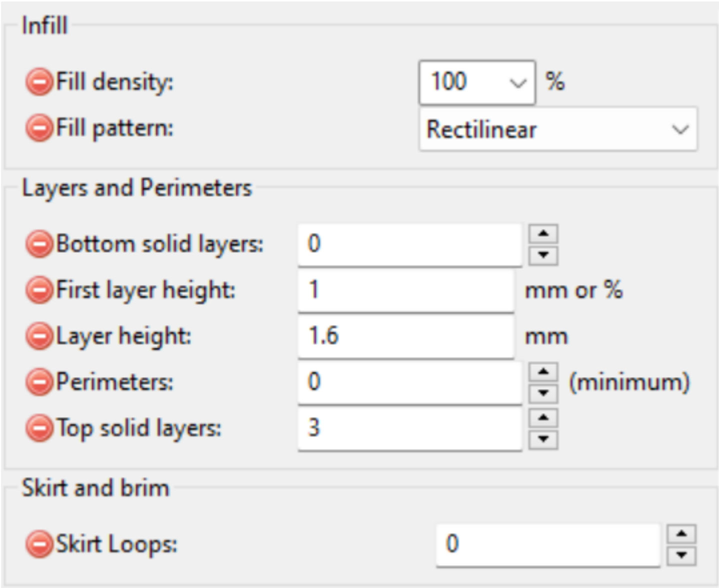

Figure 7 - Print settings.

After defining the previous parameters, the resulting printing preview is generated (Figure 8). Based on the values inserted, the software generated a five-layer circular cookie with an alternating rectilinear filling pattern.

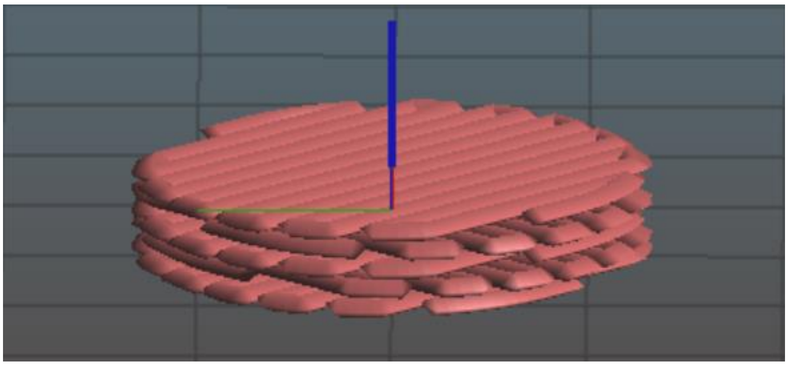

Figure 8 - Printing preview.

After setting all the parameters, the g-code file was generated and uploaded to the printer (byFlow Focus 3D Food Printer). After filling and placing the cartridge, the file was chosen and the printing started. The extrusion rate was defined as 120 % for raw flour inks, while for the extruded flour inks it was 100 %. These values were optimized based on the texture of the inks, to achieve a homogeneous printing pattern.

3.4 Shape fidelity

Shape fidelity values were obtained from the axial dimensions of the cookies. The length, width, and height of the cookies were measured with a caliper before and after baking. Axial loss values regarding STL to printed format and raw to cooked cookies were calculated according to Equation 7.

$$\%\ \text{axial loss} = \frac{\text{lenght}_{\text{initial}} - \text{length}_{\text{final}}}{\text{length}_{\text{initial}}} \times 100 \qquad (7)$$

3.5 Colour analysis

The colour of baked cookies was measured with a colorimeter (Konica Minolta CR-400). Data was analysed by converting average L*a*b* values from CIELAB colour space to RGB values (L* represents lightness from black to white on a scale of zero to 100, while a* and b* represent chromaticity with no specific numeric limits).

3.6 Texture analysis

In a texture analyser (Shimadzu AGX™-V2), a texture profile analysis (TPA) was performed using the baked cookies mentioned in 3.2.4. Cookie samples were measured with a caliper and the values were inserted in the texture analyser software (TRAPEZIUM X-V). Three samples of each ink concentration were analysed with a 500N cell (approximately 50 kg) at 40 % height compression, creating a two-bite simulation. Data was then analysed by the software, where hardness, force, springiness, cohesiveness, chewiness, gumminess, and adhesiveness were calculated according to Figure 9.

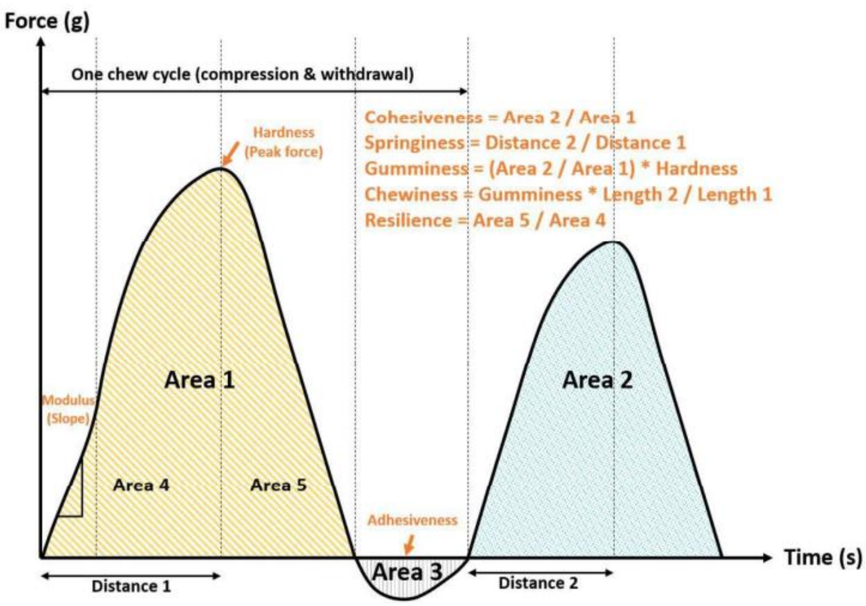

Figure 9 - TPA parameters calculation. Reproduced with permission. (Wee et al., 2018) *Copyright 2018, Royal Society of Chemistry.*

4 Results and discussion

4.1 Flour characterisation

Mesquite flour, as an underutilised ingredient in Europe, might be a valuable source of nutrition, and therefore its characterisation is essential to understand the potential uses in local culinary products. A partial characterisation was performed, mainly to assess its usability as a gluten-free protein source with antioxidant properties.

4.1.1 Antioxidant activity

DPPH assay

Antioxidant activity claims an important role in preventing the damages caused by oxidation in living beings, which might prevent the progression of various health conditions. DPPH is used as a common measure for antioxidant activity since it contains a stable free radical in its composition. In this reaction, the antioxidant (reducing agent) reacts with a central nitrogen free radical to create a secondary amine. The non-reduced DPPH presents a dark purple colour, while the reduced molecules are yellow. For this reason, and since the reaction is singular in each molecule, a proportion can be made by assessing the spectrophotometric properties of the solution.
The Trolox equivalent in millimolar per gram of flour of raw and extruded mesquite flours is presented in Table 5. When comparing both results, it is clear that the non-processed flour presents a slightly higher (7 %) antioxidant capacity. These results are not comparable with the ones presented by (Díaz-Batalla, Hernández-Uribe, Gutiérrez-Dorado, et al., 2018), in which the values are, respectively, 9.11 mg AAE/g and 9.32 mg AAE/g (presented in milligrams of ascorbic acid equivalent per gram of flour). Since the values are not greatly discrepant between the two flours, a slight error in the laboratorial method might originate an inversion of the results when comparing raw and extruded flours.

Table 5 - Trolox equivalent antioxidant capacity of raw and extruded mesquite flours.

Sample	mM Trolox · g sample^{-1}
Raw mesquite	6.236 ± 0.118
Extruded mesquite	5.837 ± 0.107

When comparing mesquite flour and wheat flour Trolox equivalent antioxidant capacities, a significant difference is observed. In (Yu et al., 2013), the Trolox equivalent antioxidant capacity of various types of wheat flour is comprised between 4.25 and 5.00 μM TEAC/g. It is worth to notice that wheat flour presents a value of TEAC within a different order of magnitude than mesquite flour: μM in the case of wheat, mM in the case of mesquite. Thus, mesquite flour, either raw or extruded, presents favourable antioxidant properties, which is a positive outcome for its usage in culinary.

Total phenolic content

The total phenolics content was assessed following the Folin-Ciocalteau method. Similarly to the previously analysed process, it relies on the change of solution colour when reduced (yellow to blue) to present an antioxidant capacity measure. The results obtained for the two samples, in milligrams of gallic acid equivalent (GAE) per gram of flour, are presented in Table 6.

Table 6 - Gallic acid equivalent antioxidant capacity of raw and extruded mesquite flours.

Sample	mg GAE · g sample^{-1}
Raw mesquite	4.916 ± 0.175
Extruded mesquite	5.544 ± 0.614

According to the results obtained, extruded mesquite flour should present a higher antioxidant capacity than the raw flour. However, in the analysis of similar samples reported by (Díaz-Batalla, Hernández-Uribe, Gutiérrez-Dorado, et al., 2018), the values for the respective flours were 6.46 mg GAE/g and 6.68 mg GAE/g, which shows a higher value in the case of raw mesquite flour. The results obtained in this study were significantly lower than the ones presented by Díaz-Batalla, who provided the flours to carry out the present work. Thus, there is a possibility that, during the dispatching process, the antioxidant properties of the flours were disrupted.

Correspondingly to the previous section, the results produced a reversed outcome, since the extruded flour presented the most antioxidant capacity in Díaz-Batalla's work. Comparing to wheat flour values for this assay, mesquite flour presents a higher GAE antioxidant capacity, in accordance with the previous section results. In Heshe et al., 2016, wheat flour presented GAE values between 3.34 mg GAE/g and 3.49 mg GAE/g, which confirms the advantage of mesquite flour in what concerns to antioxidant activity and its potential benefit in culinary uses.

4.1.2 Soluble protein

The soluble protein assay, performed by comparing a standard curve of absorbance in function of concentration of bovine serum albumin, resulted in a percentage of soluble protein between 4.8 % and 6 %, as presented in Table 7. Since these values are not related to the total content of protein in the flour samples, it is hard to infer any conclusions within that scope. Nevertheless, raw mesquite flour presents a higher soluble protein content than the extruded flour, which might suggest that some extent of protein denaturation may occur during the extrusion process.

Table 7 - Soluble protein content of raw and extruded mesquite flours.

Sample	% Soluble protein (g protein · g sample^{-1})
Raw mesquite	5.933 ± 0.602 %
Extruded mesquite	4.820 ± 0.250 %

In (Díaz-Batalla, Hernández-Uribe, Gutiérrez-Dorado, et al., 2018), the total protein content of raw mesquite flour is stated as 36.51 %, which compared to a 14.70 % wheat flour protein content present in (Ocheme et al., 2018), is significantly higher. In fact, that is a 150 % increase, which makes mesquite flour an appealing high-protein ingredient with potential uses as supplementation nutrition.

4.1.3 Ash content

Ash content, as a measure of the inorganic fraction of foods, is a parameter that usually provides useful information about bran contamination in the case of wheat flour (Saeid et al., 2015). Nevertheless, since the composition of mesquite bran is presumably different from that of wheat bran, this value is not an accurate measure of bran contamination in mesquite seed flour. Instead, it may be an assessment of mineral content, which is essential for numerous biochemical processes since those compounds are not synthesised by living beings.

The results regarding the ash content of raw and extruded mesquite flour are presented in Table 8. The ash content of the extruded flour is slightly higher than raw ash percentage, which may be caused by contamination derived from the extrusion process, since it is performed with resource to metallic tools and some erosion particles might become incorporated in the flour.

Table 8 - Ash content of raw and extruded mesquite flours.

Sample	% Ash (g ash · g sample^{-1})
Raw mesquite	3.700 ± 0.0158 %
Extruded mesquite	4.022 ± 0.1110 %

Furthermore, when comparing the hereby presented ash content of mesquite and (Ocheme et al., 2018) wheat flour ash content value - 0.70 % -, it is clear that mesquite presents a much higher mineral content. A high mineral content might be beneficial for consumers since the human body does not synthetise inorganic compounds.

4.1.4 Moisture

The water content of food samples is related to their shelf life, since invasive organisms are dependent on water to thrive. In this way, and analysing Table 9, there is a 1 % increase in moisture when comparing raw and extruded mesquite flours, which indicates a slightly greater shelf life for extruded flour. Nonetheless, both flours are in a better storage preview than wheat flour, which presents 9.1 % of water, according to (Ocheme et al., 2018).

Table 9 - Moisture content of raw and extruded mesquite flours.

Sample	% Moisture (g water · g sample^{-1})
Raw mesquite	6.2402 ± 0.0765 %
Extruded mesquite	5.2475 ± 0.0721 %

4.2 Food inks

4.2.1 Preliminary formulation of food inks

Preliminary formulations of food inks were prepared as mentioned in the previous section and their characteristics were firstly assessed visually. The results of the non-heated raw flour inks varied significantly, depending on the liquid base: sunflower oil presented a runny suspension of flour particles, while the other two water-based liquids created a non-runny paste. The oil suspension created two phases, a stiffer phase where the majority of flour was suspended, and a more liquid phase with less flour quantity. Commercial oat milk produced a stiffer ink, compared to the ink sample using water.

When heated to 86 ºC, the oil suspension remained constant, as no further dissolution happened. The water-based samples became slightly hardened when compared to the previous non-heated equivalents. Nevertheless, the water-based ink presented a runnier consistency than oat milk ink. Figure 10 presents a photograph of the ink samples abovementioned.

Figure 10 – Preliminary ink samples produced with raw mesquite flour.
(From left to right: sunflower oil, sunflower oil (heated), water, water (heated), oat milk, oat milk (heated))

Identical tests were performed using the extruded mesquite flour, excluding the sample containing the sunflower oil, because of the non-encouraging results obtained previously with the raw mesquite flour. When extruded flour was combined with water-based beverages, it created a stiffer paste than the one obtained with the raw flour, presenting little to no syneresis. Heated samples created an even stiffer and darker ink that also presented no signs of syneresis. Ink samples produced with extruded flour and water or homemade oat milk are presented in Figure 11.

It is important to mention that in the first assessment with raw mesquite flour (Figure 10), a commercial oat milk was utilised. Since its behaviour could be dependent on the additives present in the oat milk composition, two new samples were prepared with homemade oat milk (two samples on the right in Figure 11). No significant difference was observed.

Figure 11 – Preliminary ink samples produced with extruded and raw mesquite flour.
(From left to right: water, water (heated), oat milk, oat milk (heated), raw flour + oat milk, raw flour + oat milk (heated))

4.2.2 Preliminary printability tests

A printability assessment was performed with all the previously prepared ink samples, which presented expected results after the visual evaluation of the samples.

Raw mesquite flour

Regarding the ink made with raw flour and water, some level of syneresis is visible (shining appearance), and there is little to no distinction in the stacked dots (Figure 12, left). This ink produces a good filament, which holds decently in a freestanding position and presents a good separation of layers in a hand-drawn square shape. When heated to 86 ºC for 30 minutes, this ink improves its printability characteristics, as seen in Figure 12 on the right. The filament is less silky and presents a better separation when stacked. This behaviour is presumably explained by the higher absorption of water by the flour, caused by the temperature rise. This temperature works as an enhancer in this case, since it is not near the water boiling point nor protein denaturation threshold.

Figure 12 - Printability tests with raw flour. Water sample (left); water sample heated to 86 ºC (right).

The ink prepared with homemade oat milk presented similar characteristics to the water-based ink, considering that cereal milks are comprised predominantly by water (75 % (w/w), in this case). Correspondingly to the previous results, when heated to 86 ºC, the ink hardens slightly and reduces the visible syneresis. Nevertheless, comparing the heated water and heated oat milk samples, a better result is seen in the latter. From Figure 13, it is clear that the heated filament is stackable in dots and presents a very good visible separation.

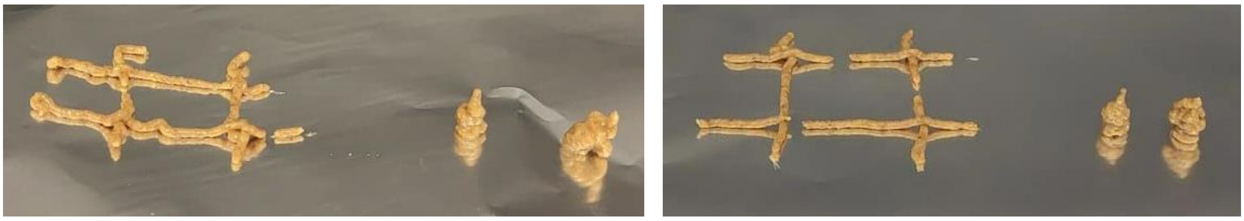

Figure 13 - Printability tests with raw flour. Oat milk sample (left); oat milk sample heated to 86 ºC (right).

The lastly considered liquid for ink preparation - sunflower oil - produced a very fluid, non-homogeneous suspension. Heating caused little to no alterations, as depicted in Figure 14.

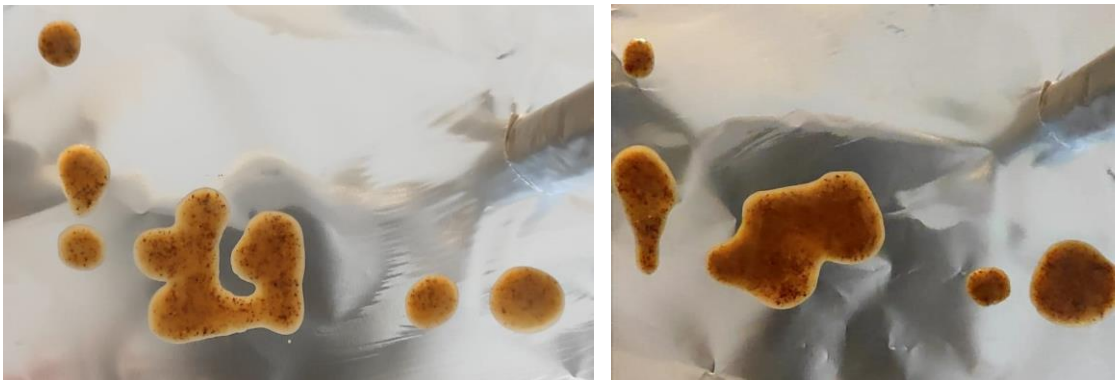

Figure 14 - Printability tests with raw flour. Oil sample (left); Oil sample heated to 86 ºC (right).

Extruded mesquite flour

Extruded mesquite flour inks made with water-based liquids present favourable characteristics for printing, since even without heat, the water absorption rate is enough to show no significant signs of syneresis. In Figure 15, the difference between heated and non-heated is shown, regarding to water-based ink. A good separation from dot to dot is visible, and there is a good layer distinction in the square in both cases.

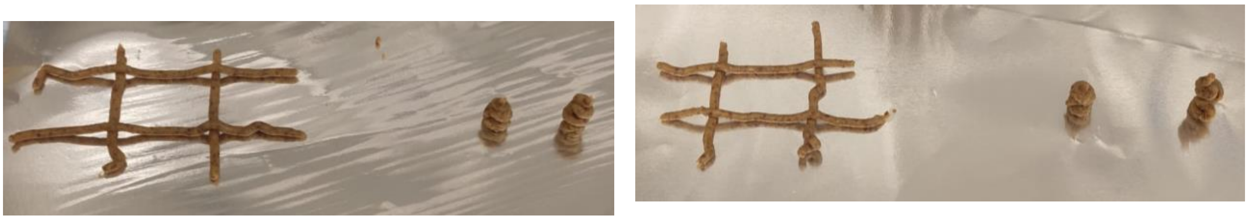

Figure 15 - Printability tests with extruded flour. Water sample (left); Water sample heated to 86 ºC (right).

When made with oat milk, the inks generate similar results as the previously mentioned water inks. Heating does cause a slight hardening of the paste, although it isn't of great magnitude. Figure 16 depicts the oat-milk-based inks.

Figure 16 - Printability tests with extruded flour. Oat milk sample (left); Oat milk sample heated to 86 ºC (right).

Tests with sunflower oil were not performed with extruded flour, since the results regarding the raw flour oil-based ink were not positive. When compared, raw mesquite flour presents a lower capability of absorption of water-based liquids than extruded flour, for what the latter is preferable for 3D printing purposes.

4.2.3 Syneresis assay

Figure 17 represents the percentage values of non-absorbed liquid (in grams of liquid per gram of ink sample). As expected, extruded flour inks did not exhibit any extent of syneresis, nor the heated samples, nor the non-heated ones. Regarding raw flour samples, when not heated, water and oat milk weight per sample are very similar. Nevertheless, after heating, most of the oat milk is absorbed, while a half of the water remains unabsorbed. According to the results, heating does effectively enhance absorption, despite its percentage depending on the liquid used. Oat milk, when compared to water, is more capable of being reabsorbed after heating possibly due to alterations of some oat constituents, such as protein degradation, which may consequently allow a better integration in the ink matrix.

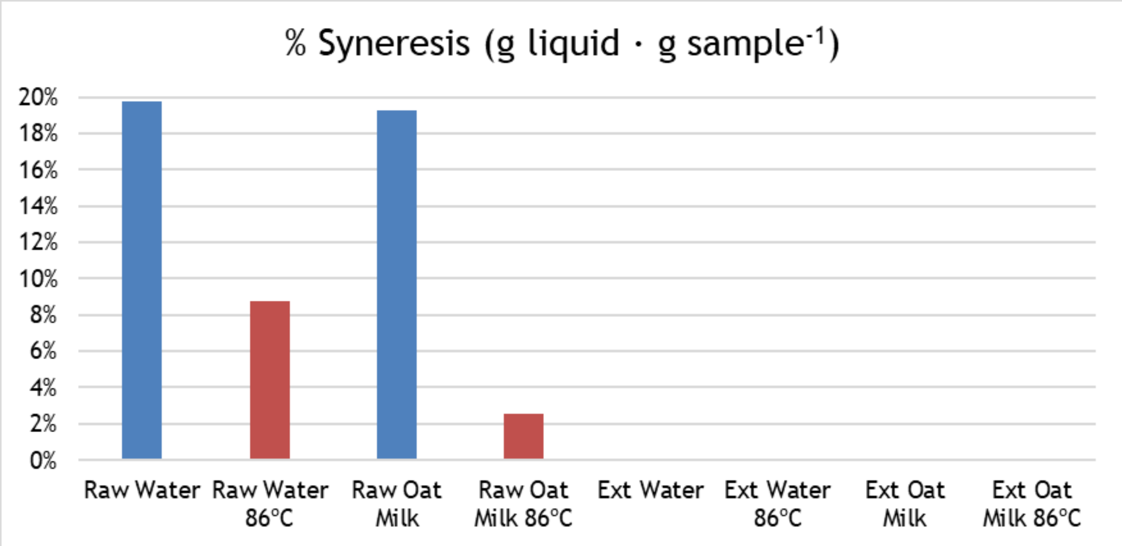

Figure 17 - Syneresis percentage of water and oat milk ink samples.

4.3 3D Printing

4.3.1 Raw mesquite cookies

The 3D printing process, based on the vertical disposition of various layers of matter, is only possible when certain conditions are attained. The filament should not be breakable, non-adherent or too elastic. However, in some of the following cases, those aspects were not observed which compromised the quality of the obtained cookie. This assay was performed to evaluate the flour concentration effect on the ink printability.

When printing the cookies with raw mesquite flour formulations, the conditions of the two most concentrated doughs did not allow the execution of the printing process to go as planned.

The most concentrated dough presented a similar behaviour to a common dough with wheat flour, which presents typical characteristics created by gluten. The filament was elastic and presented visible signs of syneresis, which didn't allow a proper fixation on the printer bed to build the first layer. The following layers, despite being attached to the previous, did not sustain the desired shape since the elastic filament was drawn back after each line change (Figure 18).

The dough formulation with a 35/65 flour to milk ratio presented a similar printing result as the previoulsy analysed dough (Figure 19). However, since milk quantity was superiour, the elastic properties weren't as intense as in the case of the most concentrated dough. Additionally, there was a higher rate of syneresis for the previous reason.

The least concentrated dough, with a flour/milk ratio of 30/70, produced a cookie with the desired shape, opposing to the previous cases. Nevertheless, the filament was very fragile and had a tendency to break (Figure 20). It didn't present elastic properties, for what the filament dragging behaviour wasn't present in this case. The levels of syneresis were very high, as expected in a high liquid concentration dough.

These results were partially expected, since the syneresis assay (4.2.3.) predicted the non-adherence to the base, but the elastic filament had no predecents to explain such behaviour. A possible explanation might rely on the addition of stevia and salt, which may have caused a structural alteration.

Figure 18 - Printed raw mesquite cookie (40/60).

Figure 19 - Printed raw mesquite cookie (35/65).

Figure 20 - Printed raw mesquite cookie (30/70).

4.3.2 Extruded mesquite cookies

The cookies made with extruded mesquite flour presented a vastly distinct outcome than the abovementioned raw cookies. The doughs presented no signs of syneresis, as predicted by the assay in 4.2.3.. The difference in the flour concentrations did not affect the printing ability. The three doughs were able to create very accurate depictions of the CAD model, and all present a great distinction of filament lines and layers (Figures 21, 22, 23).

Figure 21 - Printed extruded mesquite cookie (40/60).

Figure 22 - Printed extruded mesquite cookie (35/65).

Figure 23 - Printed extruded mesquite cookie (30/70).

4.3.3 Shape fidelity

When printing objects or foods, in this case, several factors can impact the outcome of the printed item. The extrusion rate had to be adjusted in order to allow an adequate performance of the printer, by creating a steady filament with no breakage or lack of material.

Raw cookies, throughout the printing process, had the extrusion rate set for 120 % to create the beforementioned conditions. Therefore, for augmenting the extrusion rate, the final dimensions of the cookie should be larger than those set for the CAD model. This is verified by the values presented in Table 10, where it is clear that all the losses are negative, implying that an increase of size did indeed occur. In fact, since the two most concentrated doughs created an uneven shape (Figures 18 and 19), the height measurement in those conditions was absurdly greater than the value specified in the STL file, getting to a 125 % variance in the 35/65 concentration cookies. Since the values were discrepant between concentrations and didn't present an evident tendency, it is not possible to conclude the effect of concentration in shape fidelity.

Table 10 – Average raw mesquite cookies axial variation values (STL to printed).

Sample	X loss	Y loss	Z loss
Raw 40/60	-9.85 %	-27.39 %	-89.79 %
Raw 35/65	-9.49 %	-16.67 %	-125.07 %
Raw 30/70	-6.88 %	-11.97 %	-57.92 %

Regarding the extruded flour cookies, the dimensions were considerably more accurate than the raw cookies, presenting lower error values in all the axes. The extrusion rate was set as 100 %, which partially justifies the improved values. Additionally, the extruded flour dough, as mentioned in section 4.3.2., created a consistent filament, which allowed a precise execution of the printing. Despite the nozzle diameter and filament diameter being set as 1.6mm in the software, the height of the cookies was still larger than the set value, which might indicate that some deviation occurred in the printing process. Table 11 presents the percentual values of axial variation of the extruded flour cookies, in which it is possible to verify that all the concentrations produced a similar extent of deviation when printing, for what it is possible to infer that the concentration does not affect shape fidelity in this case.

Table 11 – Average extruded mesquite cookies axial variation values (STL to printed).

Sample	X loss	Y loss	Z loss
Extruded 40/60	-5.17 %	-8.17 %	-31.53 %
Extruded 35/65	-6.09 %	-9.45 %	-31.68 %
Extruded 30/70	-5.71 %	-9.84 %	-33.96 %

4.4 Cooking

4.4.1 Raw mesquite cookies

Raw cookies, after baking at 185 ºC for 8 minutes, essentially maintained their shape, except for the least concentrated one. In the latter, the filament distinction vanished and the excess oat milk created a dry halo around the cookie. Figures 24 to 26 illustrate the previous statements.

Figure 24 – Cooked raw mesquite cookie (40/60).

Figure 25 - Cooked raw mesquite cookie (35/65).

Figure 26 - Cooked raw mesquite cookie (30/70).

4.4.2 Extruded mesquite cookies

Extruded mesquite cookies, when baked at 180ºC, presented a different behaviour when comparing to the raw mesquite cookies. All three concentrations created a resulting cookie that rose in height, in a way that is similar to wheat bread (Figures 27, 28, 29). Nevertheless, these cookies do not contain any rising ingredients, since no yeast or baking powder was added, which makes this behaviour a non-predicted result. A potential explanation could rely on the liquid absorption capacity alteration of the extruded flour, which allows the creation of internal bubbles.

Figure 27 - Cooked extruded mesquite cookie (40/60).

Figure 28 - Cooked extruded mesquite cookie (35/65).

Figure 29 - Cooked extruded mesquite cookie (30/70).

4.4.3 Shape fidelity

In the baking process, raw flour cookies had a tendency to nearly maintain their volume, having a variation of under 5 % in all the axes. Table 12 presents the values regarding raw flour cookies axial measurements, in which it is not possible to observe a linear tendency between the different concentrations, excepting the Y axis. This tendency is quite possibly a coincidence, since the cookies were very irregular and a linear behaviour is unlikely.

Table 12 – Average raw mesquite cookies axial variation values (raw to cooked).

Sample	X loss	Y loss	Z loss
Raw 40/60	-1.19 %	4.80 %	0.55 %
Raw 35/65	4.28 %	3.21 %	-1.76 %
Raw 30/70	0.40 %	-0.64 %	-8.19 %

Extruded flour cookies, as visible in Table 13, had a tendency to rise. The values for the X and Y axes were constant througouht all the concentrations, balancing around 10 % reduction in length and width. The height of the cookies sustained a more intense alteration, where the percentual values were between 70 % and 100 %. This suggests that the extrusion process provides a different baking capability to the mesquite flour, although the reasons have still to be asssessed.

Table 13 – Average extruded mesquite cookies axial variation values (raw to cooked).

Sample	X loss	Y loss	Z loss
Extruded 40/60	10.38 %	10.15 %	-70.28 %
Extruded 35/65	10.28 %	12.26 %	-98.56 %
Extruded 30/70	7.88 %	11.92 %	-99.60 %

4.5 Colour analysis

In the previous subchapter, the Figures 24 to 29 display a clear visual difference between the cookies made with raw flour and extruded flour. Cookie colours and their respective RGB codes are displayed in Table 14. The three numerical values correspond to each base colour intensity: red, green and blue; thus, when all values are maximum (R=255, G=255, B=255), the corresponding colour is white, and when all values are zero, the colour is black.

Extruded mesquite cookies generally present lower values of all RGB parameters when compared to raw mesquite cookies, which is evidently correlated to the darker colour. Within each table line, it is possible to observe a decadent tendency in darkness, which is supported by the rising RGB values. This behaviour is explained by the proportions of flour and milk: milk presents a lighter colour within the spectrum, subsequently its higher concentration implies an increase in each colour parameter.

In general, these results are satisfactory since the colours fall within the spectrum of the desired colour scheme for cookies. These colours develop mainly due to the flours' original characteristics, but also due to the occurrence of Maillard's reaction.

Table 14 - Cookie colour analysis.

Sample	40/60	35/65	30/70
Raw	RGB (117, 89, 56)	RGB (118, 92, 59)	RGB (137, 114, 70)
Extruded	RGB (88, 75, 58)	RGB (95, 80, 60)	RGB (103, 90, 69)

4.6 Texture analysis

The physical parameters of a food product are an essential measure in the creation of one. TPA parameters correlate with mastication and breakdown of the product when eaten by a consumer. Hardness, presented in Newton, relates to the force needed to compress the product in the first bite. As such, the higher the value, the harder the sample. Springiness is a measure of restoration of shape, while chewiness and gumminess relate to the rate of breakdown and effort to masticate until ready to swallow. Cohesiveness is a measure of structural integrity, and adhesiveness if the work required to overcome the adhesive forces between the food product and the consumer's oral cavity.

4.6.1 Raw mesquite cookies

In Table 15, the parameter values for the raw mesquite cookies are presented. The first parameter, hardness, should be related to the concentration of the cookies. Nevertheless, while the two most concentrated cookies present a logical behaviour, the third is inconsistent. The high value of hardness for the 30/70 cookie is possibly an error related to the hardness of the apparatus base being sensed in the probe. In this case, it is safe to infer that an increase of concentration implies a greater hardness value.

Springiness values are mostly concomitant with each other, despite the 40/60 cookie presenting a slightly higher value. Most likely, the lower the concentration, the lower the springiness value should be, for what if a fourth cookie concentration was added, its springiness value should be inferior since liquid content would be greater.

Cohesiveness values are reasonably consistent throughout all concentrations, presenting a lower value in the 30/70 concentration. Similarly to springiness, lower concentrations should yield a lower cohesiveness value, which is confirmed by the values depicted in the table.

Chewiness and gumminess do not present a linear behaviour, for what the base influence on the probe is a reasonable justification, once more.

Finally, the adhesiveness values tend to increase in absolute value, which is justified by the increasing wetness of the samples. Higher liquid content relates to adhesiveness in a way that is explained by the surface tension rise when liquids such as water are present, causing the sample to stick to the probe and base surfaces.

Table 15 – TPA parameters of raw mesquite cookies.

Sample	Hardness (N)	Springiness	Cohesiveness	Chewiness (N)	Gumminess (N)	Adhesiveness (mJ)
Raw 40/60	97.8867	0.8370	0.6738	55.671	65.535	-0.0871
Raw 35/65	82.2273	0.6582	0.6770	36.6585	55.3689	-0.1925
Raw 30/70	154.2123	0.6696	0.6131	63.7257	94.3588	-0.2433

4.6.2 Extruded mesquite cookies

Regarding extruded mesquite cookies, the results of the TPA are presented in Table 16. Hardness values decrease according to the formulation of the cookies. A larger quantity of milk implies a reduction in hardness. When comparing to the raw mesquite cookies, these values are significantly higher, being 2.5 times greater regarding the 40/60 concentration. This implies that the extrusion process performs physical alterations to the flour matter (e.g. moisture reduction) which create a hardened material.

Springiness presents constant values for all the concentrations, while cohesiveness, chewiness and gumminess decrease with the concentration reduction. The decreasing values all have a common explanation: the rising concentration of milk in the cookies. The material becomes less cohese with higher quantities of milk, which allows a reduced effort to break down the cookies when chewing. Aditionally, chewiness and gumminess values, when comparing to the equivalent numbers in the previous subchapter, are once again 2.5 to 3 times greater.

Adhesiveness, in this case, remains very reduced, approximating zero in all concentrations. This behaviour is supported by the syneresis assay results in chapter 4.2.3., where a zero syneresis percentage is reported for all extruded flour samples.

Table 16 - TPA parameters of extruded mesquite cookies.

Sample	Hardness (N)	Springiness	Cohesiveness	Chewiness (N)	Gumminess (N)	Adhesiveness (mJ)
Extruded 40/60	253.4165	0.9950	0.6412	163.424	164.252	-0.00023
Extruded 35/65	117.4327	0.9958	0.4413	51.0368	51.1735	0.000163
Extruded 30/70	43.7415	0.9985	0.3645	15.6578	15.6687	-0.00002

5 Conclusion

The present work, being developed initially to attain beneficial chemical and physical characteristics in a sweet snack made with mesquite flour, returned satisfactory results.

The nutritional characteristics of the developed snack weren't assessed, however, based on the characterisation of the utilized mesquite flours, an estimate can be performed. The flours presented very good nutritional aspects when compared to wheat flour, namely higher protein content, better antioxidant capacity and greater mineral content. Shelf life is also improved when compared to wheat, based on the moisture values obtained for mesquite. Comparing the two mesquite flours, raw and extruded, the latter presents superior nutritional characteristics in general.

The printability of both flours was assessed, and from those assays, it is possible to conclude that extruded mesquite inks produced further positive outcomes. Syneresis isn't present in the latter mentioned inks, either when at room temperature or when heated to 86ºC, hence the better printability results. However, raw mesquite inks created a matrix similar to gluten when printing, which might be a constructive quality in future culinary uses.

When printing the cookies, the shape fidelity values were discrepant between the two flour inks. Raw mesquite cookies became unshaped due to the high levels of syneresis, which didn't allow a first layer to be set in the printer bed, but also due to the elastic behaviour of the raw ink, which should be researched in future work developments. Extruded mesquite inks produced a more accurate shape, despite being slightly larger when compared the CAD model dimensions.

In the baking process, raw mesquite cookies maintained their shape, while extruded mesquite cookies rose in a way that is similar to wheat bread. This behaviour isn't explained by the tests performed in this work, for what further investigation is needed.

When baked, raw mesquite flour yielded softer and lighter-coloured cookies, while extruded mesquite created harder, darker ones. Although extruded cookies are chewier and gummier, the adhesiveness in these is almost inexistent; in contrast, regarding the raw mesquite cookies, the adhesiveness is significant (mainly anticipated by the syneresis effect) but the chewiness and gumminess are less substantial.

These results may be the basis for the future construction of a snack that is possibly accessible to every consumer, based on the good nutritional characteristics of both flours and variable physical behaviour between them. Depending on the envisioned product, raw or extruded flour may be chosen to accomplish the desired properties.

6 Assessment of the work done

6.1 Objectives Achieved

The nutritional characterisation was partially developed, instead of its totality. This characterisation is timely, costly, and requires specific materials that weren't available at the time of work development. The creation of various inks with different sweetening and salting ingredients was also not feasible, for the quantity of flours available wasn't sufficient for all the possibilities. Nevertheless, a good textural analysis was performed, as well as a satisfactory printability assessment.

6.2 Final Assessment

The works developed in the scope of the dissertation were personally satisfactory, although not many results were obtained. Mesquite flour is a new ingredient in our continent and subsequently not researched by other institutions and/or people, for what the laboratory assays needed several attempts to achieve adequate results. Overall, this work was gratifying and allowed the opening of personal horizons inside the scope of engineering.

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