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TÍTULO: Vegetal Natural Fiber for Surgical Suture Applications

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Dedication

This thesis is dedicated:

To my parents Ruth and Alberto who with their love, patience and effort have allowed me to come to achieve a dream, thanks for inspiring me of overcoming and without fearing the adversities.

To my sisters Gabriela and Valeria for their love and unconditional support, for being fundamental pillars in my life.
My grandparents Mercedes and Luis for their advice and words of encouragement made me a better person and in one way or another they accompanied me in my educational process.

Finally, I want to dedicate this thesis to all my friends at the university, especially to my friends Veronica and Nigel for supporting me in difficult moments, I always carry them in my heart.

Dedicatoria

Esta tesis está dedicada:

A mis padres Ruth y Alberto que con su amor, paciencia y esfuerzo me han permitido llegar a alcanzar un sueño, gracias por inspirarme en superar y sin temer las adversidades.

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Abstract

In Ecuador, the surgical suture is a topic of medical relevance due to the percentage of infections, according to the International Nosocomial Infection Control Consortium (INICC) in its report of 2015, where include Ecuador, the infections acquired in the hospital have great importance due to their high mortality of 14% to 38%.

For this reason, there is a need for new biopolymers approaches for medical sutures based on cellulose to improve the quality of the material suture. We worked with two types of Ecuadorian fibers of plant origin, they were called Fibers F2 and F4, which were purified. Once the F2 and F4 fibers were purified, different tests were carried out to verify that F2 and F4 fibers are appropriate for surgical suture applications. According to SEM morphology, F2 fiber has a porous surface, internal spaces, and a non- compact structure, in the case of F4, it has a uniform structure without internal spaces in its morphology. It was found that the F4 138.84 MPa of UTS presents mechanical properties closer to surgical threads based on natural fiber such as silk (564.78 MPa of UTS, 26.6% Elongation and 6.73 GPa of Young's Module). The biodegradability of the fibers has the same behavior, the main difference is that the F2 fibers have a disintegration of 65.95% in the test period, the F4 fibers have a percentage of loss of weight is less with a maximum of 21.31%. The antibacterial test was negative to both fibers, but F4 avoids biofilm formation, this characteristic is an advantage to decrease the likelihood of infection. In conclusion, the F4 fibers are the best option to possible use in suture application.

Key-words: biopolymers, fibers, biodiversity, antimicrobial, bacteria fiber, suture

Resumen

En Ecuador, la sutura quirúrgica es un tema de relevancia médica debido al porcentaje de infecciones, según el Consorcio Internacional de Control de Infecciones Hospitalarias (INICC) en su informe de 2015, donde incluye a Ecuador, las infecciones adquiridas en el hospital tienen gran importancia debido a su alta mortalidad entre 14% al 38%.

Por esta razón, existe la necesidad de nuevos enfoques de biopolímeros para suturas médicas basadas en celulosa para mejorar la calidad de la sutura del material. Trabajamos con dos tipos de fibras ecuatorianas de origen vegetal, llamadas fibras F2 y F4, que se purificaron. Una vez que se purificaron las fibras F2 y F4, se realizaron diferentes pruebas para verificar que las fibras F2 y F4 son apropiadas para aplicaciones de sutura quirúrgica. Según la morfología SEM, la fibra F2 tiene una superficie porosa, espacios internos y una estructura no compacta, en el caso de F4, tiene una estructura uniforme sin espacios internos en su morfología. Se encontró que la fibra F4 posee un UTS de 138.84 MPa, presentando propiedades mecánicas más cercanas a los hilos quirúrgicos basados en fibra natural como la seda (564.78 MPa de UTS, 26.6% de elongación y 6.73 GPa de Young's Module). La biodegradabilidad de las fibras tiene el mismo comportamiento, la principal diferencia es que las fibras F2 tienen una desintegración del 65.95% en el período de tratamiento, las fibras F4 tienen un porcentaje de pérdida de peso menor con un máximo del 21.31%. La prueba antibacteriana fue negativa para ambas fibras, pero F4 evita la formación de biopelículas, esta característica es una ventaja para disminuir la probabilidad de infección. En conclusión, las fibras F4 son la mejor opción para su posible uso en la aplicación de suturas.

Palabras clave: biopolímeros, fibras, biodiversidad, antimicrobianos, fibra bacteriana, sutura.

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Introduction: Theoretical framework

1.1 Suture Basic Principles

Sutures are used in the area of medicine for tissue restoring after surgery, injury, wound or mutilation. The surgical suture is composed of two main elements; a needle to allow penetrate the tissue and a thread which is used to heal and protected the tissue. Sutures can be used to restore both topical wounds and sub-dermally to promote rapid tissue restoration in the body.

All sutures are foreign bodies and provoke an inflammatory response in the patient dermis. Peak inflammatory response is seen between second and seventh days with an abundance of polymorphonuclear leukocytes, lymphocytes, and large monocytes in the dermis (Kudur, Pai, Sripathi, & Prabhu, 2009).

There are several suture types based on their physical properties including material, construction, absorbability, size, tensile strength, length, and color. There are two different classifications depending on their absorbability and the number of filaments that make up the surgical thread. Based on the absorbability of the surgical thread, it is categorized into absorbable and non-absorbable, which refers to biodegradability in the body. Based on surgical suture structure, the number of threads which may be monofilament and polyfilament. In Table 1, some examples of commercial surgical sutures are classified on their absorbability and structure (Bayl et al., n.d.)(Pillai & Sharma, 2010).

1.2 Absorbable vs Non-Absorbable Surgical Thread

Absorbable sutures are manufactured to degrade over time via controlled enzymatic reactions or biological processes, which involve a patient's reaction to surgical thread. The aim of absorbable materials is the preservation of the material function for a short – term; for this reason, they are frequently used in deep tissues and quick healing tissues such gastrointestinal tract and bladder (Haschek, Rousseaux, Wallig, Bolon, & Ochoa, 2013). In the case of absorbable sutures, they lose their tensile strength within 60 days (Ethicon Inc., 2007).

In contrast, non-absorbable sutures are made of resistant material to avoid absorption. Unlike the absorbable surgical threads, these are used to provide long-term tissue maintenance, do not lose their strength and maintain tensile strength for longer than 60 days. This type of suture thread is used for slow healing tissues, such as skin, fascia, and tendon (Tsugawa & Verstraete, 2012). Another special characteristic of non-absorbable suture material is less inflammatory than absorbable suture materials, but in the majority cases origin a severest inflammatory response (silk is an example of a very inflammatory suture material).

1.3 Monofilament and Multifilament

The monofilament suture is formed by a single strand of the material; this structure type offers lower risk of infection and poor handling/mechanical properties. The multifilament suture is composed of several strands braided together to form a structure; this suture type is stronger and has better handling - mechanical properties compared to the simple strand. Due to the presence of numerous fibers, multifilament sutures exhibit greater capillarity and better knot security (Langley-Hobbs, 2013). The surface of the multifilament sutures has roughness and internal spaces, causing a greater risk of infection and a higher probability of bacterial attachment and colonization.

Table 1 Principal characteristics of commercial suture material

Suture	Material	Type	Thread structure	Duration at maximum strength (days)	Complete absorption time (days)
Natural Suture					
Catgut (Tsugawa & Verstraete, 2012)	Sheep's intestine submucosa	Absorbable	Multifilament	3-4	45 -60
Silk (Kudur et al., 2009)	Silk	Non - absorbable	Multifilament	70% after 14 d; 50% after 30 d	N/A
Synthetic Suture					
Dexon(B. Joseph, George, Gopi, Kalarikkal, & Thomas, 2017)	Polyglycolic acid	Absorbable	Multifilament	10 – 14	90 – 120
Vicryl (B. Joseph et al., 2017)	Polyglactin 910	Absorbable	Multifilament	14- 21	90
Polydioxanone (Tsugawa & Verstraete, 2012)	Polydioxanone	Absorbable	Monofilament	74% after 14 d; 58% after 28 d; 41% after 42 d	180

Nylon (Kudur et al., 2009)	Polymers of nylon	Non - absorbable	Monofilament /Multifilament	15−20% of tensile strength every year	N/A
Prolene (Srinivasulu & Kumar, 2014)	Polypropylene	Non - absorbable	Monofilament	N/A	N/A
Hexafluoropropylene (Kudur et al., 2009)	Polyvinylidene fluoride	Non - absorbable	Monofilament	N/A	N/A

1.4 Suture Size

The diameter of the suture will affect its handling properties and tensile strength. The diameter of the suture material is measured numerically, this numbering has been defined by the United States Pharmacopeia (U.S.P - ww.usp.org). The classification according to U.S.P is showed in Table 2, divided in absorbable and non-absorbable surgical suture.

The optimal repair time of the injured tissue depends on the characteristics of the sutured area, its location, morphology and is level of healing. On the other hand, if the sutured tissue is exposed to a great tensile force, a larger suture diameter can be used; for example, abdomen aponeurosis supports a large tension due its location and movements therefore should be sutured with thick surgical threads, as the zero or one gauge (The United States Pharmacopeial Convention & May, 2007).

Table 2. Comparison between the diameter of the absorbable and non-absorbable surgical threads.

USP designation	**Synthetic absorbable diameter (mm)**	**Non-absorbable diameter (mm)**
11-0		0.01
10-0	0.02	0.02
9-0	0.03	0.03
8-0	0.04	0.04
7-0	0.05	0.05
6-0	0.07	0.07
5-0	0.10	0.10
4-0	0.15	0.15

3-0	0.20	0.20
2-0	0.30	0.30
0	0.35	0.35
1	0.40	0.40
2	0.50	0.50
3	0.60	0.60
4	0.60	0.60
5	0.70	0.70
6	-	0.80

Source: The United States Pharmacopeia - www.usp.org

1.5 Suture Properties

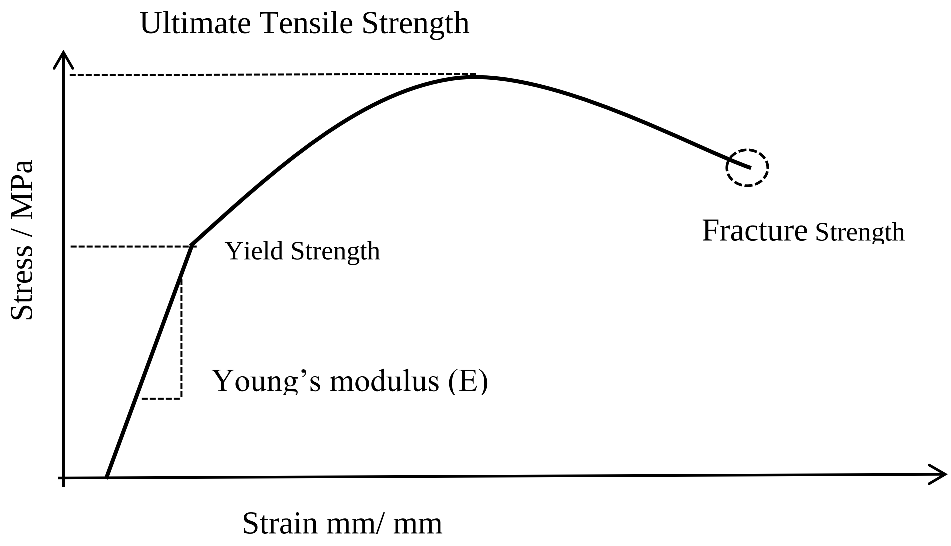
A suture must keep certain characteristics to be considered optimal or ideal; the suture must be easy to handle, sterilized, flexible, produce a minimal drag of the tissue, ideal mechanical resistance, biocompatibility and the necessary force to support the abrasion until the growth of the new tissue (Muffly, Boyce, Kieweg, & Bonham, 2012).

1.5.1 Physical and mechanical properties

Mechanical properties, such as elongation percentage, modulus of elasticity, and ultimate tensile strength are measured usually to evaluate the possible applications in the surgical suture of the fibers.

In the representation of engineering stress-strain can identify the mechanical properties, such as Young’s modulus, ultimate tensile strength, fracture point and yield strength deduced in the Graph 1. In the stress-strain curve, two distinct regions of the elastic region and plastic region represent different material behavior of the fiber (Graph 1).

Graph 1: Representative graph of stress vs. strain with the main mechanical properties.



In the representative Graph 1, one observes a change in the slope of the curve that characterizes the mechanical property defined as yield strength which, in turn, describes the limit of the elastic behavior and the beginning of the plastic behavior of the fiber. Once the fiber or material exceeds its yield point, it will permanently deform. In other words, this resistance defines how much force this material can resist to without changing its shape and length (Shackelford & Alexander, 2000). The elastic stage is related to proportionality constant *E*, called Young's modulus or the modulus of elasticity, which is one of the most important mechanical descriptors of a material. It measures the ability of fiber to withstand length changes caused by tension or comprehension force without altering its original length, it can be deduced from the graph by calculating its slope (Budynas & Nisbett, 2014).

This research mainly considers the ultimate tensile strength (UTS) to measure the efficiency of the fibers. UTS is defined as the breaking load divided by area of the cross-section of the fiber represented in units of stress N/m^2 (also called Pascals, or Pa), for practical purposes of the scale MPa is used. UTS is the maximum load that a fiber can withstand (Brown, 2018).

Elongation is measured as the displacement that a suture can experience before breaking during the tensile testing (Abellán, Nart, Pascual, Cohen, & Sanz-Moliner, 2016). The amount of elongation exhibited by a fiber sample under tension during a test provides a value for the ductility, it is commonly expressed as a percentage of the elongation and calculated using the final longitude and original longitude according to the following formula (Pytel & Singer, 1994):

$$\% \, elongation = \frac{l_f - l_o}{l_0} \times 100\%$$

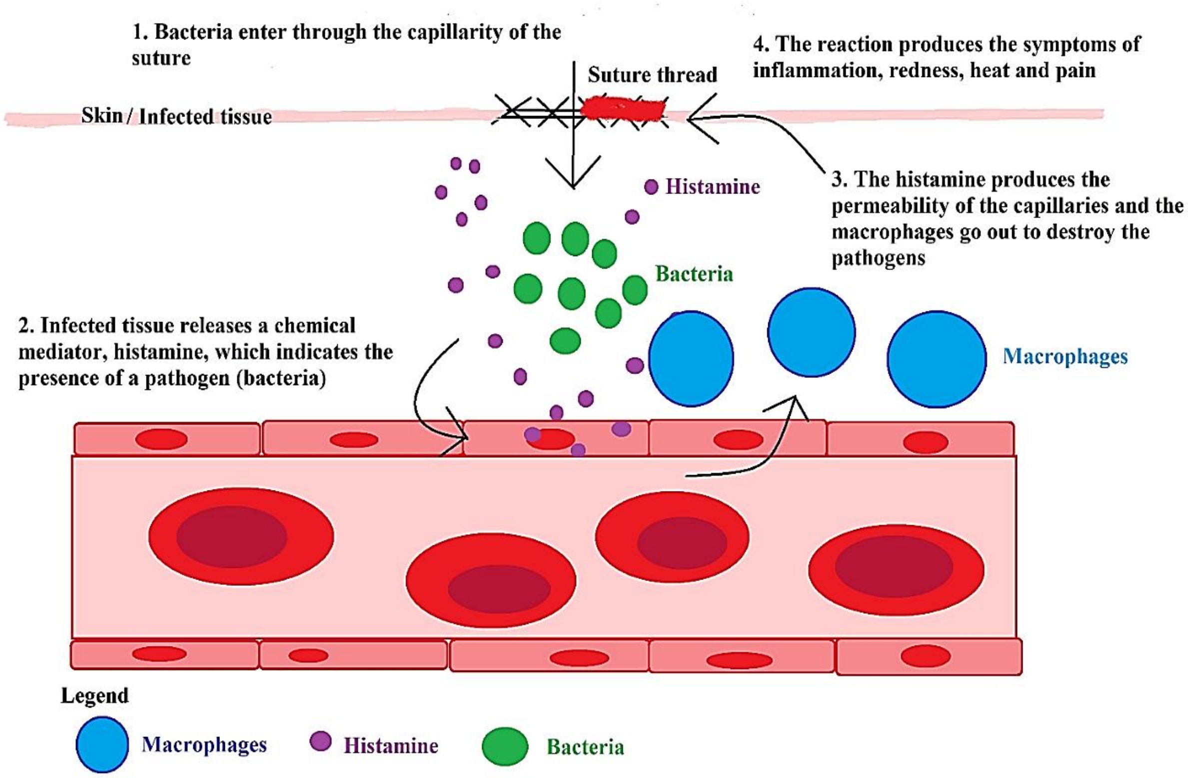
Table 3 describes the basic mechanical properties (UTS, % Elongation at break and Young's Modulus) of some examples of commercial surgical sutures, highlighting the two main types of commercial sutures based on natural sources: silk and catgut.

Table 3 Mechanical properties of commercial suture thread

Commercial Suture	**UTS (MPa)**	**Elongation at break (%)**	***E* (GPa)**
Natural Suture			
Silk (Abiri et al., 2016)	564.78	26.60	6.73
Catgut (Kreszinger et al., 2018)	212.42	44.90	1.53
Synthetic Suture			
Polypropylene (Fraunhofer, Storey, & Masterson, 1988)	446.97	18.90	3.65
Polydioxanone (Abiri et al., 2016)	478.99	30.90	1.00
Nylon (Naleway, Lear, Kruzic, & Maughan, 2014)	508.85	62.10	4.36
Vicryl (Paez, Martin, Sestafes, Millhn, & Navidad, 1994)	624.33	38.50	0.67
Polybutester (Naleway et al., 2014)	527.59	40.00	0.95

Another important physical property of the suture is the capillary effect and subsequent absorption of fluid by suture thread (Gazivoda, Pelemiš, & Vujašković, 2015). The multifilament suture has internal spaces of tubular shape, it can transport and capture fluids by the effect of capillarity. On the other hand, the unifilated structure hinders the capillarity process and is not able to absorb fluids. This physical characteristic of capillarity is a key factor in the contamination probability of the suture by microorganisms, resulting in reduced wound healing (Larena & Frosch, 2005). For example, bacterial contamination of silk sutures produces an abscess with the conglomeration of inflammatory cells around the suture producing symptoms of inflammation, such as redness, pain, increased temperature and swelling (Scheme 1).

Scheme 1 Steps of the inflammatory response caused by the surgical suture (foreign body)



1.5.2 Biological properties

The biological response of tissues against sutures can be influenced by different factors, such as biodegradability, biocompatibility, absorbability, configuration, and size. While the suture remains in the tissue, this may influence different host reactions in living tissues, specially the activation of the inflammation cascade through diverse pathways, such as abrasion, foreign body reaction, allergic reaction, or degradation byproduct (Guadarrama, Scougall, Morales, Sánchez, & López, 2015).

Any biological response to the suture material should be limited and controlled because numerous inflammatory reactions delay or avoid tissue healing, cause scar formation, and predispose to several infections.

Another biological property of the suture is the existence of bacterial growth or bacterial contamination of surgical suture. This is produced by the creation of a biofilm or bacterial adherence in the surgical thread material. This property will depend on the microbial species, the composition, and the thread structure, the multifilament sutures having greater bacterial colonization than the monofilament ones (Henry, Hess, Barnes, Dunny, & Wells, 2010).

The formation of a biofilm is typically due to a defense mechanism to achieve an environment capable of retaining nutrients and ensure the survival of bacteria. It can be schematized as a series of cyclical steps beginning with the initial fixation (reversible and irreversible), which consists of an initial interaction that may be transient due to weak interactions between bacteria and the surface. Bacteria adhere to the surface of surgical sutures by physical forces or by appendices of the bacterium, such as the pili or flagella (Joo & Otto, 2012)(Gupta, Sarkar, Das, & Bhattacharjee, 2015). Following by this first adhesion, comes the aggregation and accumulation in multiple cell layers. In this phase, the adhered cells grow and mature when interacting with each other, the pathogenic cells begin the secretion of the EPS (Extracellular Polymeric Substances) that forms a barrier to protect the biofilm from antibacterial agents (Veerachamy, Yarlagaddab, Manivasagam, & Yarlagadda, 2014). Finally, the dispersion / detachment occurs. This stage defines the future projections of the biofilm and therefore the infection; this is the process by which the bacteria expand from one region of the body to another, repeating this process cyclically (Khatoon, Mctiernan, Suuronen, & Mah, 2018).

The formation of biofilms in surgical sutures between the first 4-6 hours of suturing the wound is fundamental for the development of SSI. In addition, the initial stage of bacterial adhesion on the surgical suture is considered the most important virulence factor of *Escherichia coli* and *Staphylococcus aureus* (Ercan et al., 2018).

Antibiotics can treat bacterial infections but in the long term produce side effects and begin to be inefficient due to the development of resistance to antibiotics. A new alternative to antibiotics consists in the use of organic or inorganic compounds as coverings for the surgical threads to decrease the bacterial filtration (Dennis et al., 2016).

There are qualitative and quantitative methods to evaluate the antibacterial potential or facility of bacterial growth if it is on the surface the surgical threads. Some studies indicate that there is bacterial growth inside surgical threads at a moderate, medium or high level. Table 4 describes the number of CFU / cm^2 (CFU = colony forming units) on multifilament and monofilament sutures using an *in vitro* model of contaminated soft tissues with *S. aureus* bacteria.

Table 4 Bacterial concentration of multifilament and monofilament commercial surgical sutures.

Type of suture	Structure	Bacterial concentration CFU / cm^2
Vicryl - Polyglactin 910 (Fowler, Perkins, Buttaro, & Truant, 2013)	Multifilament	213 000
PDS -Polydioxanone (Fowler et al., 2013)	Monofilament	101 000
Silk (Qadri et al., 2017)	Multifilament	500 000
Polypropylene (Zamora, Granizo, Esteban, Zafra, & Celdran, 2007)	Monofilament	~ 126 000

In Table 4, surgical sutures with a multifilament structure have a higher number of colonies than monofilament suture. Moreover, silk suture has the highest bacterial concentration. Therefore, the creation of a new type of suture with monofilament structure and natural origin would be an option to conventional synthetic sutures and a solution to the problem of infections caused by the suture of natural origin, such as silk.

There are several methods to incorporate antibacterial properties into the fibers, among which the use of silver nanoparticles (AgNPs) is the most common. AgNPs have long been shown to have broad-spectrum antibacterial effects and anti-inflammatory properties with possible surgical applications. In several studies, AgNPs are deposited by the method of layer by layer; these studies have implemented tests with surgical sutures of various types (absorbable, non-absorbable, monofilament and braided), such as Vircyl, silk, and catgut, providing evidence of the antibacterial and anti-inflammatory potential of AgNPs (Zhang, Liu, Wang, Peng, & Kenneth, 2013) (Chung & Um, 2014)(Wang & Zou, 2015).

1.5.3 Handling properties

The handling properties are defined by parameters that are involved in the improvement of the surgical thread, they include mainly forces of friction, bending, compression and knot mechanical properties. The interaction between these forces within the suture knot directs the displacement mode and deformability of the suture thread within the knot (Xiaojie Chen, Hou, Tang, & Wang, 2015).

The suture handling characteristics include flexibility that is defined as the easiness with which a suture can be folded. While the friction coefficient is defined as a measure of the sliding ability of the suture.

Sutures with a high friction coefficient have a high level of complexity to pass through the tissue and cause significant tissue damage. However, these sutures are easier to handle and preferable for tying knots (Debbabi & Abdessalem, 2018). When comparing multifilament sutures with monofilament counterpart, the former has a higher friction coefficient causing a problem in tissue recovery, this can be reduced by using coatings with wax, silicone, and Teflon (Pfenninger & Fowler, 2010).

1.6 The Current Innovation of Suture Threads

Progress in this area can be anticipated by manufacturing processes of strong and elastic threads made of biocompatible absorbable natural polymers, such as chitin, chitosan, and alginate.

Chitin and chitosan (the most important derivative of chitin) are defined as polysaccharides in nature with linearity in arrangement and which remain in a random distribution. These biopolymers are synthesized by an enormous number of living organisms, and considering the amount of chitin produced annually in the world, it is the second most abundant polymer after cellulose (Rinaudo, 2006). Both polymers have biomedical implications in the treatment of many diseases in humans (Ali-Komi & Hamblin, 2016) due to their coagulant property and used as hemostatic agents to accelerate the healing pace of wounds in humans (Ganguly, 2013). Another important feature of these two biopolymer in biomedical applications is their antibacterial property; chitosan can inhibit the growth of some microbial species by the cationic amino groups probably by binding to anionic groups of microorganisms and prevent their growth (Qu, Guo, Tian, & Lu, 2014). For all these advantages, chitin and chitosan are used to make strong surgical and tensile threads that have high biodegradability and wound healing properties. An example of the industrialization of chitosan as a surgical thread is the German company that sells a suture made of chitosan from crab shells called Heppe Medical Chitosan.

Some investigators have employed a method of creating nanofibrillar cellulose-alginate (NFCA), The alginate is a natural anionic polymer that is typically obtained from algae, and generally used for biomedical applications due to its biocompatibility, low toxicity and relatively low cost (Lee & Mooney, 2013). The investigators used the alginate in the formation of suture threads and coatings for possible applications in surgery. They projected that NFCA coated surgical sutures are viable on accessible areas, such as the skin and other surfaces where its removal would not cause further complications. NFCA coated sutures show potential as cell carrier systems integrated with surgical suture processes (Somersalo, Pitka, Lou, & Urtti, 2017).

Alginate, chitin, and chitosan are examples of new approaches for the manufacture of surgical threads based on natural resources, such as plants including and animals (shell of the crabs).

1.7 Comparison of plant and commercial sutures for a viable surgical suture application

Natural fibers, like silk and collagen, have undergone treatments to obtain a viable commercial product, sterilized and ready for use. In the case of natural silk, it is commercially sold in different sizes with appropriate characteristics for the application as surgical sutures, such as excellent handling and mechanical characteristics, ease of use, and ideal knot security (Xiaojie Chen et al., 2015). The silk suture thread is very resistant and undergoes slow proteolytic degradation, losing its strength after 1 year (Spelzini et al., 2007). In its natural state, it contains up to 25% of natural gum composed of sericin, so must be removed to avoid an immune response after its implantation in the human body (Sahoo, Ramana, Satyanarayana, & Bhongir, 2017). In this process, chemical treatment and braiding of silk are used, followed by with its pigmentation using non-toxic dyes (for its visualization), and, finally, the silk fibers are sterilized (Altman et al., 2003). These processes contribute to optimizing these surgical threads that are characterized by their high flexibility with Young's module of 6.75 GPa and their ultimate tensile strength of 564.75 MPa (Table 3) do to silk the suture material used mostly in several types of interventions including use in cardiovascular, ophthalmic and neurological procedures, it is indicated for use in approximation and / or soft tissue ligation in general.

The sterilized surgical catgut is also a surgical thread of natural origin, it is manufactured from the intestinal mucous tissue, which consists of a strand prepared from collagen derived from mammals, which are chemically and mechanically cleaned (Chellamani, Veerasubramanian, & Balaji, 2013). Then, by an electronic screwing process, a monofilament fiber is created with reliable force and retention power. Finally, the fiber is meticulously polished to produce a smooth and uniform suture; the process being terminated by, the sterilization (ethylene oxide) (Sahoo et al., 2017).

There are two types of sutures: plain and chromic. Chromic catgut is treated with chromium salts (brown) that decrease the process of absorption in the body and minimize the reaction of tissue in the surrounding tissues.

Plain catgut commonly has strength retention for about 7 days once interacting with the tissue while chromic catgut has approximately twice the retention time. Finally, catgut sutures are packaged in a solution of alcohol (ethanol or isopropanol) or glycerin to prevent drying out, since catgut becomes stiff when dry and produce handling problems (Chu, 2013) (Rajendran, Anand, & Rigby, 2016). These methods contribute to retaining its flexibility with Young's module of 1.53 GPa and its ultimate tensile strength of 212.42 MPa (Table 3). With these mechanical properties, this type of suture is usually used for wounds in areas where tissues have rapid regeneration. The catgut tends to lose its resistance to traction (within days of surgery) in most internal tissues due to digestion by enzymes or degradation by hydrolysis and these sutures may break down even faster when the pH is low. A decreased pH may result from infection, medications or metabolic disorders (Suzuki & Resnik, 2018)(Karabulut et al., 2010).

As detailed in Table 3, the synthetic and natural-origin suture threads found in the market have similar mechanical properties, but the silk suture threads have a much greater elasticity modulus than the other fibers that provide greater stretch without losing their original properties. Silk (non-absorbable) and catgut (absorbable) having a natural origin and possessing mechanical parameters sufficiently effective for use in the surgical suture, especially the silk material is taken as a basis for comparing possible natural fibers of plant origin that could be an alternative in the surgical application.

To select of the primary source of the fibers, a comparison of mechanical, physical and biological properties was made with natural materials used usually in the surgical suture. The resistance of the surgical thread must be present both in the operation and in the postoperative period since it must stand until the healing process ends.

The main mechanical properties define a standard to find another natural fiber derived from plants that simulate the same characteristics of the raw materials of surgical threads derived from natural sources (silk). Table 5 presents the mechanical properties (UTS, % Elongation and Young's Modulus) of four types of silk fibers derived from various sources. Then, silk is compared with other fibers obtained from plants (Table 5 and Graph

2) for the selection of the most optimal fibers to achieve the desired features in materials for surgical suture.

Table 5. Mechanical properties of *Bombyx mori,* twisted *Bombyx mori, E. bauhiniae* and Tussah silk fibers

Fiber samples	UTS (MPa)	Elongation at break (%)	*E* (GPa)
***B. mori* silk** (Heung, Lau, Ho, & Mosallam, 2009)	208.45	19.55	6.10
Twisted *B. mori* silk (Heung et al., 2009)	165.27	20.57	3.82
***E. bauhiniae* silk** (Teshome, Onyari, Raina, Kabaru, & Vollrath, 2012)	247.70	20.8	4.32
Tussah silk (Heung et al., 2009)	248.77	33.48	5.79

For four silk fibers, an average of their mechanical properties was estimated, based on the bibliography, the silk has an ultimate tensile strength of 217.55 MPa, an elongation percentage of 23.60% and a Young's Modulus (E) of 5.00 GPa.

Table 6. Mechanical properties of plant-based natural fibers.

Natural fibers	UTS (MPa)	Elongation at break (%)	*E* (GPa)
Flax (P. V Joseph, Joseph, & Thomas, 1999)	300- 900	2.70 – 3.20	24
Jute (P. V Joseph et al., 1999)	200 - 800	1.16 – 8.00	43.80
Sisal (P. V Joseph et al., 1999)	444 - 552	2.00– 2.50	9 – 38
Pineapple (Heung et al., 2009)	170 – 1627	2.40	60 – 82

Coir (Nishino, 2004)	175	14.21 – 49.00	4 – 6
Hemp (Nishino, 2004)	690	6.00	70
Cotton (Nishino, 2004)	264 – 800	3—8	5.00 - 12.60
Wool (Heung et al., 2009)	120 - 174	25 – 35	2.30 – 3.40

Graph 2: Comparison of the mechanical properties of silk fiber with other natural fibers.

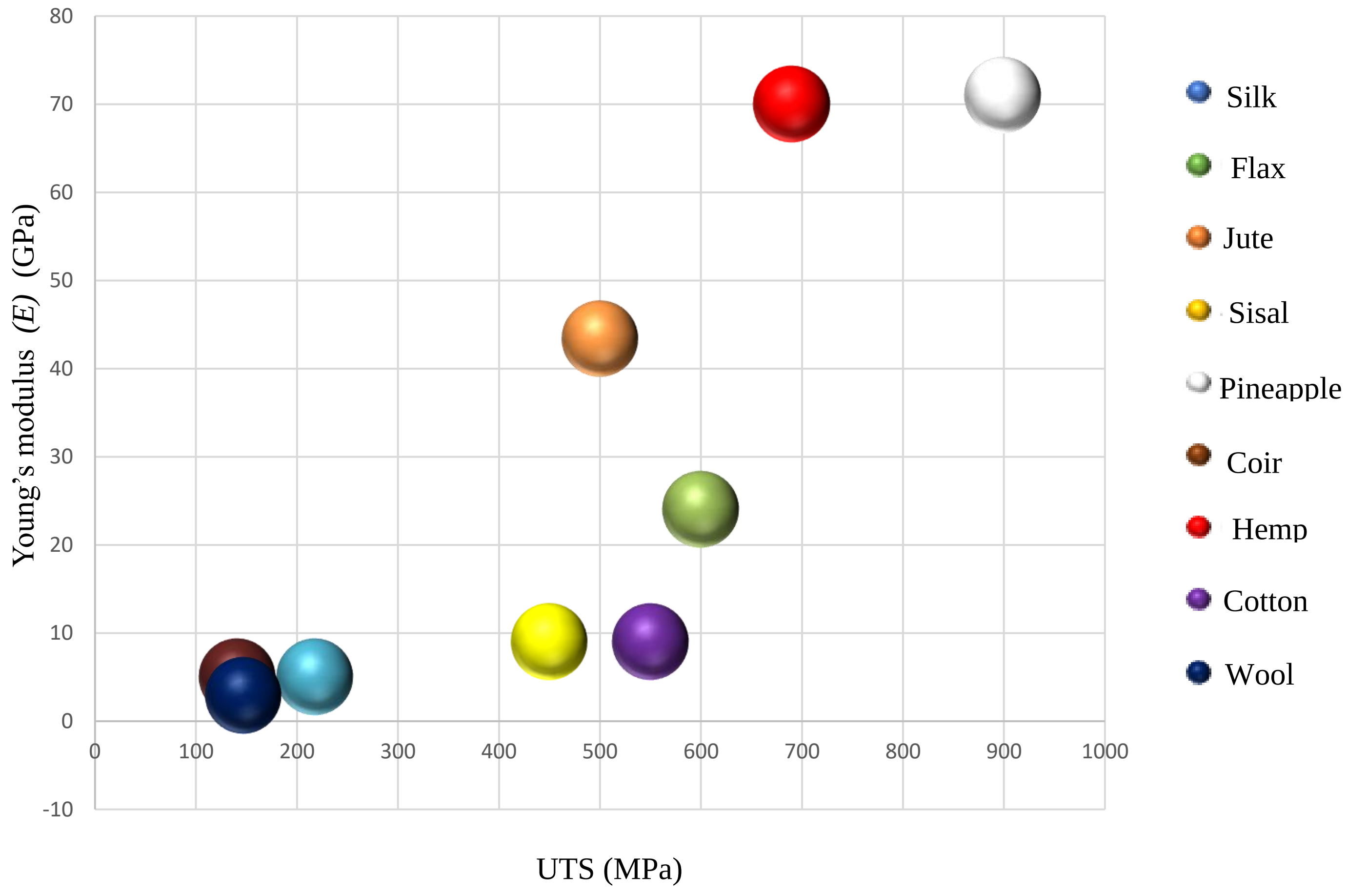


Table 6 and graph 2 support that some natural fibers could have application in surgical suture, as the coir, sisal, and wool are the most accurate options as they approximate the mechanical properties of natural silk. As a second option could be the jute, flax, and cotton. However, the use of pineapple and hemp is totally ruled out.

On the other hand, the biological properties are relevant to evaluating a suture thread due to its contact with humans and, the characteristics that will be considered principally is its biodegradability, absorbability, and biocompatibility.

The biodegradability (chemical nature of the material) and absorbability can be measured by the absorption times of the suture materials. This parameter can only be measured in absorbable sutures because silk and other nonabsorbable synthetic materials do not have this biological characteristic due to their compositions. The catgut, due to its structure, facilitates its decomposition by the body since it is constituted by 98% of collagen, thus proteolysis plays an important role in the biodegradation process (Rajendran et al., 2016). Catgut is an excellent example of biodegradable suture material, with low immunological activity and inert character; for this advantage, it is used in ophthalmological surgery and in tissues of rapid regeneration. In addition, it has having a lower absorption rate in comparison with other sutures. (Table 7) with a rate of 70 to 90 days for the material to be completely digested by proteolytic means (Srinivasulu & Kumar, 2014).

Table 7. Biodegradability on absorption time of absorbable suture thread. (Pineros-fernandez, Drake, Moody, Edlich, & Rodeheaver, 2004)

Suture material	**Absorption time (days)**
Plain catgut	70
Chromic catgut	90
Polydioxanone	120 – 180
Vicryl (Polyglactin 910)	70
Dexon (Polyglycolic acid)	90 – 120
Glycomer 631	90 – 110
Polyglecaprone	91 - 119

Biodegradability can be represented in weight loss when exposing the surgical threads to fluids. Table 8 shows examples of monofilament and multifilament surgical threads with their percentages of progressive weight loss per week. The monofilament surgical threads have a greater degradation than the multifilament threads; due to their structure, they lose more weight.

Table 8. Weight loss of absorbable suture thread and silk in two and four weeks.

Suture material	Structure	% Weight Loss (Two weeks)	% Weight Loss (Four weeks)
Dexon (Polyglycolic acid) (Niculescu et al., 2016)	Monofilament	28.0	33.8
Copolymer Polyglycolic acid (Niculescu et al., 2016)	Multifilament	0.0	9.4
Polydioxanone (PDS) (Mitchell, 2004)	Monofilament	2.3	6.7
Silk (Horan et al., 2005)	Multifilament	0.0	0.6
Polycaprolactone (Liu, 2008) (Molea, Schonauer, Bifulco, & Angelo, 2000)	Multifilament	1.9	2.1
Glycomer 631(Molea et al., 2000)	Monofilament	2.6	5.6
Polyglecaprone (Molea et al., 2000)	Monofilament	14.3	35.0

Another important feature to consider is the biocompatibility (inflammatory reaction, wound infection, thrombi formation) of the medical suture, and how suture provoke an immunological response. Natural materials, such silk, have been used for the closure of wounds with satisfactory results, but they are more immunogenic than synthetic materials and increase the risk of the development of infectious processes by their poor characteristic of defense against microbes (Thilagavathi & Viju, 2015; Srisuwan et al., 2008).

The structure and the number of threads forming the surgical thread are implicated in the immune response that will occur in the body. Multifilament sutures are very efficient for handling, knotting, and mechanical resistance but have a risk of infection and significant friction with the tissue in contact. On the contrary, monofilament sutures are easy to unknot but have less risk of infection in comparison with the multifilament ones.

Bacterial growth also is higher when braided sutures are used; their infection rate is five to eight times greater than for the monofilament sutures. The smooth surface of monofilaments causes less response in the host (Manavalan & Mukhopadhyay, 2009). Based on this, silk sutures are likely to cause surgical site infections as they are composed of a braided structure that produces internal spaces where microorganisms can lodge (Guadarrama-Reyes, Saraí Scougall-Vilchis, Rogelio J. Morales-Luckie & Sánchez-Mendieta, Víctor López-Castañares, 2015)(Rathinamoorthy, Sasikala, & Thilagavathi, 2009). In the case of catgut, its monofilament structure is an advantage, but it also has a localized immune response and biological reaction due to its foreign body condition and its protein incompatibility. In special, plain catgut has more likely to cause a tissue reaction more severe than the chromed (Chu, 2013). On the other hand, independently of the material or the number of the filaments, while the suture size increases, it also increases tissue reaction (Başçı, Akgun, & Barber, 2008).

A solution for the poor protection against the pathogenesis of most surgical sutures, especially multifilament sutures, consists in the use of fibers or materials of some plants that possess an antibacterial activity towards a wide spectrum of pathogens. The plant material has been used for fillers and reinforces in polymer composites for their antibacterial benefits. Natural sources, such as jute, flax, sisal, bamboo, and hemp have an antibacterial capacity that can be attributed to alkaloids, cannabinoids, essential oils, polypeptides, lectins and other bio-acid compounds that are produced by these plants, and are considered as the candidates for future applications in the surgical suture (Khan, Warner, & Wang, 2014).

2. Problem statement

Surgical site infection (SSI) or infection of the wound is defined as the presence of symptoms and signs of inflammation (heat, flushing, pain, and edema) or purulent discharge at the site of the incision (Norman et al., 2017).

The first step for the appearance of an infection is the microbial colonization. The risk of infection arises when a surgical wound is contaminated with 106 microorganisms per gram of tissue, this risk is increased when foreign material is placed (sutures, permanent devices or prosthesis), it leads to reduce the minimal infective dose of 106 to 103 microorganisms per gram of tissue (Aguilar Lopez & Obando Navas, 2013). Most of the SSIs come from endogenous sources, the flora of patients being responsible for the contamination of the surgical site. For example, almost 20% of skin bacteria are lodged in sebaceous glands and hair follicles where they cannot be eliminated by antiseptics (Grice & Segre, 2011).

The International Nosocomial Infection Control Consortium (INICC) realized a report from January 1, 2010, to December 31, 2015. It conducted a multicenter surveillance study of device-associated healthcare-associated infection (DA-HAI) in 703 Intensive care units (ICUs) in 50 countries from Latin America, Europe, Eastern Mediterranean, Southeast Asia, and Western Pacific World Health Organization regions including Ecuador (Quito). Out of all hospitals, 62% were public or academic, and the remaining 38% were private (Rosenthal et al., 2016). In this report, nine Latin American countries (Ecuador, Argentina, Colombia, Venezuela, Bolivia, Peru, Chile, Brazil and Uruguay) were studied highlights the fact that nosocomial infections, especially the SSIs, represent a serious and frequently hidden risk to the safety of the patient, and that this danger is much greater in developing countries such as Ecuador. Infections acquired in the hospital, specifically in the ICU area, are of great importance due to their association with high mortality. The associated mortality is 14% to 38%, which depends on the causal agent, the population, and the associated risk factor (García, 2016).

With this background, each operation requires the use of adequate suture material with consideration of the specific situation. For this reason, one problem that will determine the progress of modern medicine is the creation of suture materials for surgery with limited probability of triggering an immunogenic reaction and to absence of infection risk.

2.1 Surgical thread in Ecuador

According to the statistics provided by the Central Bank of Ecuador (https://www.bce.fin.ec/) during the period January to December 2012, the companies that imported surgical threads for their following distribution and commercialization in Ecuador are:

- Alconlab Ecuador S.A.
- B. Braun Medical S.A.
- Bio-In S.A Medical Systems
- Ecuasurgical S.A.
- Global Representations GBR S.A.
- Invimedic S.A
- Johnson & Johnson of Ecuador

- Palfarma Cia. Ltda.
- Saipacol S.A.
- Tecmed S.

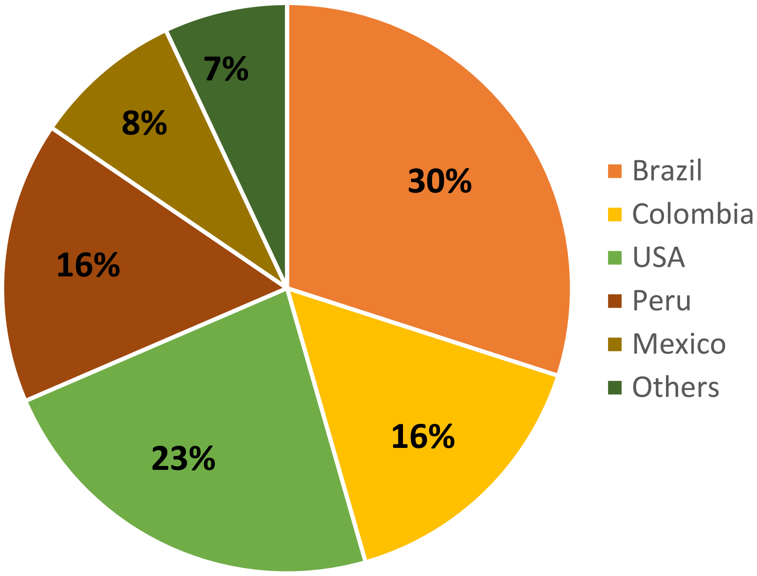
On the other hand, it should be noted that the main sources of supply of surgical threads are in the following countries: Brazil, Colombia, United States, Mexico, Peru, Holland, India, Germany, Italy, Belgium and Panama.

Table 9 Surgical suture suppliers' countries from January to July 2018

Surgical Suture Suppliers' Countries Period from January to August 2018				
	Country	**Tons**	**FOB – Dollar in thousands of USD**	**% / Total FOB - Dollar**
Sterile catgut and similar sterile ligatures for surgical sutures (including sterile resorbable wires for surgery or odontology)	Australia	0.1	52.0	1%
	Belgium	0.1	7.2	0.2%
	Brazil	4.4	919.0	**30%**
	China	0.1	2.0	0.06%
	Colombia	7.0	513.5	15.65%
	Germany	1.0	132.8	4%
	Spain	0.1	38.1	1.16%
	India	0.2	21.0	0.64%
	Mexico	3.1	280.4	8.5%
	Netherlands	0.6	17.1	0.52%
	Peru	4.4	538.1	16.4%
	USA	3.2	758.6	**23.12%**
Total:	**12**	**24.3**	**3279.8**	**100%**

Source: Central Bank of Ecuador (2018).

Graph 3. Surgical thread suppliers' countries in Ecuador and their market shares in percentage



Source: Central Bank of Ecuador (2018).

In Graph 3, it can be noted that more than 60% of the surgical threads in Ecuador are supplied by three Latin American countries: Brazil, Peru, and Colombia. With the development of the industry and the research in suture materials, Ecuador could convert into a producer of surgical threads.

3. Hypothesis, General and Specific objectives

3.1 Hypothesis

Natural vegetable fibers of Ecuadorian origin could be an alternative in the surgical suture area to avoid infection in the suture site. These fibers could exhibit mechanical characteristics comparable to silk (a natural raw material for the elaboration of surgical suture), a stable biodegradability and antibacterial properties that favor the manufacture of a surgical suture.

3.2 General objective

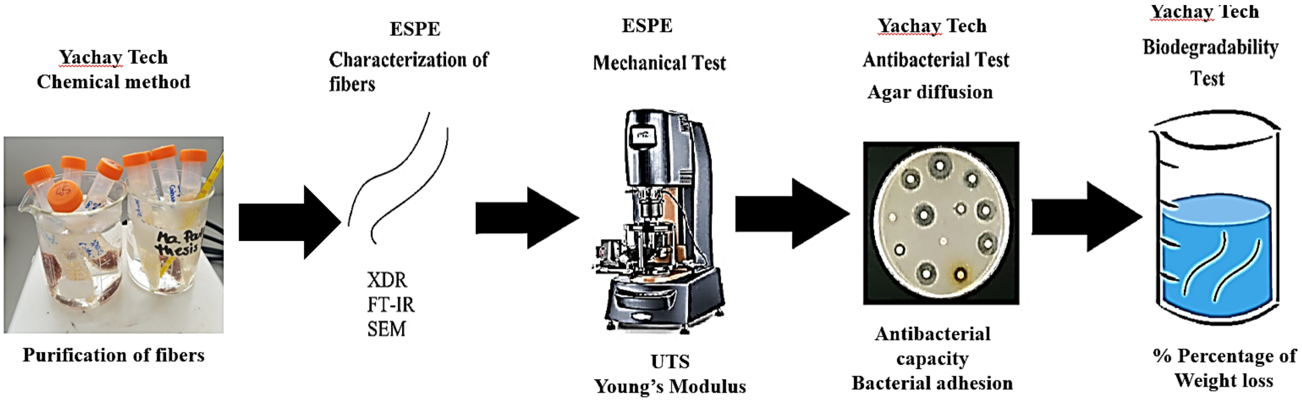
Obtain fibers from natural plant biopolymers compatible with the application in the field of surgical suture, through purification of the fibers followed by tests to check their mechanical properties, antibacterial characteristics and biodegradability.

3.3 Specific objectives (scheme 2):

- Purify fibers F2 and F4 (two vegetal sources of Ecuador).

- Perform the physico-chemical characterization of the fibers to verify their purification.
- Perform the mechanical test of fibers F2 and F4 to obtain relevant data of their mechanical properties, then compare the properties of these fibers with the mechanical characteristics of silk (commercial natural surgical thread).
- Perform an antimicrobial test with gram-negative *E. coli* bacteria to assess possible antipathogenic properties of fibers F2 and F4.
- Perform a biodegradability test to define whether fibers F2 and F4 are possible candidates to be absorbable or non-absorbable sutures.

Scheme 2. Flow- chart of the objectives of this investigation



4. Methodologies

4.1 Materials

The fibers F2 and F4 of natural origin were purchased in the city of Guayaquil, Ecuador. The raw material of F2 fibers was obtained at the Sauces IX Agricultural Market located on street Dr. Antonio Parra Velasco, between Mz 518 and 550 fronts to Sauces III, in the section of vegetables and fruits. The raw fiber material F4 was obtained in the Handicraft Market of Guayaquil located Av. Dr. Alfredo Baquerizo Moreno, Guayaquil 090313.

4.2 Sample Preparation

In total 16 samples were made, 4 samples of complete fiber for each type of fiber (4 samples of F2 and 4 samples of F4) and 4 samples of ground fiber for each type of fiber, each sample is a single fiber of different lengths between 7 to 14 cm. The fibers were previously cleaned and placed in sterilized 50 mL falcon tubes.

4.3 Fiber Characterization

Purified fibers were, characterized by several techniques such as FT-IR, SEM, and XRD.

First, it is necessary to observe the surface and structure of the fibers for the description of the result related to the antimicrobial test. The scanning electron microscope (SEM) was used in this research because it is one of the most versatile instruments available for the investigation and analysis of the microstructure morphology and chemical composition characterizations (Zhou, Apkarian, Wang, & Joy, 2007).

The X-ray diffraction is defined as an analytical technique of qualitative characterization that uses the radiation produced by X-rays to observe the crystal lattice to determine the structure of the atoms and molecules. Through this analysis, it is possible to differentiate between the structures; even if they have the same elementary profile, XRD can detect minute differences between the samples (Day, 2016).

With the XRD patterns, the crystallinity index can be calculated to evaluate the mechanical properties of the fibers based on this parameter. The crystallinity index (I_c) was calculated using the following equation:

$$I_c = \frac{I_{(002)} - I_{(am)}}{I_{(002)}} \times 100$$

where

$I_{(002)} =$ the counter reading at peak intensity at a 2θ angle close to 22° representing crystalline material.

$I_{(am)} =$ the counter reading at peak intensity at a 2θ angle close to 18° representing amorphous material (Fonsêca et al., 2015) (Skood, 2008).

Finally, the Fourier Transform Infrared Spectroscopy (FTIR) has been used in this investigation. Commonly used in biological and biomedical analyses, this technique obtains an infrared spectrum of absorption, emission or photoconductivity of a solid, liquid or gas. FTIR uses an incandescent source of light to emit a bright ray in the infrared wavelength range. The absorption of infrared radiation generates individual vibrational movements in molecules, defined as stretching and, in fact, the molecule changes its vibrational state as it passes from fundamental vibrational state to excited vibrational state (Dwivedi et al., 2017) (Alawam, 2014).

Moreover, different tests such as mechanical, biodegradability antibacterial tests are carried out with these fibers after its characterization.

4.4 Mechanical Test of Fibers

The mechanical test was performed on 4 samples of each type of fiber using a device called Hybrid Rheometer (Brand: TA instruments model: Discovery HR-1). This machine, commonly used for fluids and viscous samples, measures the rheological properties, is which describes the interrelation between force, deformation and time.

A Hybrid Rheometer has several functions. For this research, the function of Dynamic Mechanical Analysis (DMA) was used. DMA mode adds a new dimension for solid and soft-solid materials testing, this device provides the most sensitive and accurate linear data. The use of axial DMA complements solid torsion testing by contributing to measure the modulus of elasticity, or Young's Modulus (E) (TA Instruments, 2016).

The properties that can be measured using DMA include the viscoelasticity, the flow of yield, the yield stress and the behavior during stress relaxation, as well as relevant parameters of the fracture point process of the fiber, its ultimate tensile strength, Young's Module, and elongation percentage.

For the application of the rheometer, safety rules for the proper operation of the machine must be followed, remember to turn on the compressor connected to the rheometer and check that pressure level is at 30 psi, and turn on the heat exchanger to avoid any damage by the elevated temperature. Afterward, open the rheometer application in the computer and chose the type of geometry to be used; in the current case, a rectangular geometry is used since the device does not have a specific option for fibers. When the geometrical plate (rectangular) is static to the base of the electromagnet of the rheometer, perform the calibration of the equipment in this geometry. Following the calibration, load the samples between two plates of similar geometries, program and execute the experiment from the computer application to obtain the different mechanical parameters (tensile strength, percentage elongation, modulus of elasticity) of the fiber. This application exerts a rotational shear stress on the material, and stress or strain index (shear rate) is measured.

Hybrid rheometers and viscometers share the same principle of operation, but a hybrid rheometer has better sensitivity. This advantage is most evident in the precision and range in which shear stress can be applied, its support for oscillatory tests and the degree of control over normal force applied during testing.

4.5 Biodegradability Test of Fibers

Biodegradability is the capacity of the material to degenerate into smaller compounds and then in very simple compounds such as carbon dioxide, water, and oxygen (Pillai &

Sharma, 2010). When performing a biodegradability test, an important parameter is obtained for the evaluation of surgical thread behavior, in addition to predicting the long-term risks and the interaction with the human body.

In this research, a simple method was implemented to measure fiber biodegradation with potential function in the medical suture. First, 20 - 30 mg of the fibers were placed in 250 mL of water at a temperature of 37 °C in the incubator (three replicates were made for each fiber). The measurements were taken on a weekly basis to evaluate the weight loss of the fiber; this process was carried out for 3 weeks. Finally, the weight loss of each sample was evaluated using the following formula:

$$Wt = \frac{W_O - W_{(t)}}{W_o} \times 100$$

where:

W(t) = the total weight after time t (1st day, 1st week and 3rd week)

Wo = the initial reference dry weight of fiber before biodegradation (Siddiquee & Helali, 2014).

4.6 Statistical analyzes

This research is defined as an independent variable that will be controlled to the variable effects on the dependent variable. Independent variables: Temperature of 37 C, the weight of the fibers in each treatment, 250 mL of water in the samples, the use of the same type of agar, bacteria *(E. coli*) and antibiotic (ampicillin).

The dependent variables that will show the results in this research will be the mechanical characteristics of the fibers, UTS and Young's modulus, the rate of degradation of the fibers, the percentage of weight loss of the fibers and the antibacterial property.

For the statistical analysis, we used t-student with the significance level of $p > 0.05$; this test was applied to compare if there is a meaningful difference between the means of the mechanical test parameters of the F2 and F4 fibers compared to the silk. Additionally, to compare the biodegradability properties between fibers F2 and F4, IBM SPSS software was used as needed and regression analysis was performed using Microsoft Excel.

4.7 Antibacterial Test of Fibers

The method used to examine the antimicrobial capacity of fibers was agar diffusion. This technique serves to determine the antimicrobial activity involved in the scaffold, fibers, drugs, and solution (Hauck, Allen, Lees, Rowe, & Verran, 2010). For this research, the agar diffusion test is applied because it is qualitative, easy carry out, manageable, inexpensive and the materials are available in the laboratory.

In the agar diffusion method in vitro, *E. coli* Gram-negative bacteria were used for their availability in the laboratory. On the other hand, according to an investigation made in India, it was found that the most common bacteria that trigger surgical site infections (SSIs) are *S. aureus* (Gram – positive) (50.4%) was the most common organism, followed by *E. coli* (Gram - negative) (23.02%), Pseudomonas aeruginosa (7.9%) and Citrobacter (7.9%) (Negi, Pal, Juyal, Sharma, & Sharma, 2015). Another study in Saudi Arabia indicates that the bacterial infections in the surgical site are produced by organisms, such as *S. aureus* (gram – positive) (16.1%), *E. coli* (gram – negative) (12.9%), and *Acinetobacter baumannii* (9.6%), and were highly associated with surgical wound infections (AL Aali, 2016) (Subrata, 2016). Due to its high infection rate in surgical sutures and the availability in the laboratory, we worked with *E. coli*.

Bacteria cultures are diluted in single concentration and spread on Petri dishes with nutritive agar to create a lawn or layer following, a procedure that is commonly used for substances, but it can be applied to the fibers by impregnating them with a 400 uL of bacteria (bacteria is proliferated with 2 ml medium and 100 uL of bacteria for 24 hours).

Three – six small samples of the each type of fiber are placed in a Petri dish (prudentially separating one from the other) (Dwivedi et al., 2017). A plate was made for each fiber (F2 and F4), each sample was labeled to clearly define the zone of inhibition (area where the bacteria do not grow). As control of the experiment, 3 µL of antibiotic (ampicillin) diluted to 10^{-3} in ultrapure water were spread on the same plate but far from the samples to determine a control zone (Byrne, 2007).

Then, the plates were incubated for 18 to 24 h at 37 ° C and, subsequently, the growth of bacteria was determined qualitatively, that is, by the presence of antimicrobial activity in each Petri plate (Tendencia, 2004).

5. Results

5.1 Fiber Characterization

The surface structure and morphology of the different cellulose fibers were examined by using a MIRA 3 (TESCAN, CZ) field emission scanning electron microscope (FEG-SEM). As displayed in Fig 1, the external structure of F2 fibers is porous, irregular and rough, forming internal spaces in its structure. On the other hand, in Fig 2, the morphology of F4 fibers is uniform, compact and does not show any porosity.

Figure 1: SEM morphologies of fibers F2.

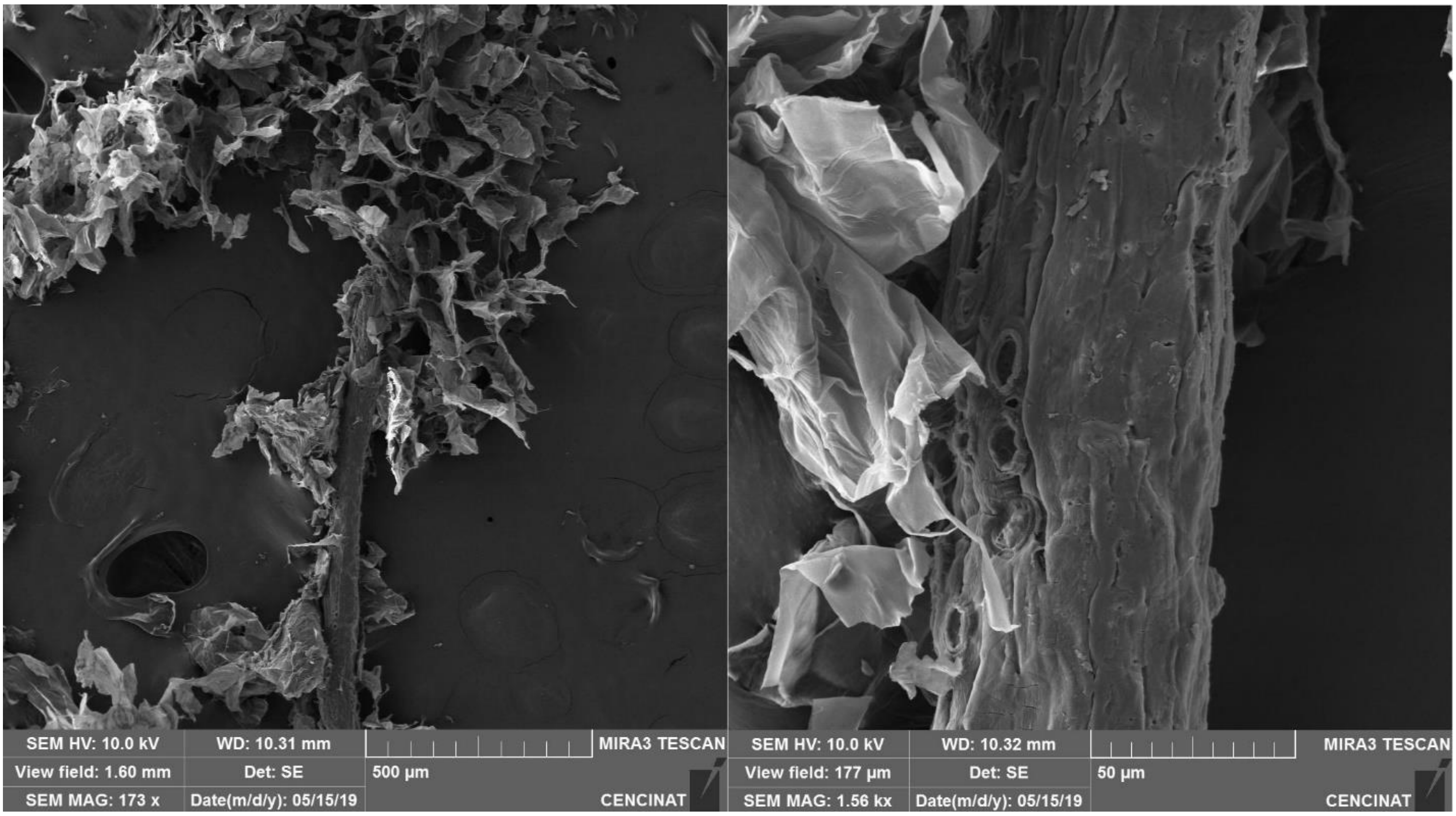
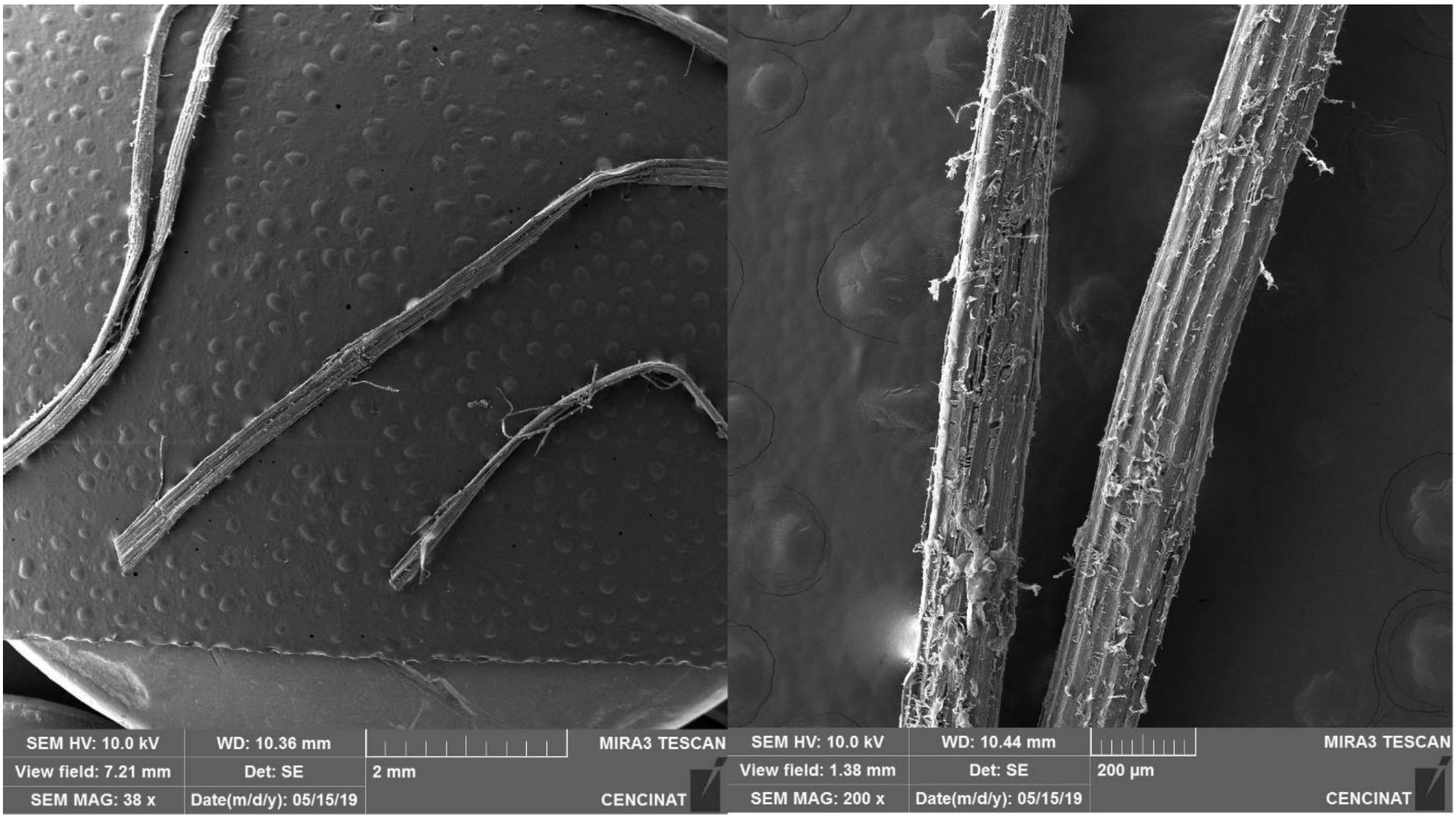


Figure 2: SEM morphologies of fiber F4.

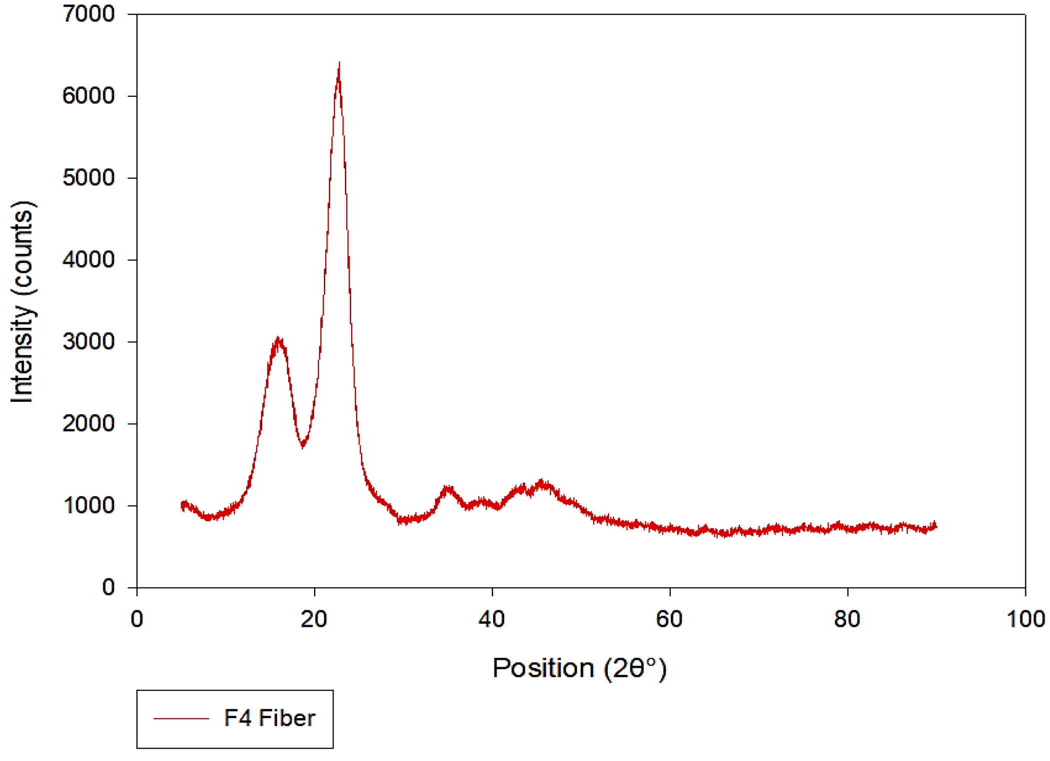


In general, cellulose includes crystalline phase and amorphous phase. The crystallinity of vegetal fibers F2 and F4 was analyzed on an EMPYREAN diffractometer (PANalytical, NL) in a Bragg-Brentano configuration at 40 kV and 45 mA and monochromatic X Rays of Cu K-α wavelength ($\lambda = 1.541$ Å) using a Ni filter.

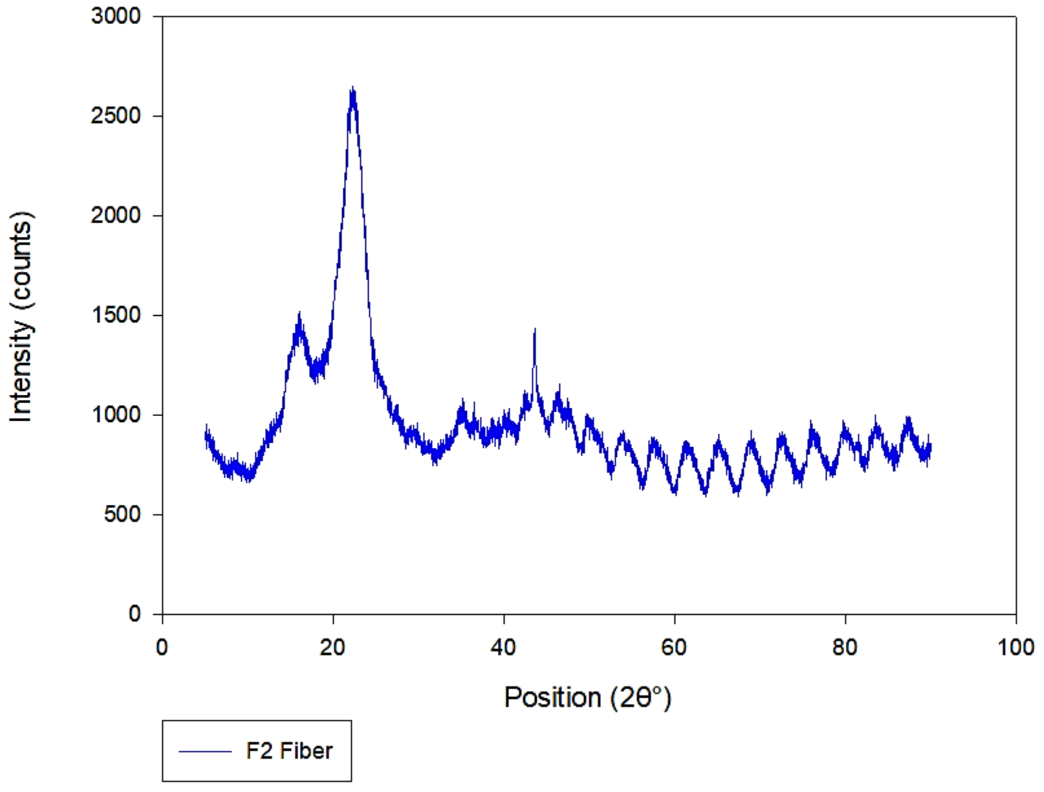
The X-ray diffraction patterns and the peaks observed in results of the F2 and F4 samples are shown in Graph 4 and Graph 5. Both graphs show well-defined main peak around $2\theta = 22$ °.

When carrying out the calculations of the crystallinity index for each fiber, it was found that fiber F2 had a crystallinity index of 56.04%. In the XRD pattern of the F4 fiber, there was a sharper peak and a higher crystallinity index of 73.65%, which meant the elimination of the amorphous phase and excellent mechanical properties of the F4 fiber.

Graph 4. XRD pattern of F4 Fibers.

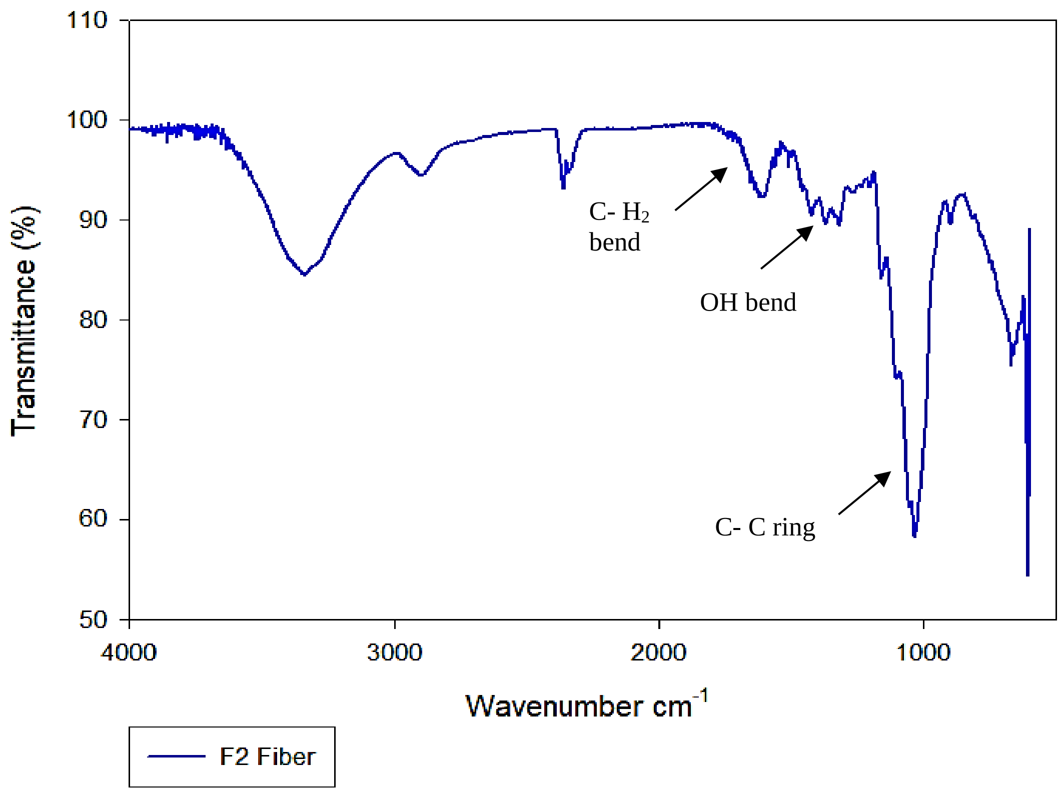


Graph 5. XRD pattern of F2 Fibers.

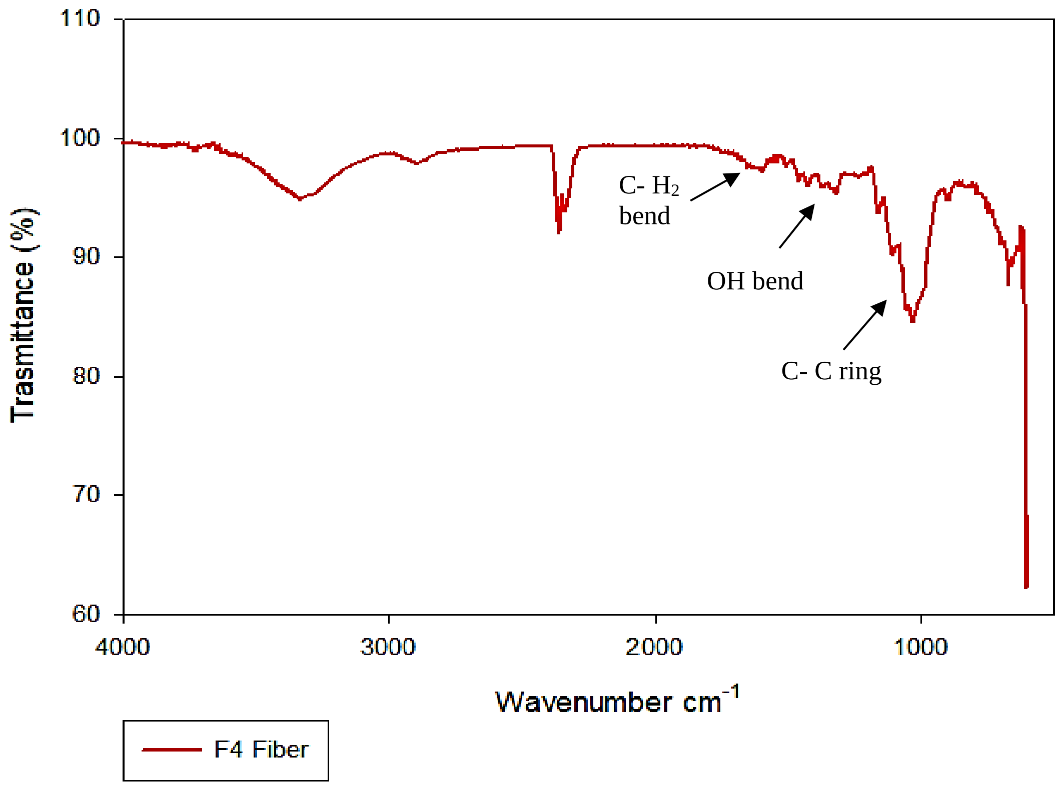


The Fourier Transform-Infrared (FTIR) spectra in Graphs 6 and 7 indicate characteristic peaks for cellulose, including C-C, C-OH, C-H ring and side group vibration bands which clearly arise at ~1100 cm^{-1}, and C-O-C glyosidic ether band notably appear at ~1150 cm^{-1}. Additionally, important peaks are evident at ~1310 cm^{-1}, ~1630 cm^{-1}, ~2900 cm^{-1}, and ~ 3300 cm^{-1} which correspond to OH bending, CH_2 rocking vibrations at C6 band, sp3 C-H stretching and OH stretching frequencies, respectively. Based on other researches (Auta, Adamus, Kwiecien, Radecka, & Hooley, 2017) (Song & Hinestroza, 2012), the FT-IR spectra of F2 and F4 are similar to commercial cellulose spectra, it confirms that both fibers are mainly composed of cellulose and do not contain hemicellulose or lignin residual contaminants, therefore, the purification method was effective.

Graph 6. FTIR spectrum of F2 Fiber.



Graph 7. FTIR spectrum of F4 Fiber



5.2 Fiber Diameter

The diameter or cross-sectional area of the fibers was estimated by an optical microscope in 4x and graph paper (reference of 1 mm/square). With these tools, the approximate diameter of each fiber was calculated with the GeoGebra program. Table 10 shows approximated diameters of the 8 samples of F2 and F4 fibers:

Table 10 Diameter in mm and USP designation of the samples of F2 and F4 fibers

Type of Fiber	**Diameter (mm)** (± 0.01 mm)	**USP designation**
F4 Fibers		
Sample 1	0.14	5-0
Sample 2	0.11	4-0
Sample 3	0.12	4-0
Sample 4	0.10	4-0
F2 Fibers		
Sample 1	0.31	2-0

Sample 2	0.29	2-0
Sample 3	0.71	5
Sample 4	0.36	0

The average diameter of the two types of the plant fibers were calculated. For F4 fibers, they were found to be fibers of more uniform longitudinal diameter with a very short standard deviation of 0.017 mm and have a mean diameter of 0.12 mm (4-0 USP), unlike the F2 fibers that have a less uniform diameter with a standard deviation of 0.197 mm and a mean diameter of 0.42 mm (1- USP) larger than F4 fiber.

Figure 3. Optical microscopy of the four samples of F4 fibers to measure the approximate diameter.

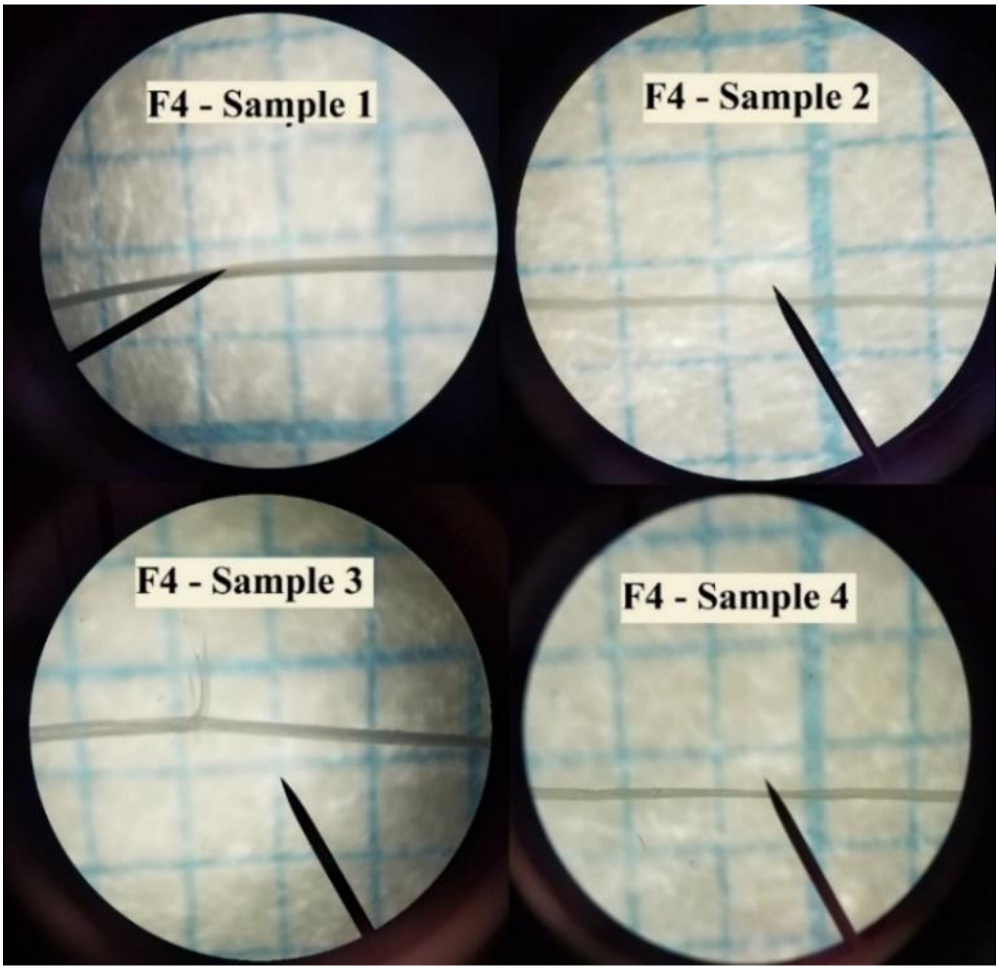
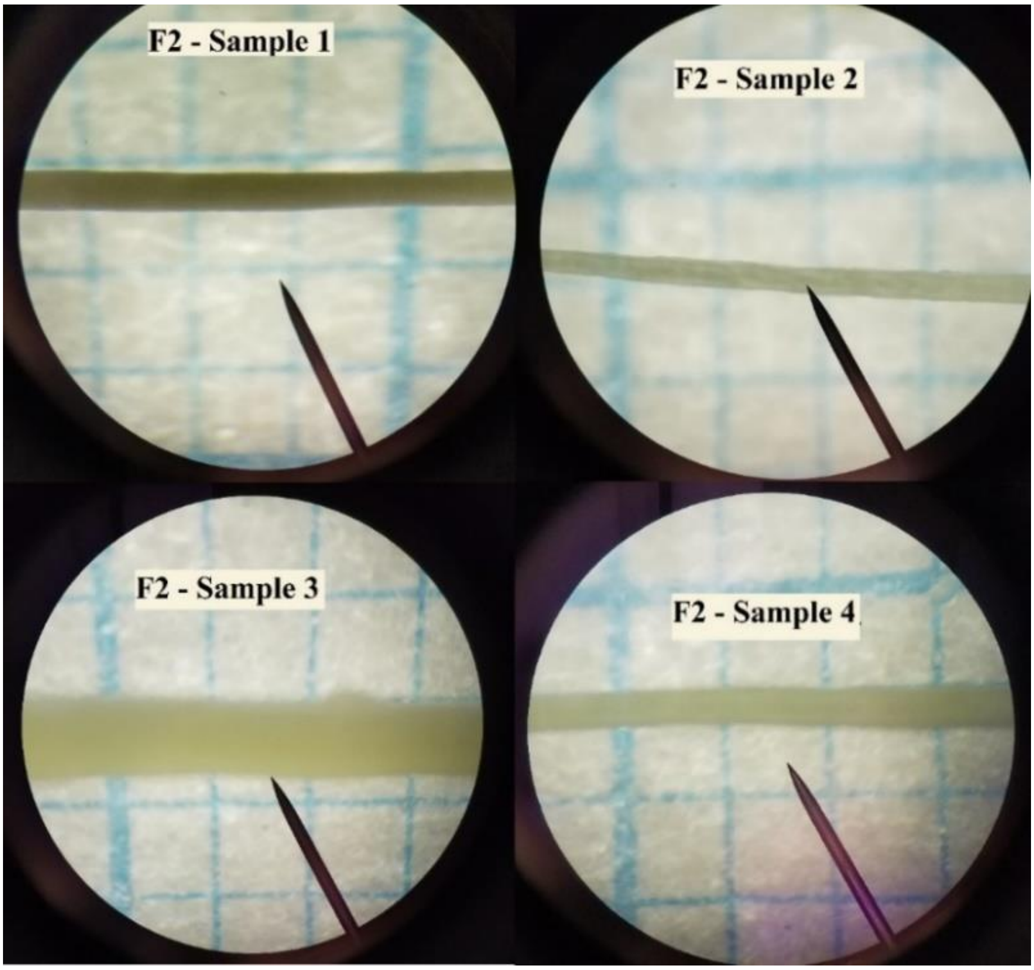


Figure 4. Optical microscopy of the four samples of F2 fibers to measure the approximate diameter.



The diameter of the fibers (Table 10, Fig 1, Fig 2) was required information for the derivation and calculation of mechanical properties described in the mechanical test, as in the stress vs. strain graphs where stress was calculated with the cross-sectional area (diameter) of the fibers.

5.3 Mechanical Tests

The rheometer resulted in raw tabulated data, with an axial force in Newton and Strain in mm, which were sorted, processed and plotted for the analysis of the mechanical properties. According to the manual, the uncertainty of the DMA mode of the axial force in the rheometer used in this investigation is 0.001 N.

In this research, the results were expressed in engineering stress-strain graphs, that consider the original cross-section and original length of fibers as data. Through Graphs 8 and 9 the mechanical properties are obtained, this research focuses on the two important parameters, ultimate tensile strength and Young's modulus.

For F4 and F2 fibers, an average of their mechanical properties was realized based on Annex B data and showed in Tables 11 and 12. According to the results in Tables 11 and 12 previously obtained based on Graphs 6 and 7 and explained in the introduction (mechanical properties). The F4 fiber has a mean ultimate tensile strength of 138.84 MPa ± 86.15, a mean elongation percentage of 2.37% ± 1.32 and mean a Young's Modulus (E) of 2.76 GPa ± 2.94, in the case of F2 fiber, it has a mean ultimate tensile strength of 18.72 MPa ± 8.72 an elongation percentage of 22.77 % ± 7.16 and mean Young's Modulus (E) of 0.04 GPa ± 0.02. The F4 fibers have a large variation between samples, UTS, and Young's modulus are considered larger than the F2 fibers results.

Table 11. Statistical analysis (mean, standard deviation and statistical significance) of main mechanical properties of F2 samples.

Parameters	Silk		F2 Fibers		Sig. prob
	Mean	Std. Deviation	Mean	Std. Deviation	
UTS (MPa)	217.55	± 39.58	18.72	± 8.10	0.00
Total Elongation (%)	23.60	± 6.61	22.77	± 7.16	0.87
Young's Modulus (GPa)	5.00	± 1.11	0.04	± 0.02	0.00

Table 12. Statistical analysis (mean, standard deviation and statistical significance) of main mechanical properties of F4 samples

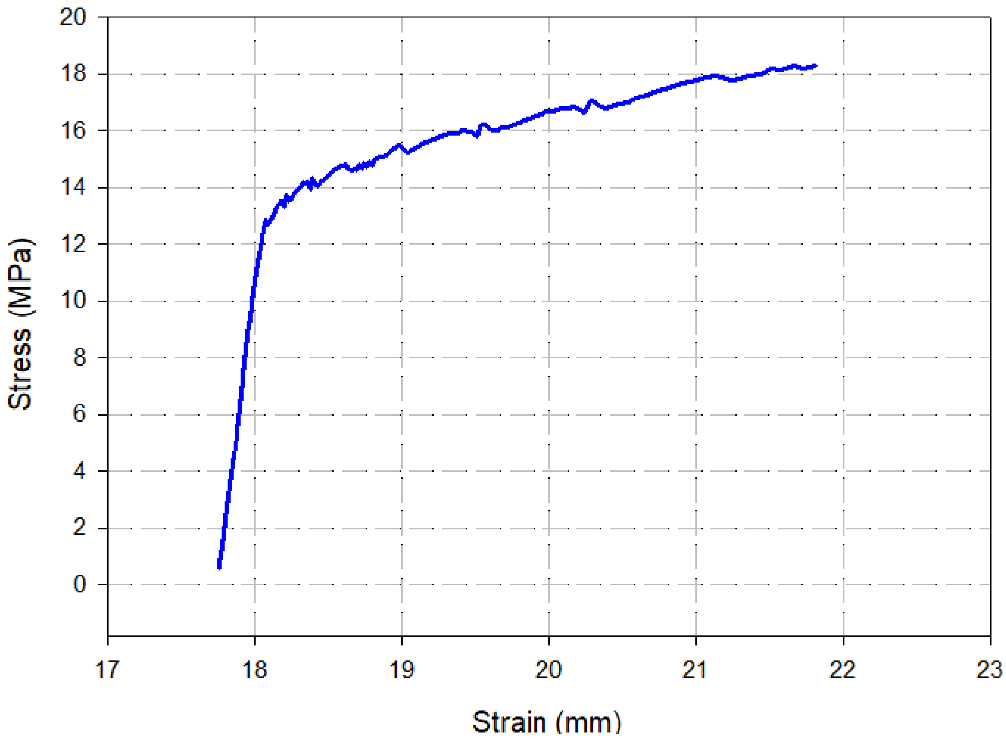
Parameters	Silk		F4 Fibers		Sig. prob
	Mean	Std. Deviation	Mean	Std. Deviation	
UTS (MPa)	217.55	± 39.58	138.84	± 86.15	0.15
Total Elongation (%)	23.60	± 6.61	2.37	± 1.32	0.00
Young's Modulus (GPa)	5.00	± 1.11	2.77	± 3.40	0.26

In Tables 11 and 12, The t- student test results show there is a significant difference between the means of the mechanical parameters of the F2, F4, and silk fibers. Previously, it was defined level significance of probability is greater than 0.05 ($p > 0.05$), in other words, if sig. p is equal to or less than 0.05, there is a statistically significant difference between the two means of the evaluated parameters, in other words, the two means are not comparable enough to have the same characteristics. When there is a smaller the p-value, it is evidence that means are divergent and statistically prove that the means are different.

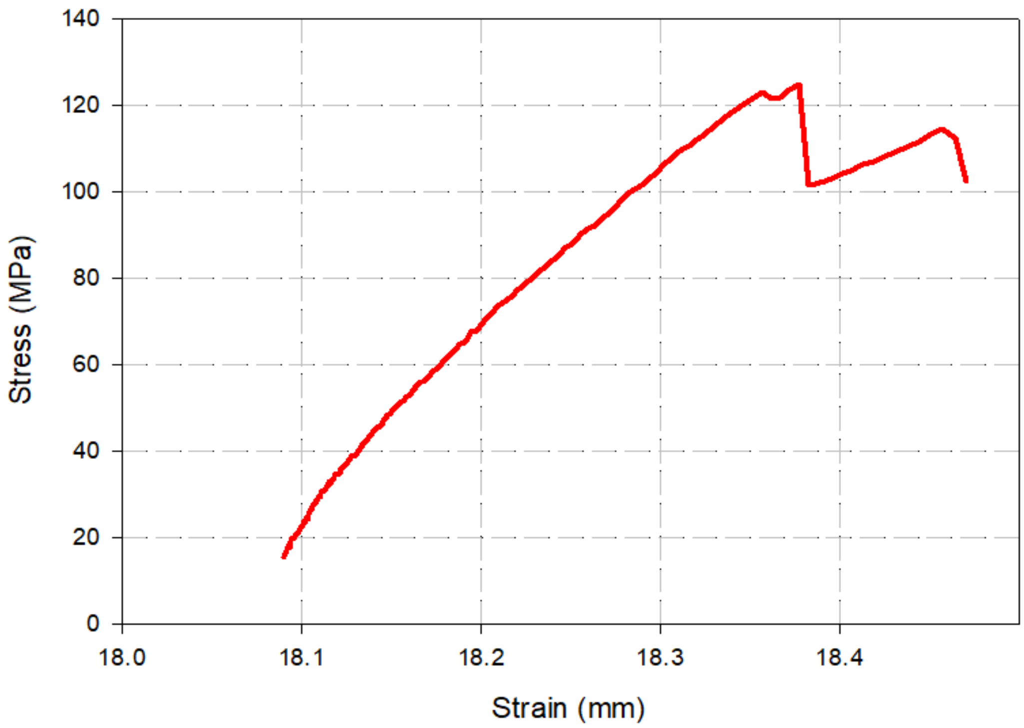
When comparing the main mechanical parameters of F2 and silk fibers, the probabilities of UTS and Young's modulus are $p=0.00$ showing a low significance, therefore, a noted difference between the means. Only one parameter has a mean comparable to the silk, percentage of elongation with a p-value of 0.87, considerably close to the probability of 1.00 (the greater the probability, the more similar are the means of the measured characteristic), that is, it is very near to the expected results of silk.

The main mechanical parameters of the F4 and the silk fibers were compared. The probabilities of UTS and Young's modulus of 0.15 and 0.26 respectively are calculated, showing a correlation between the means, therefore a similarity between the calculated parameters. The only parameter that does not approximate the characteristics of silk is the percentage of elongation with a p-value of 0.00, that is, it is very away from what was expected in the percentage of elongation silk.

Graph 8. The Relationship between stress vs strain (load and elongation) average for F2 fibers

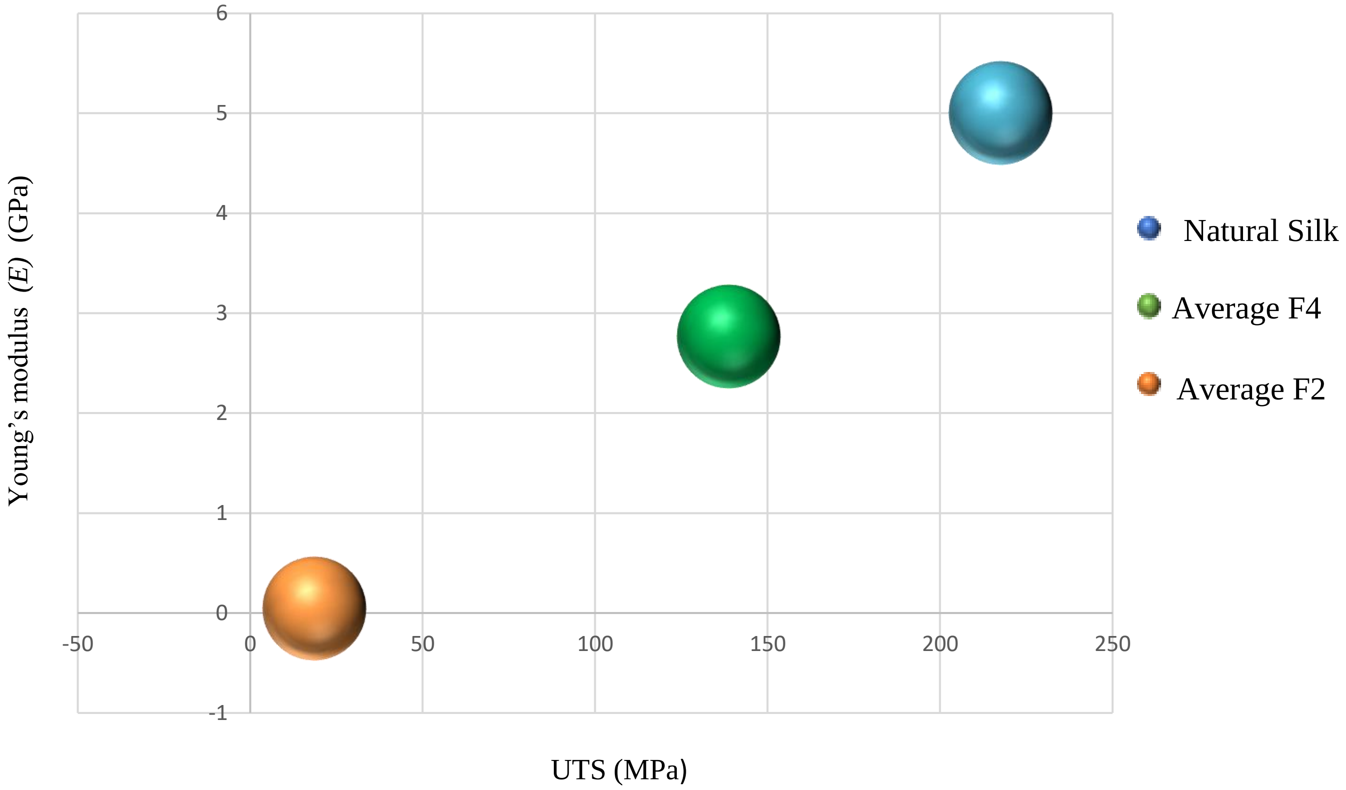


Graph 9. The Relationship between stress vs strain (load and elongation) average for F4 fibers.



In Graph 10, the comparison of UTS vs Young's Modulus of the samples of F2 and F4 fibers with natural silk fibers (raw material for the sterilized silk suture) was performed. It is evident that the samples of F4 fibers are the closest to the mechanical properties of silk, in contrast with the average of F2 fibers that are distant from the expected results with a statistically significant difference.

Graph 10. Comparison of UTS (MPa) and Young's Modulus (GPa) of natural silk fibers with average of F4 and F2 fibers.



5.4 Biodegradability Test

Weight loss measurement of the fibers were carried out before the purification treatment and after the purification (Table 13), evidence that there was a decrease in their weight due to the elimination of impurities from the fibers, only preserve cellulose in their structure. For F4 and F2 fibers, their average weight loss was 18.5% and 28%, respectively, after purification treatment, F2 having the greatest weight loss after its purification. Once the treatment is finished, the measurements of the weight loss were made one day later, one week and three weeks after the procedure was started.

Based on the purified fibers, it was measured over a period for three consecutive weeks (Graph 9). The analysis of the behavior of the fibers can be evaluated taking as a basis that initial weight is 100%. When analyzing F2 fibers, a greater weight loss is observed between the first day and the first week with an average percentage of loss of 62.01% and stabilizes in the third week of treatment with a percentage of loss much lower than 3.94%. When analyzing the behavior of F4 fibers, their slow biodegradation with a mean weight loss on the first day and in the first week of only 17.26%, F4 fibers stabilized in the third week with a percentage of weight loss of only 4.05%. Clearly, F2 fibers degrade faster than F4.

In Table 13, the weight loss of the fibers in milligrams is calculated, the purified F2 fibers had an average total weight loss of 16.2 mg which represents 65.95% from the initial weight (after the purification), weight loss of F2 fibers is greater than the total mean weight loss of the F4 fibers of 5.4 mg representing 21.31% from the initial weight (before the purification), F2 fibers present twice more weight loss than F4 counterparts.

Table 13 shows the average weight loss and its statistical analysis, evaluating the biodegradability of the fibers. At the beginning of the experiment, before the purification of the fibers, the two types of fibers have a significance probability greater than 0.05 ($p = 0.063$); after of the first day of treatment, a decrease in their significance probability is noted, which means a statistically significant difference between the means of the F2 and F4 fibers after their purification.

As result, after a period of 21 days, the comparison between two types of fibers presents a significant probability of 0.05, that is, the means are different.

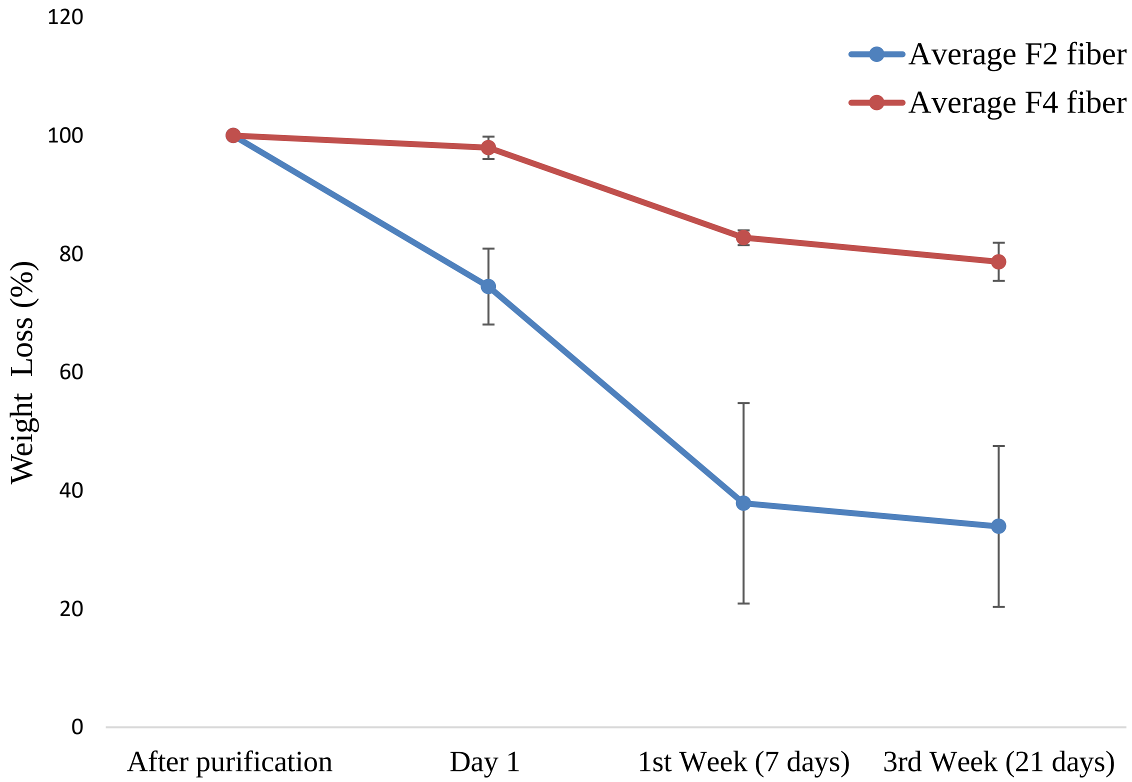
Table 13. Average weight loss of F2 and F4 fibers in a period of three weeks of treatment.

Parameters	**F2 Fiber**			**F4 Fiber**			**Sig. prob**
	Mean	**Std. Deviation**	**% Weight**	**Mean**	**Std. Deviation**	**% Weight**	
Weight initial / mg (Before purification)	34.27	1.95	-*	31.37	0.20	-*	0.06
Weight initial / mg (After purification)	24.57	0.32	100	25.50	0.36	100	0.03
Weight / mg	18.30	1.64	74.49	24.97	0.15	97.9	0.00

1 day							
Weight / mg 1st Week 7 days	9.33	4.26	37.99	21.10	0.60	82.74	0.01
Weight / mg 3rd Week 21 days	8.37	3.43	34.05	20.07	1.08	78.69	0.01

* Based on after purification

Graph 11. Average of percentage weight loss of F2 and F4 fibers in a period of three weeks of treatment.



5.5 Antibacterial Tests

The antibacterial tests were performed in triplicate using complete fibers and a dilution of the fiber material to determine the ability of the fibers to limit pathogenic growth. As shown in Fig 3 and Fig 4, a negative response was obtained for the test, that is, there was not inhibition against the bacterium *E. coli* Gram-negative. In Fig 3, it is clearly observed that there isn't bacterial inhibition of the fibers that mimic the same behavior when placing the antibiotic. In addition, in the bacterial growth test of F2 fibers are observed a bacterial plaque around the fiber (bacterial attachment), contrasting the

F4 fibers didn't have any direct bacterial plaque adhered to the fiber, but it showed a bacterial growth in the plaque (Fig 4).

Figure 5. Antibacterial Tests with fiber samples F2 and F4.

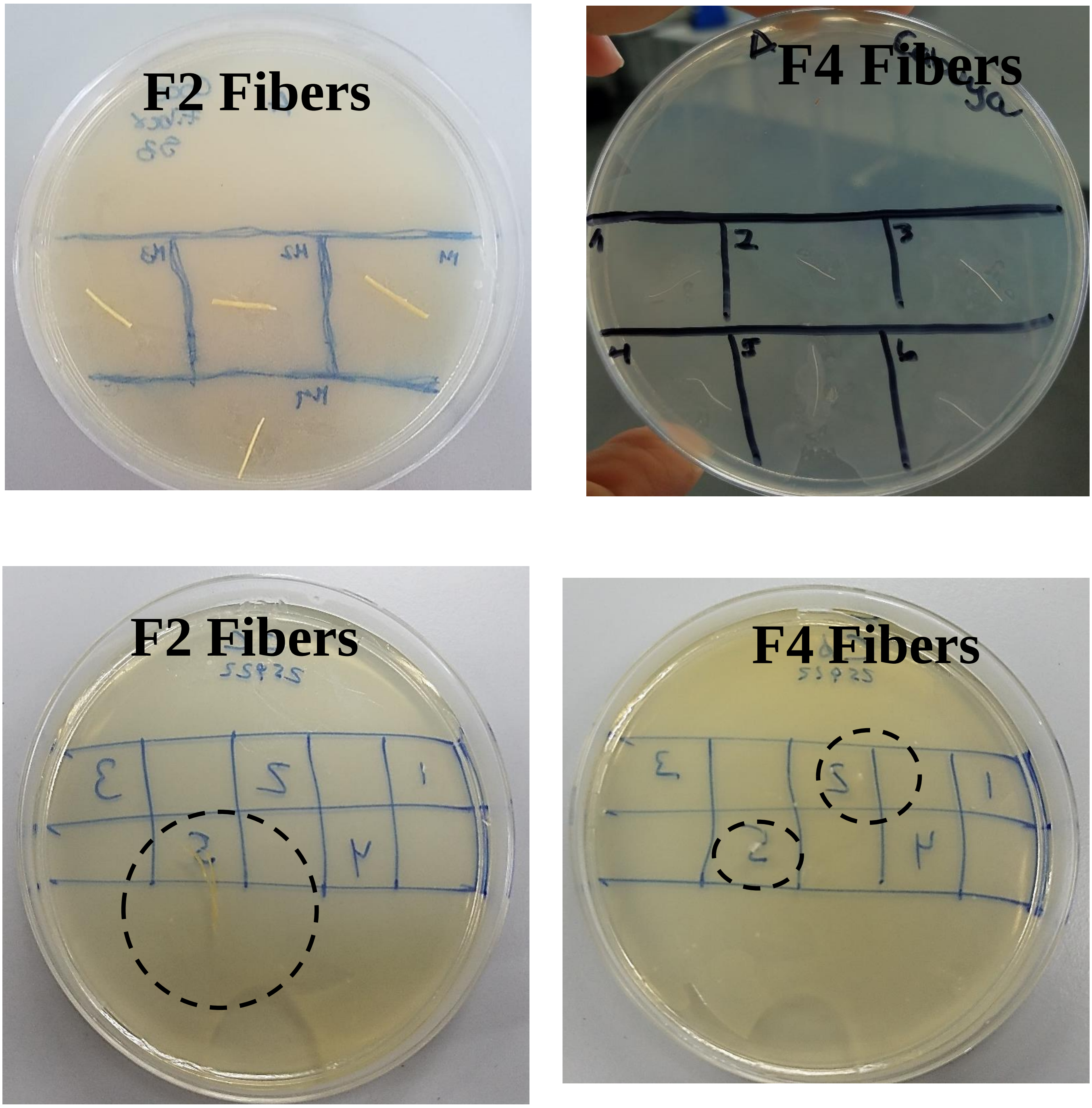
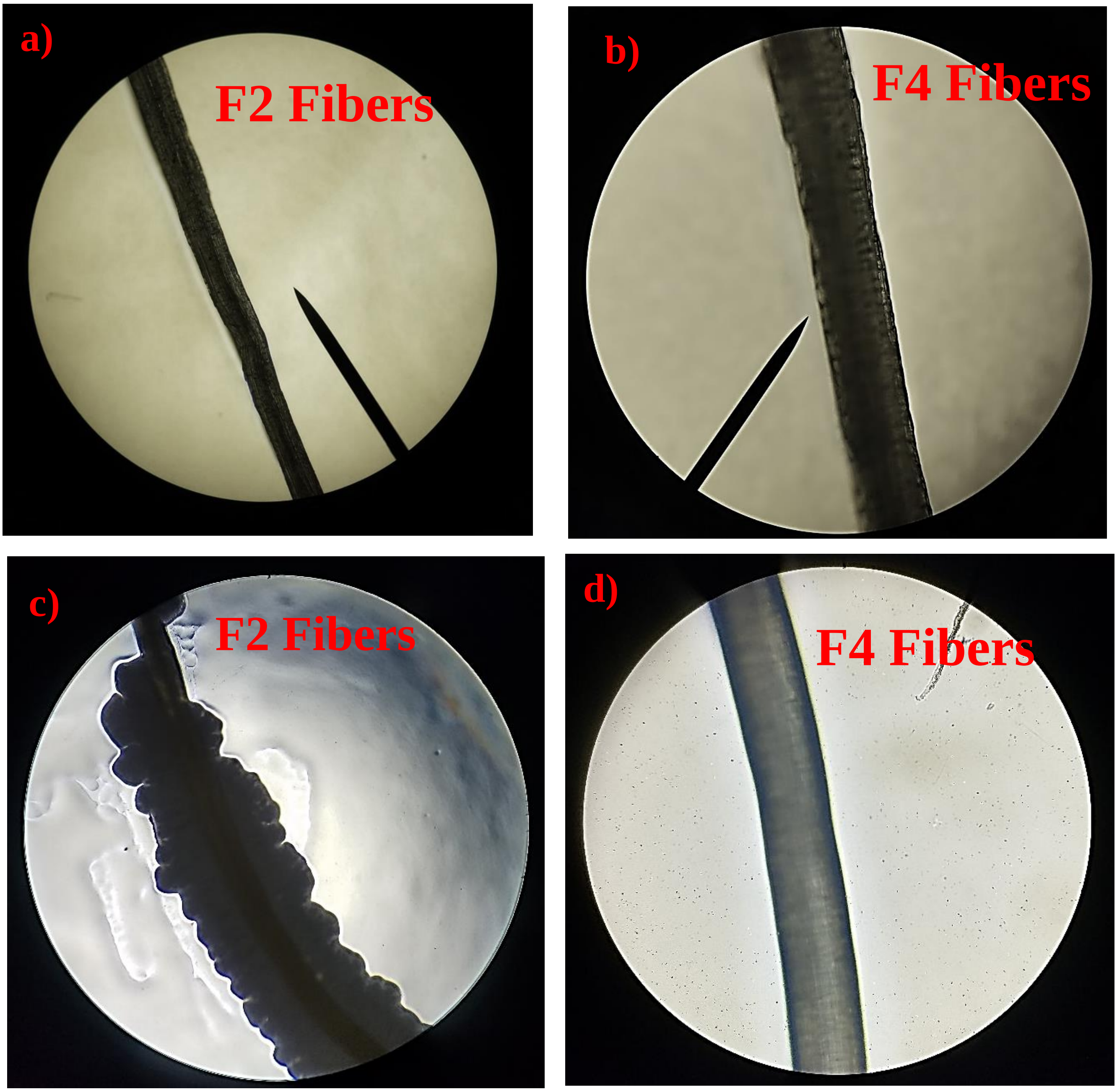


Figure 6. Antibacterial test F2 and F4 observed in the optical microscope.



a) and b) Fibers F2 and F4 before performing the antibacterial test. c) and d) F2 and F4 fibers after performing the antibacterial test.

6. Discussion

In the characterization of F2 and F4 fibers, a very high level of crystallinity was obtained for F4 (73.65%) in comparison with F2 fibers (56.04%), it could show excellent mechanical properties due to its high crystallinity. In contrast with the silk, both fibers show crystallinity indexes close to silk. The commercial sutures are characterized by having a high crystallinity index, an example is Dexon (Poly Glycolic Acid) that presents a highly crystalline (around 45-55%) stimulating excellent mechanical properties in the fibers (Pillai & Sharma, 2010). Therefore, the F2 and F4 fibers have crystallinity indices like those expected from a commercial surgical suture.

In Table 14, the mechanical properties of the F2 and F4 fibers were compared with a natural source of surgical sutures, such as silk, due to its commercial use in the surgical area and its appropriate mechanical properties mentioned above. Based on this, the UTS and Young's modulus were considered as key properties to assess fiber strength. The F4 fibers showed results closer to the UTS and Young's modulus of silk, moreover, a lower elongation percentage. F2 fibers didn't exhibit the mechanical characteristics needed to mimic the surgical suture, with a UTS and Young's module statistically different the properties of silk (control fiber). (Table 10).

The fiber structure (monofilament or multifilament) is essential to explain the mechanical and antibacterial properties of the F2 and F4 fibers. The monofilament structure of F2 fibers did not provide enough resistance to support mechanical tests efficiently, establishing them as fragile fibers. Another factor is the fibers purification since it could eliminate certain components that favor better resistance and elasticity for the F2 fibers, or the chemical structure of the fibers does not favor an adequate resistance to maintain the suture. Moreover, the mechanical properties depend on the amount of crystal structure, the crystalline phase is ordered and with high cohesion, contrary to the amorphous phase that is in disorder. Therefore, any applied stress will be very concentrated in the weak phase, so if fibers have higher crystallinity index, its mechanical properties will be excellent to suture application and it will increase its resistance (Songa, Wanga, & Wanga, 2016). The F4 fiber has a higher crystallinity index than F2 fibers

(73.65% and 56.04% respectively), then based on literature its mechanical properties are more effective than the mechanical properties of fiber F2 by its crystallinity.

Table 14 Summary table of mechanical properties, biodegradation, and antibacterial capacity for F2, F4, and silk

Parameters / Fibers	**Crystallinity index (%)**	**Average Mechanical Properties**		**Average Biodegradability (% Total weight loss)**	**Antibacterial Capacity (Yes/Not)**	**Bacterial Plaque formation (Yes/Not)**
		UTS (MPa)	**Young's Modulus (GPa)**			
Natural Silk	58 – 64 (Chung & Um, 2014) (Bhat & Nadiger, 1980)	217.55	5.00	0.60	No	Yes
F2	56.04	18.72	0.04	65.95	Not	Yes
F4	73.65	138.84	2.76	21.31	Not	Not

The biodegradability of F4 fibers have a slow behavior that resembles commercial absorbable sutures with a slow degradation of 21.31% weight loss, F2 fibers have a biodegradability of 65.95 % over the period of three weeks that could be explained by the lack of certain components eliminated in the purification of fibers or the method could weaken the fibers since in table 16 (Annex B) have same weight before purification. The rate of degradation of fiber should correspond to the healing rate development (Jasmine & Mandal, 2014). In the case of F2 fibers have an accelerated biodegradability that could be an obstacle to the application in the field of surgical suture since it will not provide enough recovery time long for the closing of the wounds.

The degree of crystallinity is critical factor that affects the biodegradability, the amorphous domains of a polymer or fiber are more prone to degradation and are harmed by the enzymes that go through the hydrolysis decomposing the fibers, so the part crystalline polymers are more resistant than their amorphous regions. With an increase in the crystallinity of the polymer decreases the degradation rate (Tokiwa, Calabia, Ugwu, & Aiba, 2009). Based on the crystallinity index, the F4 fibers have a slow degradation rate by their greater crystallinity degree (73.65%) avoid an accelerate degeneration. In the case of F2 fiber have a lower crystallization index (56.05%) causing faster degradation than F4 fibers.

The natural fibers have fluctuating moisture absorption behavior, since they have diverse interfacial bond strengths and several structures, for example, the porous configuration of bamboo fibers absorb a higher amount of moisture than common fibers such as hemp, kenaf, and flax fibers (Al-Maharma & Al-Huniti, 2019), this increased permeability and interaction with the water, causes a higher response rate biodegradability. Previous research has shown that biodegradability of the fibers depends on molecular weight and structure, if fibers have a low molecular weight and non – compact structure are more susceptible to enzymatic hydrolysis (Funabashi, Ninomiya, & Kunioka, 2009) (Zuo, Dai, & Wu, 2006). Building on these previous studies, the morphology affects to the biodegradability of fibers, in Figure 1, F2 fiber has an irregular structure with possible porosity, interspaces and roughness in its configuration, this type of morphology can origin a better permeability and a higher biodegradation rate. Therefore, water can enter and interact with fiber more easily than fiber F4, which in Figure 2 clearly shows a more compact structure without internal spaces in its morphology.

Moreover, the F4 and F2 fibers have a biodegradability more compatible with the absorption and degradation time of an absorbable suture. According to table 7 and 8, the absorption times must be at an average of 98 days or approximately 3 months, subsequently the percentages of loss of the weight of the fibers are in a maximum of 65% in 4 weeks. When compared to the silk surgical thread that is considered a nonabsorbable suture, it has a low biodegradability rate with a percentage of weight loss at 4 weeks of only 0.6%, contrasted to fibers F2 and F4 are not present a percentage close to the biodegradability of silk, so they could be defined as possible absorbable sutures.

The role of suture material as a contributing factor to SSIs has been the subject of research since the 70s, Osterberg and Blomstedt investigated that multifilament suture material is prone to produce biofilm, bacteria tend to be protected from the phagocytic activity of leukocytes through their enclosure in the interstices of the material. Therefore, this type of material can sustain and extend an infection (Kathju, Nistico, Tower, Lasko, & Stoodley, 2014). The suture structural is a crucial parameter that influences adhesion of bacteria, roughness on a nanoscale has been shown to be beneficial for pathogen adhesion biomedical material (Dhom, Bloes, Peschel, & Hofmann, 2017), the silk surgical threads due to its structural characteristics, it causes a bacteria accumulation between the grooves of its multifilament thread and at the same time an increased risk of infection. F2 fiber, with similar bacterial attachment property to silk thread, favored the formation of bacterial plaque/accumulation which can be explained by its morphology, in Figure 1 is clearly observed a porous structure with internal space in the fiber where bacteria easily can host, so F2 fiber being more prone to the production of biofilms. The F4 fibers prevented the accumulation of bacteria consequently they could avoid possible infections. This advantage is possibly originating by its smooth, compact and non-porous surface observed in SEM morphology (Figure 2).

Another parameter that interferes in the bacterial adhesion is the crystallinity of the suture. It has been shown that the formation of biofilm is affected by the crystalline phase on the surface of the biomaterial. In some investigations, it has been shown that a crystalline layer has reduced the bacterial attachment with respect to an amorphous layer, without disadvantageous effects on the cell metabolic activity (Lorenzetti, Stopar, Kalin, & Kobe, 2015). The high crystallinity of fiber F4 helps prevent the accumulation of bacteria on its surface, on the other hand, fiber F2 has a lower index of crystallinity, where its amorphous region favors the growth or formation of a biofilm.

The antibacterial test was negative for the two types of fibers F2 and F4 (Table 14), the silk as mentioned in the bibliography does not have an antibacterial potential, but researchers added certain components to produce an antipathogenic effect, some of these techniques could be used to improve the antibacterial capacity of fibers F2 and F4, for example add Nano-Ag-loaded SiO_2 antibacterial agent or coating of antibacterial substances by immersion methods as levofloxacin hydrochloride and poly ε-caprolactone (antimicrobial agents) (Xiaoli Chen & Wei, 2014) (Wang & Zou, 2015).

7. Conclusion

This research study on surgical suture based on natural vegetable fibers as an alternative to conventional synthetic and natural surgical suture, evaluating mechanical characteristics, biodegradability and antibacterial capacity (qualitative test) of two types of natural fibers called F2 and F4.

The proposed hypothesis was partially demonstrated due to its multiple parameters, the results obtained showed that F4 fibers have better mechanical properties. The F4 fiber has a mean UTS of 138.84 MPa ± 86.15, a mean elongation percentage of 2.37% ± 1.32 and mean Young's Modulus (E) of 2.76 GPa ± 2.94, in the case of F2 fiber, it has a mean ultimate tensile strength of 18.72 MPa ± 8.72 an elongation percentage of 22.77

% ± 7.16 and mean Young's Modulus (E) of 0.04 GPa ± 0.02. Based on reference material to silk, the mechanical properties of F4 fibers are those expected for use in the area of surgical suture. The F4 fibers have a large variation between samples, UTS, and Young's modulus; their standard deviations are considered larger than the F2 fibers results.

F2 and F4 fibers are biodegradable, F2 fibers degrade with a weight loss percentage of 65.95% and F4 fibers lose 21.31% of their initial weight. F4 fibers have a degradation rate similar to absorbable sutures, it is considered that F2 fibers have a very high biodegradability to resist tissue recovery time.

The last property evaluated is the antibacterial capacity of F2 and F4 fibers. The literature indicates that there is no natural surgical suture with this property, but it can be improved with the use of added antimicrobial agents. The F2 fibers were negative in the antibacterial tests, bacterial growth and the creation of a biofilm adhered to the fiber was observed. On the other hand, although the F4 fibers are negative to possess an antibacterial property, they did not show bacterial attachment in their fibers.

Therefore, it is concluded that the F4 fibers are the most optimal and the most appropriate option for use in the field of surgical suture, due to its properties close to natural sources such as silk used commercially and no bacterial adhesion. In addition, these fibers can be useful in other areas of research as a biomaterial for the textile, petrochemical or mechanical industry.

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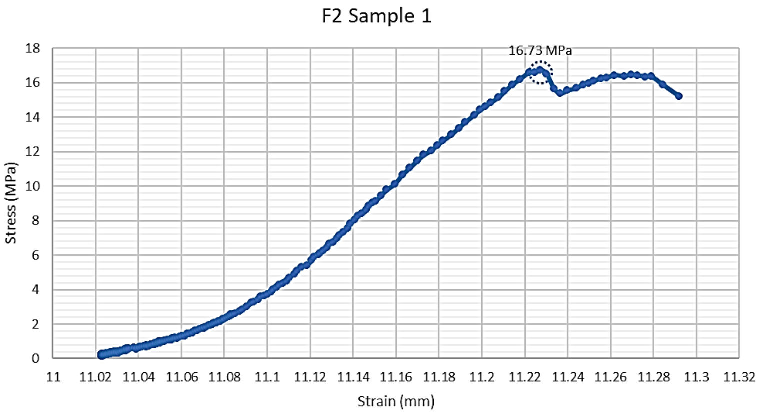
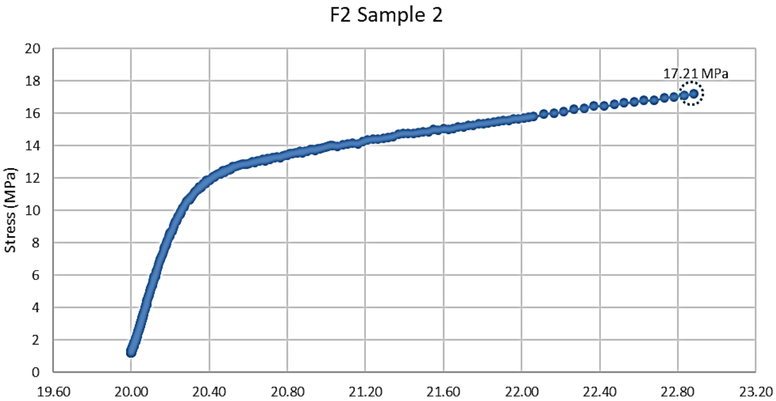
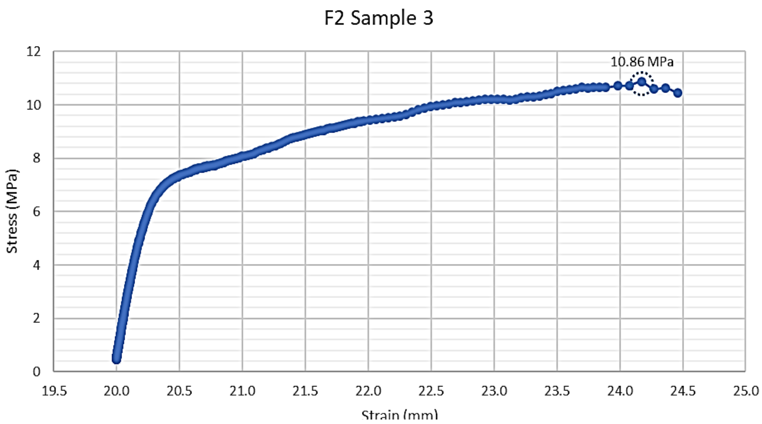
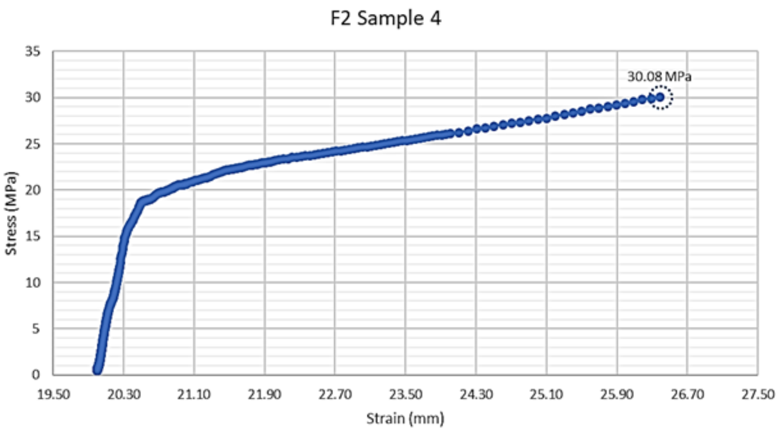
Annex A

Graph 10: The relationship between stress and strain (load and elongation) of F2 fibers.

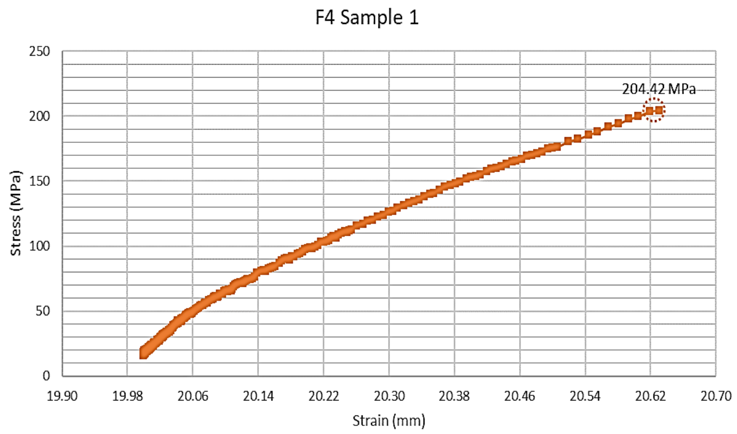


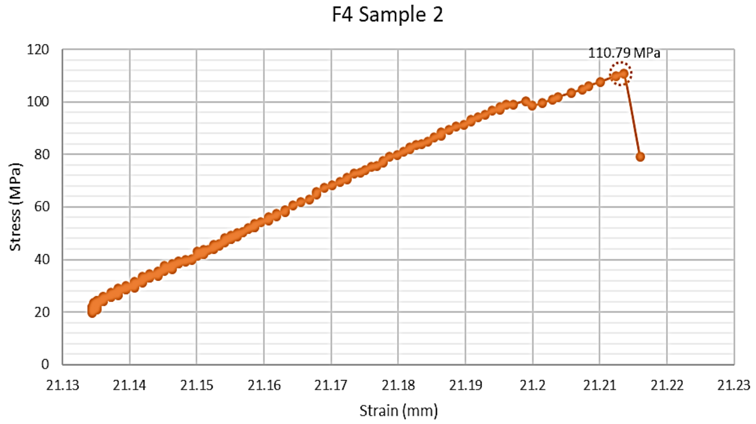


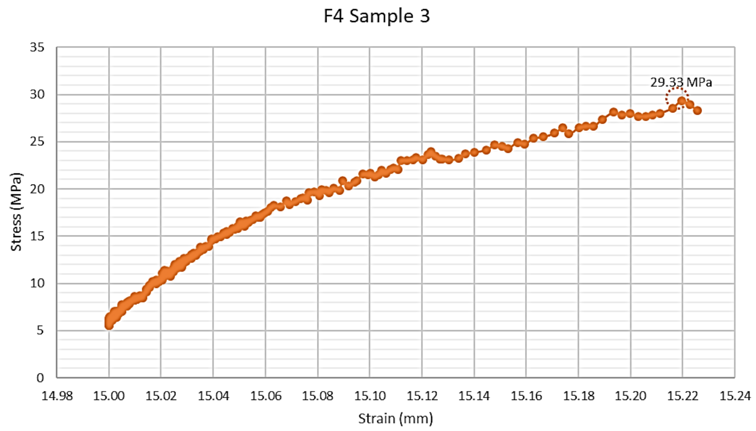


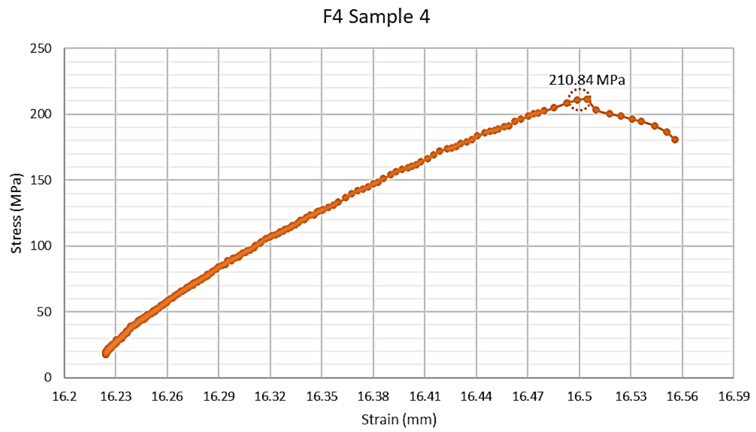


Graph 11: The relationship between stress and strain (load and elongation) of F4 Fibers.









Annex B

Table 15. Mechanical properties of the samples F4 and F2.

Samples	UTS (MPa)	Total Elongation (%)	Young's Modulus (GPa)	Fracture strength (MPa)	Yield strength (MPa)
F4 Fibers					
Sample 1	204.42	4.2	0.34	109.28	174.92
Sample 2	110.79	1.04	1.27	79.21	98.87
Sample 3	29.32	2.2	1.65	16.8	22.97
Sample 4	210.84	2.04	7.80	180.28	161.27
F2 Fibers					
Sample 1	16.73	22.46	0.08	15.54	14.88
Sample 2	17.21	14.4	0.03	17.21	10.37
Sample 3	10.86	22.3	0.02	10.44	6.27
Sample 4	30.08	31.9	0.04	30.08	18.28

Table 16. Weight loss of fiber samples F2 and F4 in the biodegradability test Weight Loss of fiber samples F2 and F4 in the biodegradability test.

	Weight initial / mg (before purification)	Weight initial / mg (after purification)	Weight / mg Day 1	Weight / mg 1st Week	Weight / mg 3rd Week
F2 Fibers					
Sample 1	32.4	24.7	20.2	9.7	8.9
Sample 2	34.1	24.2	17.4	4.9	4.7
Sample 3	36.3	24.8	17.3	13.4	11.5
F4 Fibers					
Sample 1	31.3	25.4	25.1	21.1	19.3

Sample 2	31.2	25.2	25.0	20.5	19.6
Sample 3	31.6	25.9	24.8	21.7	21.3

Annex C: Raw data of the t-student test of F2, F4 and silk fibers

	Fiber	N	Mean	Std. Deviation	Std. Error Mean
UTS	F4	4	138.8425	86.15136	43.07568
	Silk	4	217.5475	39.57996	19.78998
TElong	F4	4	2.3700	1.32358	.66179
	Silk	4	23.6000	6.60903	3.30451
Young	F4	4	2.7650	3.40148	1.70074
	Silk	4	5.0075	1.10885	.55443

		t-test for Equality of Means						
							95% Confidence Interval of the Difference	
		t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
UTS	Equal variances assumed	-1.660	6	.148	-78.70500	47.40419	-194.69888	37.28888
	Equal variances not assumed	-1.660	4.212	.169	-78.70500	47.40419	-207.74367	50.33367
TElong	Equal variances assumed	-6.299	6	.001	-21.23000	3.37013	-29.47641	-12.98359
	Equal variances not assumed	-6.299	3.240	.006	-21.23000	3.37013	-31.51911	-10.94089
Young	Equal variances assumed	-1.254	6	.257	-2.24250	1.78883	-6.61960	2.13460
	Equal variances not assumed	-1.254	3.631	.285	-2.24250	1.78883	-7.41492	2.92992

	Fiber	N	Mean	Std. Deviation
UTS	F2	4	18.7200	8.10492
	Silk	4	217.5475	39.57996
TElong	F2	4	22.7650	7.15846
	Silk	4	23.6000	6.60903
Young	F2	4	.0425	.02630
	Silk	4	5.0075	1.10885

		t-test for Equality of Means						
		t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference Lower	95% Confidence Interval of the Difference Upper
UTS	Equal variances assumed	-9.843	6	.000	-198.82750	20.20064	-248.25668	-149.39832
	Equal variances not assumed	-9.843	3.251	.002	-198.82750	20.20064	-260.39476	-137.26024
TElong	Equal variances assumed	-.171	6	.870	-.83500	4.87142	-12.75493	11.08493
	Equal variances not assumed	-.171	5.962	.870	-.83500	4.87142	-12.77330	11.10330
Young	Equal variances assumed	-8.953	6	.000	-4.96500	.55458	-6.32202	-3.60798
	Equal variances not assumed	-8.953	3.003	.003	-4.96500	.55458	-6.72881	-3.20119

	Fiber	N	Mean	Std. Deviation
UTS	F4	4	138.8425	86.15136
	F2	4	18.7200	8.10492
TElong	F4	4	2.3700	1.32358
	F2	4	22.7650	7.15846
Young	F4	4	2.7650	3.40148
	F2	4	.0425	.02630
Fracture	F4	4	96.3925	67.90534
	F2	4	18.3175	8.35369
YieldS	F4	4	114.5075	69.42633
	F2	4	12.4500	5.24127
Weight1	F4	3	31.3667	.20817
	F2	3	34.2667	1.95533
Weight2	F4	3	25.5000	.36056
	F2	3	24.5667	.32146
WeightD	F4	3	24.9667	.15275
	F2	3	18.3000	1.64621
WeightW	F4	3	21.1000	.60000
	F2	3	9.3333	4.26185
Weight3D	F4	3	20.0667	1.07858
	F2	3	8.3667	3.43123

		t-test for Equality of Means						
							95% Confidence Interval of the Difference	
		t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
UTS	Equal variances assumed	2.776	6	.032	120.12250	43.26588	14.25470	225.99030
	Equal variances not assumed	2.776	3.053	.068	120.12250	43.26588	-16.22396	256.46896
TElong	Equal variances assumed	-5.603	6	.001	-20.39500	3.63990	-29.30151	-11.48849
	Equal variances not assumed	-5.603	3.205	.009	-20.39500	3.63990	-31.57091	-9.21909
Young	Equal variances assumed	1.601	6	.161	2.72250	1.70079	-1.43918	6.88418
	Equal variances not assumed	1.601	3.000	.208	2.72250	1.70079	-2.68980	8.13480
Fracture	Equal variances assumed	2.282	6	.063	78.07500	34.20862	-5.63048	161.78048
	Equal variances not assumed	2.282	3.091	.104	78.07500	34.20862	-29.00530	185.15530
YieldS	Equal variances assumed	2.932	6	.026	102.05750	34.81195	16.87574	187.23926
	Equal variances not assumed	2.932	3.034	.060	102.05750	34.81195	-8.02666	212.14166

Weight1	Equal variances assumed	-2.554	4	.063	-2.90000	1.13529	-6.05208	.25208
	Equal variances not assumed	-2.554	2.045	.122	-2.90000	1.13529	-7.68248	1.88248
Weight2	Equal variances assumed	3.347	4	.029	.93333	.27889	.15902	1.70765
	Equal variances not assumed	3.347	3.948	.029	.93333	.27889	.15501	1.71165
Weight D	Equal variances assumed	6.984	4	.002	6.66667	.95452	4.01649	9.31684
	Equal variances not assumed	6.984	2.034	.019	6.66667	.95452	2.62560	10.70773
Weight W	Equal variances assumed	4.735	4	.009	11.76667	2.48484	4.86764	18.66570
	Equal variances not assumed	4.735	2.079	.039	11.76667	2.48484	1.45641	22.07692
Weight3 D	Equal variances assumed	5.634	4	.005	11.70000	2.07659	5.93446	17.46554
	Equal variances not assumed	5.634	2.391	.020	11.70000	2.07659	4.03013	19.36987