Functionalizing Textile Materials with Mosquito Repellents
in Melt Extrusion and Electrospinning

Functionaliseren van textielmaterialen met muggenwerende producten
via smeltextrusiematrix en elektrospinning

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Ghent, 15th July 2014.
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Table of content

Table of content

Table of content

Summary

The textile industry is constantly evolving at every phase of the supply chain, from fiber and yarn production to the final finished end product. This is because the market has been through a transition from conventional use of textile materials (clothing and upholstery) to non-conventional technical applications (personal protection, packaging, transportation, agriculture, building, etc.). The main engines driving the textile industry towards this novelty trend are the consumer demands for performance, cost, comfort, aesthetic values, ecological concerns, compliance with the set rules and regulations as well as technological advancement. This has resulted in production of novel textile materials with new functionalities for different applications.

Although techniques for developing such functional textile materials exist, there is a need for novel techniques that can produce better quality products with functionalities that can last the life time of the textile. This is important because the current techniques involve adding functionalizing compounds into textile material at finishing stage. In this case, the functionalizing agents are mostly physically or chemically bound on the material surface and are prone to harsh treatment during care and use which can affect their permanency. Moreover, the surface treatment often leads to reduced comfort during wear, quality control issues, weight add on, poor drape, etc.

Therefore, this research work focused on studying the possibility of adding the functionalizing compounds in the textile material at fiber production stage. By doing this, the functionalizing compound will be entrapped in the polymer matrix during solidification of the fiber forming polymer and may take part in crystallization process resulting in a strong bond between the polymer and additives as well as providing high resistance to abrasion. This can also lead to a slow release mechanism which can extend the functionalizing compound`s activity.

In this study, textile materials were functionalized with repellent during fiber production for control against mosquito borne diseases like malaria and dengue. Repellents used in the study were from biological sources because they are considered to be safe and are effective in repelling mosquitoes unlike synthetic repellents which can irritate the skin, can cause asthmatic reactions and environmental pollution as well as mosquitoes are developing resistance towards them. The two fiber production techniques studied were melt extrusion and electrospinning. Melt extrusion was selected because it is a common technique for producing staple and multifilament fibers in micro-scale diameters from thermoplastic polymers. Therefore, success in using this technique would mean a possibility of producing different types of mosquito repellent textiles from various polymers. On the other hand, electrospinning was chosen because it is a simple and fast technique for spinning nano-scaled fibers from various polymers. Moreover, nanofibers generally have large surface area which may give high functionality hence maximizing protection against mosquito bites.

Summary

To develop the novel functional textile material for repelling mosquitoes through melt extrusion and electrospinning, the classical functional textile materials are first reviewed and classified. Afterwards, an in-depth review on current techniques for functionalizing textile materials was discussed and is given in **Chapter 1**.

Chapter 2 reviews mosquitoes and mosquito repellent compounds. The mosquito taxonomy, life-cycle, mosquito-borne diseases and stimuli that attract mosquitoes are discussed and the different types of mosquito repellent compounds examined. This provides a base on which the repellents used in the thesis are selected. The *B. amyloliquefaciens* spores are selected for use as model repellent in melt extrusion because they are known to be resistant to extreme environmental conditions like high temperature and pressure used during extrusion. For electrospinning, Para-methane-3,8-diol(PMD), permethrin, chili and catnip oil are used because they are safe and are reported to be effective in repelling mosquitoes. However, these repellents cannot be extruded into fibers because they are volatile are not resistant to the extreme process conditions used during melt extrusion.

Chapter 3 investigates the resistance of *B. amyloliquefaciens* spores to melt extrusion process parameters and develops a model system for testing the resistance of different biological compounds to melt extrusion process parameters. This is the first step in testing the feasibility of adding biological compound in fibers during melt extrusion. The results shows that the *B. amyloliquefaciens* spores survived the melt extrusion process parameters mainly high temperature (21 200, 250 and 300° C), pressure (0.1, 0.6 and 1 Mpa) and residence time (0, 1 and 10 minutes). Additionally, the spores are successfully extruded in PET films/fibers and viability results of extruded samples further confirms the resistance and survival of spores to melt extrusion process parameters.

Afterwards, **chapter 4** reports the study on the morphology and mechanical properties of Poly(ethylene terephthalate) (PET) fibers with incorporated spores. This is done to examine if the quality of the resulting textile fibers was affected by incorporating the spores. Incorporating spores in PET fibers resulted in decreased tensile strength, Young's modulus and elongation at break. However, the reported properties are within the acceptable range thus the produced PET fibers were as good as normal PET fibers in the market only that they can have some special functionality from the incorporated spores.

Later, microcapsulated biological mosquito repellent and mosquito repellent emulsions are electrospun in poly vinyl alcohol (PVA) nanofibers as discussed in **chapter 5** and **6** respectively. In these chapters, the possibility of getting a slow release mechanism for the repellent though electrospinning is investigated. This is important because the aim of this research was to develop a textile material with incorporated repellent that can give protection for the life time of the material without any need for re-treatment.

Incorporating the biological compounds fibers during electrospinning didn't negatively affected the mechanical and thermal properties of the resulting nanofibrous structure. However, samples with higher concentrations of repellent had lower sorption rates as compared to those with lower

concentrations. The presence of the incorporated repellents in the PVA nanofibers is confirmed by Raman spectroscopy and repellency tests. The repellency tests shows that all the incorporated repellents significantly reduced the number of mosquito landings compared to the control.

Finally, **chapter 7** gives the general discussion, conclusion and recommendation for future research.

A schematic diagram showing the outline of the dissertation is shown in Figure 1 below:

Functionalizing textile materials with mosquito repellents in melt extrusion and electrospinning

Chapter 1
General Introduction

- Defination of functional textiles
- Classification of functional textiles
- Functionalization of textiles
    - Finishes applied on surface
    - Additives incorporated during fiber production

Chapter 2
Mosquito and mosquito repellents

- Taxonomy of mosquito
- Mosquito life cycle
- Mosquito borne diseases
- Stimuli that attract mosquitoes
- Mosquito repellents
- Repellent mode of action

MELT EXTRUSION | **ELECTROSPINNING**

Chapter 3
Resistance of *Bacillus amyloliquefaciens* spores to melt extrusion process conditions

Chapter 4
Morphological and material properties of PET fibers with incorporated spores

Chapter 5
Electrospinning microencapsulated biological repellents in PVA nanofibers

Chapter 6
Electrospinning mosquito repellent emulsions in PVA nanofibers

- Resistance of spores
    - Temperature
    - Pressure
    - Residence time
- Spores extruded in PET
- Exponential equation

- Morphology (optical, SEM & TEM microscopy)
- Mechanical properties
- Thermal properties
- Raman spectroscopy

- Process optimization
- Incorporation of capsules

Characterization
- Morphology (SEM)
- Raman spectroscopy
- Mechanical properties
- Thermal properties
- Repellency

- Electrospinning emulsions
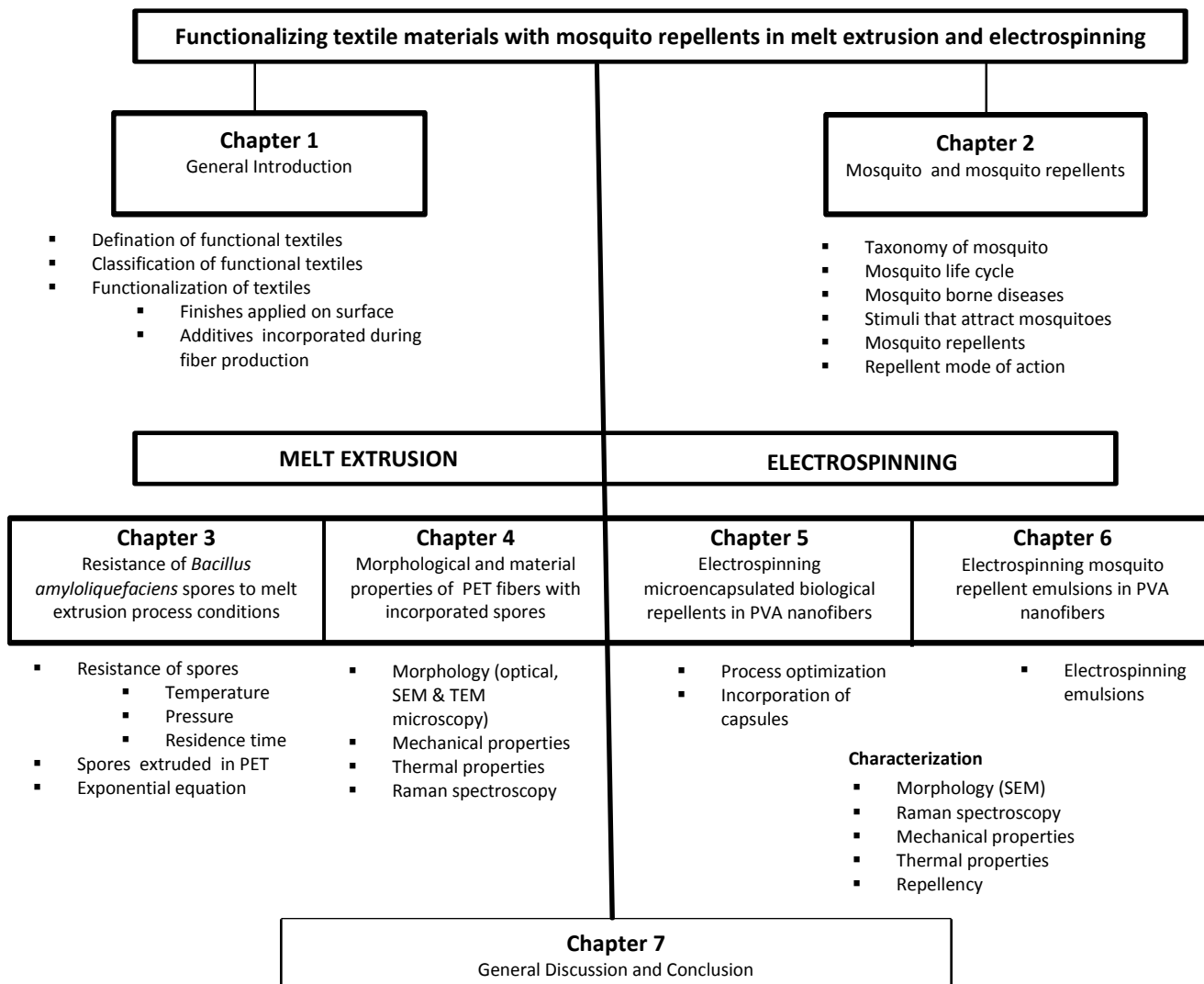
Chapter 7
General Discussion and Conclusion

Figure 1. A schematic diagram showing the outline of the dissertation

Samenvatting

Functionaliseren van textielmaterialen met muggenwerende producten via smeltextrusiematrix en elektrospinning

De textielindustrie kent in iedere fase van de toeleveringsketen een constante evolutie, van de vezel- en garenproductie tot het afgewerkte eindproduct. Dit komt omdat de markt een transitie heeft doorgemaakt van het conventionele gebruik van textielmaterialen (kleding en stoffering) naar niet-conventionele technische toepassingen (persoonlijke bescherming, verpakking, transport, landbouw, constructie etc.). De voornaamste drijfveer van die tendens in de textielindustrie is de vraag van de consumenten naar performantie, prijs, comfort, esthetische en ecologische overwegingen, conformiteit met bestaande regels en reglementering en technologische vooruitgang. Als gevolg daarvan worden nieuwe textielmaterialen geproduceerdmet nieuwe functionaliteiten voor verschillende toepassingen.

Hoewel de technieken om dergelijke functionele textielmaterialen te ontwikkelen reeds bestaan, is er nood aan nieuwe methodes die kwalitatievere producten produceren waarvan de functionaliteiten blijvend zijn gedurende de hele levensduur van het textiel. Dit is van belang omdat bij de huidige technieken de functionaliserende verbindingen aan het textielmateriaal worden toegevoegd in de eindfase. In dit geval zijn de functionaliserende agentia meestal fysisch of chemisch gebonden op het materiaaloppervlak, waardoor ze vatbaar zijn voor irriterende behandelingen tijdens wassen en gebruik, wat dan weer hun permanentie kan beïnvloeden. Bovendien leidt de oppervlaktebehandeling vaak tot verminderd comfort tijdens het dragen, tot problemen met kwaliteitscontrole, gewichtstoename, een slechte manier van vallen, enz.

Daarom werd in dit doctoraatswerk de mogelijkheid bestudeerd om de functionaliserende verbindingen aan het textielmateriaal toe te voegen in de vezelproductiefase. Hierdoor zal de functionaliserende verbinding vast komen te zitten in de polymeermatrix tijdens het stollen van de vezelvormende polymeer en deel uitmaken van het kristallisatieproces, wat leidt tot een sterke binding tussen het polymeer en de additieven en een hoge schuurweerstand zal opleveren. Het kan ook voor een traag afgiftemechanisme zorgen, wat de werking van de functionaliserende verbinding kan uitbreiden.

In deze studie werden textielmaterialen tijdens de vezelproductie gefunctionaliseerdmeteen insectenwerend middel ter afweer van door muggen overgedragen ziektes zoals malaria en dengue. De repellents gebruikt in deze studie zijn van biologische oorsprong omdat die als veilig worden beschouwd en doeltreffend zijn in het afweren van muggen, in tegenstelling tot synthetische repellentsdie de huid kunnen irriteren, astmareacties kunnen veroorzaken, milieuverontreinigend zijn en waartegen muggen een resistentie aan het ontwikkelen zijn. De twee bestudeerde vezelproductietechnieken zijn smeltextrusie en elektrospinning. Smeltextrusie werd geselecteerd omdat het een gebruikelijke techniek is om van thermoplastische polymeren stapelvezels en multifilamentvezels te produceren in microschalige diameters. Vandaar dat een succesvol gebruik van deze techniek zou betekenen dat het mogelijk is om verschillende types van muggenwerend textiel te produceren vertrekkende van verschillende polymeren. Elektrospinning daarentegen werd gekozen omdat het een eenvoudige en snelle techniek is om vezels op nanoschaal te spinnen vertrekkende van verschillende polymeren.

Samenvatting

Bovendien hebben nanovezels over het algemeen een groot oppervlak, wat een hoge functionaliteit kan opleveren en dus een maximale bescherming tegen muggenbeten.

Om nieuwe functionele muggenwerende textielmaterialen via smeltextrusie en elektrospinning te ontwikkelen, werden eerst de klassieke functionele textielmaterialen bekeken en geclassificeerd. Daarna werden de huidige technieken om textielmaterialen te functionaliseren grondig behandeld in **Hoofdstuk 1**.

In **Hoofdstuk 2** worden muggen en muggenwerende verbindingen benaderd. De muggentaxonomie, de levenscyclus van de mug, door muggen overgedragen ziektes en de stimuli die muggen aantrekken worden behandeld en de verschillende soorten muggenwerende verbindingen onderzocht. Dit leverde de basis waarop de in deze scriptie gebruikte repellents werden geselecteerd.De*B. amyloliquefaciens* sporen werden geselecteerd als modelrepellent in smeltextrusie omdat ze resistent zijntegenextrememilieuomstandigheden zoals hoge temperaturen en druk tijdens extrusie.Voor elektrospinning werden PMD, permethrine, chiliolieen olie van wild kattenkruid gebruikt omdat ze veilig zijn en doeltreffend blijken in het afweren van muggen. Deze repellents konden echter niet worden geëxtrudeerd in vezels omdat ze vluchtig zijn en niet bestand tegen de extreme verwerkingsomstandigheden tijdens de smeltextrusie.

Hoofdstuk 3 belicht de resistentie van *B. amyloliquefaciens* sporentegen de procesparameters van smeltextrusieeener wordt een modelsysteem voorgesteld ontwikkeld om de resistentie te testen van verschillende biologische verbindingen tegen de procesparametersvansmeltextrusie. Dit was de eerste stap in het testen van de haalbaarheid om biologische verbindingen toe te voegen aan vezels tijdens de smeltextrusie. De *B. amyloliquefaciens*sporen overleefden de hoge procesparameters, nl. de voornamelijk hoge temperaturen (21, 200, 250 en 300° C), druk (0.1, 0.6 en 1 Mpa) en levensduur (0, 1 en 10 minuten). Bovendien werden de sporen met succes geëxtrudeerd in PET films/vezels. De levensvatbaarheidresultaten van de geëxtrudeerdestalen bevestigden eveneens de resistentie en de overlevingskans van de sporen tegen de verwerkingsparametersvan de smeltextrusie.

In **Hoofdstuk 4** werden de morfologie en de mechanische eigenschappen bestudeerd van PET-vezels met geïncorporeerde sporen. Dit was om na te gaan of de kwaliteit van de resulterende textielstof beïnvloed werd door het incorporeren van de sporen. Het incorporeren van de sporen in PET-vezels leidde tot een verminderde treksterkte, deelasticiteitsmodulus en rek bij breuk. Deze eigenschappen bevinden zich echter binnen het aanvaardbare bereik, waardoor de geproduceerde PET-vezels even goed zijn als normale, op de markt beschikbare PET-vezels, alleen kunnen zij een speciale functionaliteit bezitten dankzij de geïncorporeerde sporen.

Later werden de micro-ingekapselde biologische muggenwerende emulsies via elektrospinnen verwerkt tot nanovezels van polyvinylalcohol (PVA), wat wordt behandeld in de **Hoofdstukken 5** en **6**. In deze hoofdstukken wordt de mogelijkheid bestudeerd om via elektrospinning een traag afgiftemechanisme van de repellent te verkrijgen. Dit is belangrijk omdat het doel van dit onderzoek eruit bestaat een

textielmateriaal te ontwikkelen met een geïncorporeerde repellent die bescherming biedt gedurende de hele levensduur van het materiaal zonder dat er extra behandelingen nodig zijn.

Het incorporeren van de biologische verbindingen in de vezels tijdens het elektrospinnen had geen negatief effect op de mechanische en thermische eigenschappen van de resulterende nanovezelstructuur. Stalen met een hogere concentratie aan repellents hadden echter lagere sorptiegehaltes in vergelijking met die met lagere concentraties. De aanwezigheid van geïncorporeerde repellents in de PVA-nanovezels werd bevestigd via Raman spectroscopie en repellency tests. Derepellency tests toonden aan dat alle geïncorporeerde repellents het aantal muggenlandingen aanzienlijk verminderden in vergelijking met het controlestaal.

Tenslotte wordt in **Hoofdstuk 7** een algemene discussie, een conclusie en een aanbeveling voor toekomstig onderzoek beschreven.

In Figuur 1 wordt een schema weergegeven met een overzicht van de thema's van deze dissertatie.

List of Abbreviations

BSA	Bovine serum albumin
Bt	*Bacillus thuringiensis*
CFU	Colony forming unit
CEN/TC	Commission for European Normalization/Technical commission
DMF	Dimethylformamide
DCS	Differential scanning calorimentry
DVS	Dynamic vapor sorption
DEET	N,N-diethi-3 Methylbenzamide
DNA	Deoxyribo Nucleic Acid
DPA	Dipicolinic Acid
EPA	Environment protection agency
FT-IR	Fourier transform infrared
IR3535	Ethylbutyl acetyl aminoproprionate
ITN	Insecticide treated nets
IRS	Indoor residual spraying
n	Number
OM	Optical microscopy
PET	Poly(ethylene terephthalate)
PVA	Poly vinyl alcohol
PMD	Para-methane-3,8-diol
RH	Relative humidity
r.p.m.	Revolution per minute
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy
USDA	United states department of agriculture
UV	ultraviolet
WG	Work group
WHO	World health organization

List of Abbreviations

Units

Cm^3/g	Centimeter cub per gram
CFU/g	Colony forming unit per gram
Cm	Centimeter
cP	Centipose
°C	Degree Celsius
°C/min	Degree Celsius per minute
g	Gram
h	hours
kV	Kilovolt
MPa	Mega Pascal
mm	Millimeter
ml	Milliliter
ml/m	Milliliter per minute
mlh^{-1}	Milliliter per hour
m	Meter
m/min	Meter per minute
mS/cm	Microsiemen per centimeter
nm	Nano meter
N/tex	Newton per tex
r.p.m	Revolution per minute
RH	Relative humidity
s	Seconds
µl	Microliter
Wt%	Total weight percentage
%	Percentage

List of publications

Journal Articles

1. **Lucy Ciera,** Lynda Beladjal, Xavier Almeras, Tom Gheysens, Vincent Nierstrasz, Lieva Van Langenhove & Johan Mertens (2014) Resistance of *Bacillus amyloliquefaciens* spores as a function of melt extrusion process parameters. Fibers & Textiles in Eastern Europe, Vol. 22, 2(104): 102-107.

2. **Lucy Ciera**, Lynda Beladjal, Xavier Almeras, Johan Mertens, Tom Gheysens, Lieve Van Landuyt, Vincent Nierstrasz & Lieva Van Langenhove (2014) Morphology and mechanical properties of Poly (ethylene terephthalate) (PET) fibers embedded with *Bacillus amyloliquefaciens* spores. Fibers & Textiles in Eastern Europe, Vol. 22, 4(106): 29-36.

3. **Lucy Ciera**, Lynda Beladjal, Lieve Van Landuyt, David Menger, Maarten Holdinga, Johan Mertens, Lieva Van Langenhove, Karen De Clerk and Tom Gheysens. Electrospinning biological mosquito repellents in polyvinyl alcohol nanofibers. In preparation.

Conference and poster presentation

1. **Lucy Ciera**, Lynda Beladjal, Xavier Almeras, Tom Gheysens, Johan Mertens, Vincent Nierstrasz & Lieva Van Langenhove (2013) A model system to study the viability of bio-aggregates to melt extrusion process parameters. 13th AUTEX World textile conference.

2. **L. Ciera**, Tom Gheysens, Lynda Beladjal , Xavier Almeras, V.A. Nierstrasz, L. Van Langenhove (2013) Protection against mosquito borne diseases by use of textiles incorporated with mosquito repellents. The Africa Platform symposium (GAP).

3. **Lucy Ciera**, Lynda Beladjal, Xavier Almeras, Tom Gheysens, Johan Mertens, Vincent Nierstrasz & Lieva Van Langenhove (2013) Resistance of biological compounds as a function of melt extrusion process parameters. Knowledge for growth conference.

4. **L. Ciera** , Tom Gheysens, Xavier Almeras, V.A. Nierstrasz & L. Van Langenhove (2012) Bio-based mosquito repellent textiles to fight malaria and dengue The Africa Platform symposium (GAP).

5. **L. Ciera**, V.A. Nierstrasz & L. Van Langenhove. NO BUG: Biobased mosquito repellent Personal protective equipment (PPE) (2012) Knowledge for growth conference.

6. **L. Ciera**, V.A. Nierstrasz, K. De Clerck & L. Van Langenhove (2011) NO BUG: Biobased mosquito repellent textiles. The training school, 'Synthesis of hybrid organic-inorganic nanoparticles for innovative nonstructural composites'.

7. **Lucy Wanjiru Ciera**, Vincent Nierstrasz, Lieva Van Langenhove & Karen De Clerck (2011) Incorporation of bioactive materials in nanofibers during electrospinning. Ghent University 12 FEA PhD symposium.

8. Vincent Nierstrasz, **Lucy Wanjiru Ciera**, Karen De Clerck & Lieva Van Langenhove (2011) NO BUG: novel release systems and biobased utilities for insect repellent textiles Polymer and Textile Biotechnology, 7th International conference.

9. Vincent Nierstrasz, **Lucy Wanjiru Ciera**, Karen De Clerck & Lieva Van Langenhove (2011) Novel release system and biobased utilities for insect repellent textiles 150 Years of innovation and research in textile science. p.846-847

10. Vincent Nierstrasz, **Lucy Wanjiru** Ciera, Karen De Clerck & Lieva Van Langenhove (2010) Novel insect repellent textiles Chemistry and application fields: book summaries. p.5-5.

11. Wangari Njuguna and **Lucy Ciera** (2014) Organic Cotton in Ethical Fashion as a Future for Development of African Apparel Industries. Annual International Conference on Cotton, Textiles and Apparel Value Chain in Africa (CTA) May 2 & 3, 2014.

12. Fredrick Nzioka, J.I. Mwasiagi, J. Wamboi, **Lucy Ciera** (2014) Comparison of Re-Usable and Disposable Sanitary Pads (A Case Study of Kenyan Rural Women) Annual International Conference on Cotton, Textiles and Apparel Value Chain in Africa (CTA) May 2 & 3, 2014.

Chapter 1

General Introduction

Generally, the textile industry is regarded as a sector for producing materials for conventional uses like clothing and upholstery. However, the current textile industry has drifted from these traditional textile materials and applications to high–tech materials with new added-values and functionalities also known as functional textile materials.

In this chapter, functional textile materials are defined and classified according to their different uses. Moreover, current techniques for functionalizing textile materials will be discussed in detail to give an in-depth understanding of how to obtain these materials which is the main focus of this dissertation.

1. General Introduction

1.1. Definition of functional textile materials

According to the European Standardization technical committees responsible for publishing textile standards for textile products (CEN/TC248) and work group that deals with standardization of smart textiles (WG 31), functional textiles are textile or textile products that possess additional intrinsic functional properties not normally associated with traditional textiles. The functional textile materials defined in these committees includes electrical, thermal and optical conductive materials, emissive, fluorescent and phosphorescent materials and materials releasing substances [1]. These are materials tailored and engineered to give preferred properties suitable for a specific application. This involves transforming the classical textile material by adding new values or functions or by improving their physical, chemical or biological properties resulting into innovative high performance textile materials aimed for a specific end use like personal protective equipment, medical applications, packaging, etc. [2, 3].

The main driver towards the development of functional textile materials has been the change in the market demand, shifting from conventional textile uses to high performing functional textile materials. The ultimate consumer needs are diverse and often complex, spanning from safety to comfort which has led to development of materials with unique and novel properties like; biocompatibility, hydrophilicity, antimicrobial, self-cleaning, moisture absorption, flame-resistance, UV light resistance, insect repellency, warmer in winter and cooler in summer, environmental friendly, etc. Due to complexity involved in developing such materials, an interdisciplinary approach is needed and covers research fields like nanotechnology, biotechnology, computing, electrical engineering, etc. A scheme demonstrating diversification of functional textile materials is given in Figure 2.
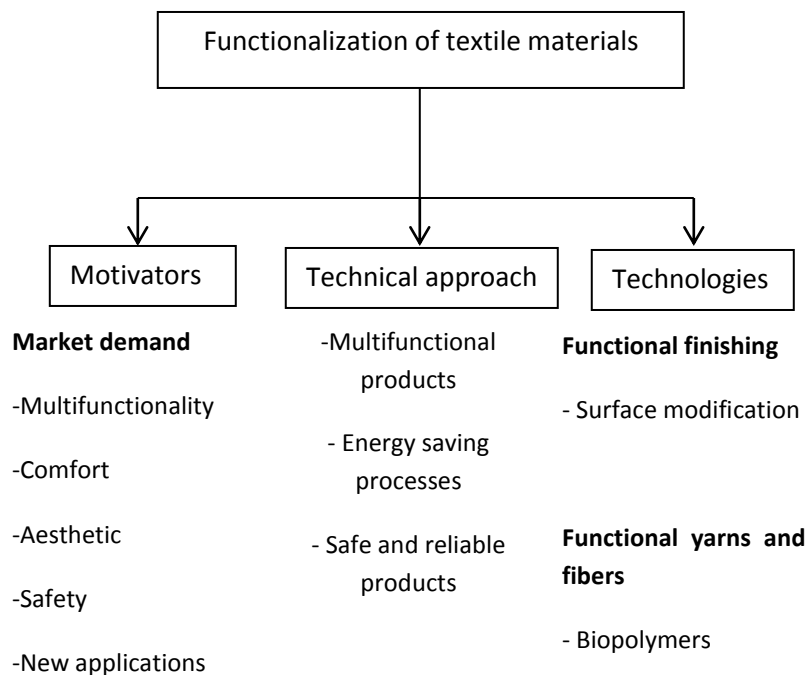
Figure 2. A scheme demonstrating the development of functional textile materials

1.2. Classification of functional textile materials

Functional textile materials can be classified according to their field of application. The main classifications/applications that textile materials are functionalized in are given below:

a) *Protective and safety*

These materials offer protection against various hazards such as biological and chemical hazards, environmental hazards, ballistic and severe impact ranging from explosions to stab wounds [3, 4]. This class is further sub-divided into various classes depending on the threat from which protection is needed. The three main important categories are:

i. ***Environmental hazards protective textiles:*** They protect against extreme environmental conditions such as dust, extreme cold or heat, wind, UV radiation, etc. Examples of such functional textiles include fire fighters suits, personal heating and cooling vests, etc.

ii. ***Biological and chemical hazards protective textiles:*** These types of textiles protect the skin from getting into contact with hazardous chemical and biological compounds. They include functional textiles used in health facilities like clothing for doctors and health workers and industrial clothing like gloves and lab coats.

iii. ***Injury hazards protective textiles:*** These functional textiles protect against injuries caused by ballistic, blunt and other mechanical impacts or occupational cuts. They include jackets, aprons, gloves, sleeves, trousers, etc. These textiles are designed from multilayers of high performance fibers like steel, aramids or ceramics, metal sheets and composites.

b) Medical

These materials are used in the medical and health care sector. They range from a simple suture, gauze or bandaging for wound dressing to materials for developing tissue cultures, like scaffolds and prostheses designed for permanent implantation into the body. Some desired characteristics of such materials include: biocompatibility, nontoxic, sorption capacity, draping quality, ability to be sterilized, air permeability, high tensile properties, elasticity, etc. [5]. Based on their end use, medical functional textile materials can be divided into several categories like:

i. ***Extracorporeal functional materials:*** These devices support the functioning of major organs like lungs, liver, kidney, etc. They include artificial liver, mechanical lung (blood oxygenator) and artificial kidney (dialyser) [6].

ii. ***Implantable functional materials:*** They consist of artificial body parts to repair or replace damaged body parts. They include sutures, prosthesis, vascular grafts, artificial ligaments, etc. [5].

iii. ***Non-implantable functional materials:*** These materials are mainly used for external application like wound care, therapeutic and rehabilitative applications. They include bandages, pressure garments, plasters, orthopedic belts, adhesive tape, etc. [3].

iv. ***Bio-sensing/Telemedicine functional materials:*** This category involves integrating electronic sensors into textiles to track the recovery of patients by monitoring various physiological parameters like temperature, heart beat rate, pulse rate, glucose levels, etc. [6, 7]. Measurements are forwarded to remote stations through wireless transmission (off body mode) or on-body mode [8, 9]. Some of the medical devices made from these materials are bio-sensors to measure the conductivity and pH of sweat and immune-sensors fixed in wound dressing materials to check specific proteins in body fluids [10].

c) Agro

These materials are used in agricultural and horticulture mostly for covering and protection but also in fishing for making nets and ropes. Some examples includes lightweight spun bonded fleeces used for making biodegradable pots for transplanting, materials for thermal insulation, shading and weed control [11]. Moreover, they include heavily constructed materials used for hail and wind protection, capillary nonwoven matting for moisture distribution to growing plants and polypropylene flexible intermediate containers for storage [4]. Some desirable characteristics for such materials include high tensile strength, biodegradable, stiff and resistant to extreme weather conditions.

d) Building and civil engineering

These materials are mainly used in building construction and civil engineering for constructing temporary structures like tents, awnings and marquees. Other applications include insulation, concrete reinforcement, protection against sunlight, interior architecture, etc. [12, 13]. Desired properties in such

materials includes high tensile strength, lightness, resistance to various factors like sunlight, creep, chemicals, fire, rain and pollution in the air, etc. [13].

e) Transport

These are textile materials that have been functionalized for use in cars, aerospace, trains and ships in the form of carpeting, safety belts, tyre cord, airbags, seat covers and furthermore, as reinforcements for composites to build automotive and aircraft bodies [14, 15]. Such applications require materials with high tensile properties, flame retardant, light weight, resistant to sunlight, chemicals, non-elastic, etc. Through the use of textiles, the transportation industry have benefited from improved aesthetic and comfort, reduced weight of car, ship and aircraft bodies, improved safety, etc. [16].

f) Sports and leisure

These materials are mainly used for producing sportswear including sports shoes and other accessories like artificial turf, sail cloth, parachute, cycle and racquet frames, etc. Various value-added sports textiles are already in the market with properties like modified surface morphology to reduce air and/or water permeability which lowers the aerodynamic drag [16]. Additionally, some textiles can apply compression on specific body muscles to increase blood flow while others have unique properties to manage moisture, reduce odor, regulate temperature, etc. [3].

g) Packaging

Important application of functional textile materials in packaging include production of wrappings, bags and sacks like lightweight nonwovens bags for tea and coffee as well as knitted net packaging for fruits and vegetables. Others include strong lightweight spun bonded paper-like materials mainly used for courier envelopes while woven stripes have replaced wires and metal bands which were previously used with packed bales [16]. Currently, the main drive in developing functional textile materials for packaging is the increasing environmental concern that demands biodegradable or recyclable materials [3, 16].

h) Clothing

This category of functional textiles covers specific components used in clothing and shoe manufacturing like elastic narrow fabric tapes, waddings, sewing threads, interlinings, umbrella cloth, insulations, shoe laces, labels, fasteners like hook and loop, etc. The main function of these components is to assure an accurate fit and excellent wear comfort [16]. Desirable material properties for these applications are durability to regular wash, stretchability, soft feel, shrink resistance, etc.

i) Industrial

These are functional textiles used in industries for purifying and separating products, transporting materials between different stages during processing, cleaning effluents, etc. They range from filtration materials, conveyor belts, abrasive sheets and printed circuit boards to computer printer ribbon [16].

j) Home furnishing

These are materials used in home furnishing like furniture covers, interior decoration, protection against sun, fire proofing, etc. Some of the products made from these materials include seat cushions, mattress,

pillow, blinds, carpet backing, wall coverings, curtains, etc. Current developments in this sector are development of nonwoven materials that kill dust mites in beddings, materials with antimicrobial, self-cleansing, dirt resistance and flame retardant properties.

k) Geotechnical

This group covers functional textile materials that can be used in activities pertaining soil, earth, rocks, etc. They involve woven, knitted and nonwoven fabrics used at or below the ground level for separation, protection, drainage, reinforcement and support. Their main applications are soil erosion control, rail-track bed stabilization, slope stabilization, embankment protection and many other areas [13]. Suitable properties for these materials include high tensile strength, low moisture absorption, thickness, resistance to toxic compounds and durability.

l) Ecological protection

These functional textiles are designed to protect the environment mostly in waste management and pollution reduction. Examples of their application include air cleaning, sealing the ground, protecting against soil erosion, cleaning water, protecting landfills against leakages, etc. [16].

1.3. Current trends of functionalizing textile materials

Over the years, various approaches for functionalizing textile material have been developed. The selection of a method or approach depends on the type of fiber, polymer characteristics, functionality required and the characteristics of the modifying agent.

There are two approaches that are popularly used of which one involves applying the modifying agent on the material surface in an after-treatment process while the other approach consists of incorporating the modifying agent in the polymer matrix during fiber production. The two approaches are here discussed in detail:

1.3.1. Applying modifying agents on the material surface in an after-treatment process

This involves applying the modifying agent on the material surface via surface treatment techniques like sol-gel technology, plasma treatment, nano-coating, enzyme immobilization, spraying, padding, coating, etc. [17, 18]. These modifying agents are mostly physically or chemically bound on the material surface and are prone to harsh treatment during care and use which can affect their permanency. Additionally, some surface treatments are often accompanied by loss of mechanical properties, reduced comfort during wear, weight add on, poor drape and loss of feel. Moreover, there are various safety issues relating to the use and disposal of the chemicals used during the finishing treatment as well as quality control issues concerning uniformity of the coats [19]. Researchers therefore are continuously looking for alternative techniques which are cost effective, easy to use, durable, ecofriendly and do not adversely affect the material properties while providing optimum functionality to the intended application. The recent advancement in this area has been to incorporate modifying agents in polymer

matrix during fiber production; this technique will be discussed in depth because it is the method used in this work.

1.3.2. Incorporating modifying agents in the polymer matrix during fiber production

Incorporating additives during the fiber production process entraps the additives in the polymer matrix during solidification of the fiber forming polymer and it may take part in the crystallization process resulting in a strong bond between the polymer and additives as well as providing high resistance to abrasion [20, 21]. In addition, the entrapped additive could slowly migrate to the surface of the fiber acting as a slow release mechanism which guarantees an extended period of additive activity [22]. The release rate is commonly influenced by the physical and chemical characteristics of the polymer in relation to the characteristics of the additive [23, 24].

Most synthetic fibers are produced through an extrusion process which involves forcing a thick viscous solution through small holes (spinneret holes) to form continuous polymeric filament fibers [25]. The fiber forming polymers are initially in solid state pellets hence they have to be converted into a fluid state for extrusion to take place. Normally, this is achieved by melting for thermoplastic synthetic polymers or alternatively by dissolving in suitable solutions if a non-thermoplastic polymers [26]. There are many different techniques for producing fibers and they include wet extrusion, dry extrusion, gel extrusion, melt extrusion and electrospinning. Melt extrusion and electrospinning are the main techniques that have been focused on in this dissertation and are here discussed in detail.

a) Melt extrusion

Melt extrusion is a process that involves applying heat and pressure to melt a polymer which is then forced through a spinneret in a continuous process. It is one of the most widely applied technologies for processing plastics and rubber. It is even used in pharmaceutical and food industries to produce products ranging from bags, sheets, pipes, fibers, films, foams, etc. [27, 28].

The melt spinning equipment is made of an extruder, monitoring devices and downstream auxiliary equipment [29]. The schematic diagram showing the melt extrusion equipment is presented in Figure 3.
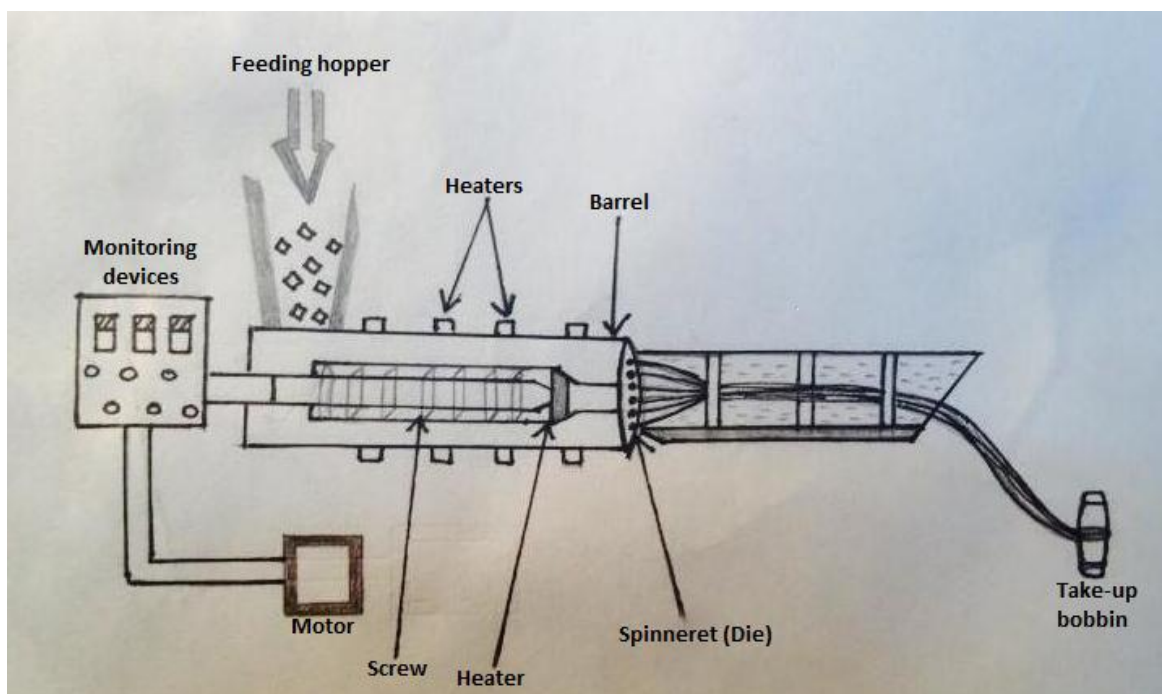
Figure 3. The schematic diagram showing the melt extrusion equipment (© drawing Wangari Njuguna)

Extruder:

The extruder includes a feeding hopper through which the polymer enters the barrel and screw which melts transports and mixes the material. Melting of the polymer in the extruder is achieved through different ways, e.g. frictional heating within the barrel, shear stress between the rotating screws of twin screw extruder and friction between the screws and the barrel wall as the polymer is conveyed [30]. Additionally, the barrel is installed with heaters which also facilitate in melting the polymer and is divided into three main sections namely polymer conveying (transport), melt (compression) and melt pumping (metering) (Figure 4). The temperature of each of these sections can be individually controlled to obtain optimal viscosity that allows proper mixing and easy conveying of the polymer melt while avoiding thermal degradation of the polymer [31]. The melted polymer is then forced through the die also known as the spinneret which shapes the melt polymer to the desired extrusion profile. There are different types of dies like sheet and film dies, co-extrusion dies used in reservoir, fiber dies for textile filament fibers and shape dies for blow molding [32].

Downstream auxiliary equipment:

Downstream auxiliary equipment includes conveyor belts for moving the extruded polymer from the die to the end of the processing line passing through the following processing steps: water bath or cooling air, rollers for stretching the filaments, strand cutters for cutting the filaments in case of staple fibers and bobbins or spool for winding up filament fibers [31].

Monitoring devices:

The monitoring devices include screw-speed controller, extrusion torque monitor, pressure and temperature gauges. These devices are used to control the quality of the end product.

Theoretically, the process can be divided into four main stages:
1. Feeding the extruder with the polymer through the hopper
2. Transport the polymer, melting, pumping, mixing and kneading
3. Forcing through the die (spinneret)
4. Extrusion from the die followed by down-stream processing e.g. stretching, applying finish, cutting staple fibers etc.

According to the number of the screw, there are two main types of extruders: the single screw extruder which has one rotating screw and the twin screw extruder with two screws which either co-rotates or counter rotates (Figure 4).
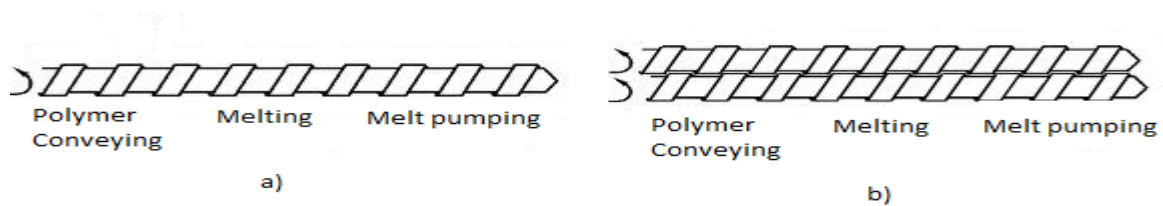

Figure 4. Cross section of extruder barrel. a) Single-screw extruder, b) Twin-screw extruder

The single screw-extruder extrusion system is simple and suitable for general extrusions of common polymers while a twin screw extruder gives better mixing possibility and is more versatile in terms of process manipulation and optimization. Hence, twin screw extrusion can accommodate a wide range and combination of materials. Additionally, twin screw extruder has a self-wiping screw profile mechanism, meaning that the flight of one screw wipes the root of the other screw on the shaft next to it minimizing polymer wastage [25, 29].

Melt extrusion process parameters

Some of the melt extrusion process parameters that determine the success of the extrusion process and the quality of the produced fibers include extrusion temperature, pressure, speed, residence time, polymer properties, etc. Some interrelationship between these process parameters includes [33]:

- Increase in the temperature results in reduction in the pressure required for extrusion
- The flow stress is reduced if the temperature is increased
- The greater the length of the barrel, the higher the extrusion pressure will be required
- The extrusion pressure usually remain fairly unchanged when extrusion speed is increased within normal limits

Some of the melt extrusion process parameters are discussed in details below:

1) High Temperature

Melt extrusion is normally carried out at elevated temperatures. High temperatures are used to melt the polymer which is later forced through spinneret to produce fibers [27]. It is important to ensure that the required temperature for a given polymer is provided to adequately melt the polymer which allows easy conveying of the polymer melt in the extruder [31]. However, overheating of the polymer should be avoided because it can lead to the degradation of the material [34]. In the extruder, the heat for melting the polymer granules is normally generated either by applying external heat from heaters attached to the barrel or internally by friction as the polymer goes through the barrel [31].

2) Pressure

The polymer melt is usually under pressure at all places in the extruder as that is what pushes it as it moves through the barrel [35]. The amount of pressure required during extrusion depends on polymer used, the temperature levels and the product to be made which is normally determined by the die design and the lip opening [36]. High viscous polymer melt yields higher pressure than less viscous ones. However, pressure doesn't have a direct effect on the quality of the resulting product as the melt doesn't remember its pressure once it leaves the die. Nevertheless, the processing pressure in the extruder affects the mixing and the melt temperature which may affect the product properties like insufficient mixing may affect the product appearance (lumpy surface and streaks) while excessive temperature may result in polymer degradation [35].

3) Residence time

Residence time is an important process parameter during melt extrusion which has an influence on the quality of the obtained extrude. While materials that are sensitive to shear and heat may decompose when left in the heated extruder for long, a given optimal residence time is required for sufficient melting and mixing in order to produce quality products [37].

4) Polymer properties

Molecular and molecular weight distribution influences the process of melt extrusion and the properties of the resulting fibers. Fibers extruded from high molecular weight polymers have superior mechanical properties than fibers from low molecular weight. High molecular weight leads to an increase in the melt strength leading to low processing rates [38].

On the other hand, the chemical nature of the polymer determines the processing parameters such as the melting temperature and glass-transition temperature as well as the mechanical and thermal properties of the resulting fibers [39].

5) Screw speed

The response of the polymer to the extrusion process conditions can be influenced by the speed of deformation. Increasing the screw speed can lead to an increase in the extrusion temperature and pressure [39, 40]. This increase can be explained by the fact that the strain rate is directly proportional to the screw speed and the magnitude of the generated heat is proportional to the stain rate [40].

6) *Additives*

Incorporating additives during extrusion can affect the various melt extrusion variables like temperature, pressure, feed rate, etc. This effect is dependent on the additive's nature, chemical nature, concentration, etc. [38, 39]. High concentration of additives may affect the polymer viscosity which may result in increase in extrusion pressure, temperature and shear stress. Additionally, the nature of the additives like particle size and high loadings can results in formation of agglomerates which can cause problems during processing by blocking the melt filters. Furthermore, the mechanical properties of the resulting product may be affected negatively as the agglomerates remaining in the final product can act as an imperfection under mechanical load [41].

7) *Feed rate*

The feed rate determines the amount of polymer in the extruder which is critical to the extrusion process as it determines other process parameters like screw speed, pressure, shear stress, etc. [39]. A constant feed rate ensures that a constant amount of the material is supplied in the extruder.

b) *Electrospinning*

Electrospinning is a simple and efficient technique for producing fibers with a diameter ranging in the micro to nanometer scale from polymer melts and solutions using an electric field. A typical electrospinning set-up consists of a syringe which holds the polymer solution that is connected to a blunt needle tip that works as a spinneret. A syringe pump ensures that the polymer solution is fed at a controllable and constant rate. One electrode from the high voltage supply (basically in the range of 0 - 30 kV) is connected to the blunt needle to charge the polymer solution in a way that the surface of the polymer solution droplet is held by its own surface tension hence getting electrostatically charged at the needle tip [33, 46]. In this way, the polymer solution droplet undergoes two electrostatic forces: the Coulomb force from the external electric field and the electrostatic repulsion between the surface charges elongating the droplet into a conical shape typically known as a Taylor cone [47]. Once the intensity of the electric field reaches to a given critical voltage value, the repulsive force in the charged solution overcome the surface tension of the droplet and a jet erupts from the Taylor cone droplet at the needle.

At the beginning, the jet from the droplet follows a linear trajectory which later changes to chaotic trajectory due to bending instability that is dependent on the critical distance from the needle tip. At the start of the bending instability, the polymer solution jet takes a diverging helical path and as the jet coils towards the grounded collector, a higher instability occurs which leads to a disordered trajectory [47, 48]. In the electric field, the jet is stretched and the solvent evaporated resulting in a randomly oriented nonwoven mat of polymeric nanofibers that covers the grounded collector [49-51]. A schematic diagram of the electrospinning process is given in Figure 5.
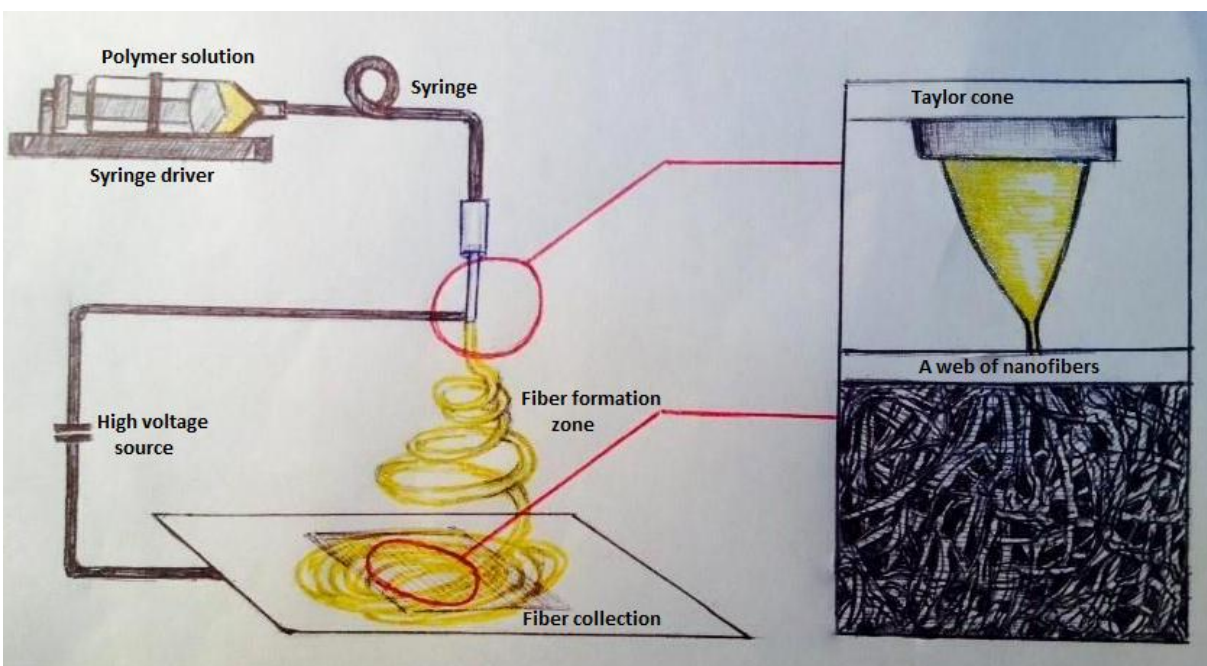
Figure 5. A Scheme of electrospinning setup with indication of the process parameters (© drawing Yared Zekarias)

The stability of electrospinning process and the quality of nanofibrous structure is influenced by a number of factors that can be categorized in three main groups: polymer solution parameters, process parameters, ambient parameters and their interaction.

Polymer solution parameter:

Electrospinning requires a polymer to be melted or dissolved in a suitable solvent to make a solution that can be spun into fibers. Hence, the properties of the polymer solution are the combination of the specific properties of the solvent and polymer used. Such properties include viscosity, molecular weight, conductivity and surface tension. These properties are interconnected and varying one parameter can greatly affect the other parameters. For instance, varying the molecular weight may affect both the solutions viscosity and the surface tension of the solution.

a) **Solvent:** Getting a suitable solvent for a specific polymer is important for a successful electrospinning process. The solvent must have a reasonable evaporation rate in that it must be high enough to allow the fibers to maintain their integrity once they get on the collector and in the same time not too low that fibers don't dry up before landing.

b) **Viscosity**: Solution viscosity which can be controlled by the polymer concentration, solvent and molecular weight is a major parameter that determines the fiber diameter and morphology of the polymeric nanofibers. Low viscosity results in bends and droplets a process which is characteristic for electro-spraying rather than spinning. Moreover, the resulting fibers have

joints and bundles because of wet fibers landing on the collector. When the viscosity is incrementally increased, the shape of the beads slowly changes from spherical to spindle–like and finally to smooth fibers. However, extremely high viscous solutions restrict the free flow of the solution through the syringe needle. This may cause the solution to dry up at the needle tip before the jet can be formed which prevents spinning of the fiber [52, 53]. Additionally, in some cases, high viscosity produces unstable jets resulting in fibers with non-uniformed diameters. Therefore, a suitable viscosity of the solution should be determined for every polymer in order to have a successful electrospinning process producing quality fibers.

c) **Molecular weight**: The polymer's molecular weight characterizes the polymer chain length which influences the viscosity of the solution [47].Just like with high viscous solution, a relatively high molecular weight gives high polymer chain entanglements in the solution promoting a stable solution jet during electrospinning resulting in uniformed spun fibers.

d) **Surface tension**: For an electrospinning process to be successful, it requires that the charged polymer solution overcomes its own surface tension. The impact of the surface tension on the spun fiber properties has been investigated by many researchers and it has been reported that lower surface tension of the polymer solution produces smooth, uniform and bead-less fibers [54-57]. Some ways to reduce the surface tension of polymer solutions include the use of ethanol, polydimethyl siloxane, surfactants, etc.

e) **Conductivity:** Because electrospinning involves stretching the solution by two opposing forces on the solution droplet surface, the solution conductivity determines the amount of charges carried by the electrospinning jet which influences the jet's stability and fiber diameter. A solution with low solution conductivity disables the stretching of the droplet resulting in fibers with beads while solutions with zero conductivity produce no fibers and a solution with high conductivity may result in an unstable jet. Tuning the polymer conductivity to an optimal level can produce more uniformed fibers without beads [58]. Different approaches for improving the solution conductivity have been investigated and they include the addition of salt, alcohol, polyelectrolyte, ion surfactants (like triethyl benzyl ammonium chloride), etc. [47, 53]. When these additives are administered, the increase in charge in the solution causes increased droplet stretching resulting in smooth fibers with smaller diameters due to the increased jet path. The increased charge can also lead to a higher bending instability of the jet resulting in an increased deposition area [56]. However, the interaction between the polymer solution and the additives used to improve the solution conductivity may influence the qualities of the resulting fibers like fiber diameter. Therefore, the additive used should not extensively affect the expected quality of the resulting fiber but should rather promote the production of the desired fibers.

f) **The dielectric constant of solvent:** Generally, a high dielectric solution produces small uniformed fibers without beads due to the increased solution charge. This results in increased droplet stretching and long jet path that produce smooth fibers with smaller diameters.

Solutions like N, N Dimethylformamide (DMF) can be used to increase the dielectric value of the solution. However, addition of such solvents does not always result in better fibers due to the possible negative interactions between the additive and the solution [57].

Process parameters:

Various external factors exerted on the electrospinning jet influence the stability of the process as well as the morphology of the resulting fibers. Such factors include applied voltage, the feeding rate, type of collector, diameter of the needle tip and the distance between needle tip and collector.

a) ***Applied voltage:*** The applied voltage is an important electrospinning parameter which charges the spinning solution and provides an electric field for creating a polymer jet. Both the applied voltage and electric field affects the fiber morphology because they influence the acceleration and stretching of the polymer jet [55]. Generally, a relatively low voltage produces a stable jet and forms uniform and bead-less fibers due to the longtime of flight which allows the fibers to stretch before landing on the collector. Nevertheless, a very low applied voltage produces fibers with droplets caused by a too low electric charge unable to overcome the surface tension of the solution leading to no formation of a Taylor cone. Sometimes, high voltage results in more stretching of the solution caused by great Columbic forces in the jet and strong electric field which can lead to fibers with smaller diameters [47]. High voltage may also promote faster evaporation of the solvent resulting in drier fibers without beads. However, an increase in voltage while spinning low viscous solution reduces the volume of the droplet at the needle tip causing an unstable Taylor cone which produce secondary jets resulting in non-uniformed fibers. Additionally, a further increase in the applied voltage makes the Taylor cone to disappear and this may lead to formation of fibers with beads, with the bead shape changing from spindle-like to spherical-like with increasing voltage [58].

b) ***The feeding rate:*** The feeding rate usually determines the quantity of solution available for electrospinning. Generally, a low feeding rate results in smaller fiber diameters while a high flow rate produce fibers with beads because the jet do not get enough time to evaporate all the solvent before landing on the collector. A relatively lower feeding rate is preferred because the solvent will have enough time to evaporate producing bead-less fibers. Additionally, the collected wet fibers may merge resulting in inter-fibrillar and inter-layer bonding [47].

c) ***Distance between needle tip and collector:*** The distance between the needle tip and the collector influence the time of flight and the electric field strength which directly affect the evaporation rate of the solvent and stretching of the jet. A short distance will result in one hand in a short time of flight for the jet leaving not enough time to evaporate the solvent and on the other hand a high electric field which will accelerates the jet to the collector resulting in wet fibers with large diameters. When the distance is long, the electric field will decrease reducing the stretching of the jet and resulting into fibers with a large diameter or no fibers at all depending on the solution properties. For that reason, an optimal distance is determined with the best compromise between the time of flight and the electric field strength.

d) **Type of collector:** Different types of materials have been used as collectors in electrospinning like metal, methanol and water reservoir, as well as a variety of geometries like parallel plates, frame collectors, conductive collection rings, etc. [53, 55, 59]. Because an electric field between the needle and the collector is essential, a conductive material is used as the grounded collector to assure a stable potential between the collector and the needle. When a non-conductive material is used, charges from the spinning jet usually accumulate on the collector resulting in a loosely packed nano-structure [59]. The properties of the collector, like porosity, also affect the resulting fibers. For example, a smooth collecting material like aluminum foil, produces highly packed nanowebs because the smooth material allows the accumulation of solvents on the fibers. As a result, the accumulated solvent diffuses through the fibers drawing them together resulting in a closely packed structure. On the other hand, porous collecting materials, like wire mesh, gives loosely packaged nanowebs. These porous materials promotes faster evaporation during spinning and encourage further drying even after the fibers have landed on the collector making the fibers repel one another resulting in a loosely packed structure [47, 60]. The collector can either be stationary or moving (rotating cylindrical drums). Rotating collectors gives the solvent more time to evaporate hence producing more dry structures and better fiber alignment [61].

Ambient parameters:

The interaction of the polymer solution with environmental conditions like temperature and humidity also affect the stability of the process and the morphology of the resulting fibers.

a) **Humidity:** At high humidity, water can easily condense onto the collected fibers resulting in formation of pores in the surface of the fibers. The pores increase in size with increasing humidity forming large, uneven shaped pores. Humidity also influences the evaporation rate of the volatile solvents during spinning. At low humidity, an imbalance between the evaporation of the solvent and the flow of the solution through the needle nozzle is created which can result in a blocked needle tip [62].

b) **Temperature:** The ambient temperature can affect the viscosity of some polymer solutions resulting in an unstable process or poor fiber morphology. Studies by Park et al. [56] reported that an increase in temperature from 25 to 60° C resulted in a decrease in fiber diameter of polyamide-6 caused by a change in viscosity of the spinning solution. However, very few studies have been conducted to examine the effect of ambient parameters on the electrospinning process.

1.4. Applications of melt extrusion and electrospinning

1.4.1. Melt extrusion

Melt extrusion technique is extensively used in the plastic, food, textile, pharmaceutical and rubber industry.

a) Plastic industry

In the plastic industry, melt extrusion has been used to produce different packaging materials and various containers via film extrusion, blow molding and injection molding. During film sheet melt extrusion, the polymer melt is forced through a long slit die on polished cooled rollers which form and wind the extruded sheet [51, 63]. In blow molding, the melted polymer is forced into an open mold with air pressure blowing the molten polymer into the desired shape of containers [64]. In contrast, in injection molding, the melt polymer is usually injected into an open mold and left to solidify after which the mold is opened and the product removed [44].

c) Food industry

In the food industry, melt extrusion technology has become an important manufacturing process playing different functions like conveying, separating, cooling, heating, mixing, shaping, co-extruding, shearing, venting volatiles, etc. [65, 66]. Additionally, extrusion cookers are also available and are used to cook food at high temperature and pressure with a short residence time thus restricting unwanted denaturing of important compounds like vitamins, proteins, enzymes, starches and amino acids [67]. Extrusion cooking is also applied in the animal feeds industry generally in the production of palletized feeds [68].

d) Textile industry

In the textile industry, melt extrusion is used in fiber production. Textile fibers are the basic units from which complicated textile structures are developed hence are important in the textile industry. Through melt extrusion, both filament and staple fibers are produced from different thermoplastic polymers. Such fibers have found applications in various fields like in medical sector, agriculture, civil engineering, personal protection equipment, etc.

e) Pharmaceutical and veterinary fields

In the pharmaceutical and veterinary industry, melt extrusion has been used for various applications. Such applications include production of implants and the control or modification of drug release. Additionally, melt extrusion is also used in enhancement of dissolution rate and bioavailability of poorly soluble drugs by forming a solid solution or solid dispersion and covering the bitter taste of some drugs [28, 29].

1.4.2. Electrospinning

Electrospinning is a simple and versatile technique that can fabricate ultrathin fibers from various polymers. Moreover, the morphology, fiber diameter and structure of the electrospun nanostructures can be tailored for a specific application. Due to such flexibility, electrospun fibers have found applications in different fields like biomedical, filtration, protective clothing, electrodes, sensors, reinforcement in composites etc.

a) Biomedical applications

Nanofibers are used in tissue engineering for making structures such as scaffolds which can mimic the nano-scale properties of the extracellular matrix. Additionally, biocompatible 3-D nanometer-sized

structures made from different proteins are developed from electrospun fibers for application in repairing and replacing body organs [69]. Ramachandran & Gouma [70] has given a thorough review on patents and applications of electrospun nanofibers for tissue engineering. Various other reports on developing scaffolds from natural or synthetic nanofibers have been published [71 - 73].

Moreover, electrospun structures are used in treatment of wounds such as burns and abrasions because they are porous and promote exchange of liquid and gases as well as they offer a fibrous structure that protect the wound from infection [70]. Electrospun structures for wound dressing normally have pore sizes of 500 to 1, 000 nm which cannot be penetrated by bacteria hence offering maximum protection [74]. Additionally, the high surface area of the structure is important for fluid absorption while a handheld electrospinning device can be used to apply the nanofibers directly on the wound [69, 74]. Nanofibers have also been used in drug delivery based on the principle that the dissolution of drugs increases with increase in surface area of both drugs and corresponding carrier.

b) Filtration applications
The superior properties of electrospun structure like high porosity, low density, high specific area and good surface adhesion qualified them for a wide range of filtration applications. Several researches are being conducted to further improve and develop new electrospun structures for filtration applications.

c) Composite reinforcement
Fibers are used as reinforcement in composites to enhance the mechanical strength of the product. Since nanofibers have better mechanical properties than traditional macroscopic fibers like higher impact strength, high Young's modulus and a higher surface area to volume ratio, they are now being used to develop novel nano-composites. By using nanofibers as reinforcement, the interaction between the fibers and the matrix material is improved which results to better mechanical properties.

d) Protective clothing
Due to the unique structure of ectrospun nano-fibrous materials, they can give chemical protection without interfering the water absorbency or air permeability of the material. Additionally, they also provide high surface area that can be functionalized to protect against different hazards like insect bites, chemicals and toxic gases. Studies by Huang et al. [75] have demonstrated that nanofibers have minimal impendence to moisture vapor diffusion and are better than traditional materials in trapping aerosol particles.

1.5. Problem statement

Although mosquitoes are an important source of food for fish, frogs, birds, etc. and helps in pollination of various plants, mosquitoes are nuisance and annoying insects which not only make outdoor activities unbearable but are also notorious in causing diseases like malaria, dengue, yellow fever, Encephalitis, etc. These diseases have caused millions of deaths, the world health organization reported 627 000 malaria deaths in 2012 and 207 million cases while 2.5 billion people are at risk of dengue with 50 million people being infected annually [76-78]. The effects of mosquitoes are mainly felt in tropical and sub-tropical regions but the risk is spreading to other parts of the world as an effect of global warming.

Moreover, millions of people are travelling yearly to tropical areas (South-America, Africa, and Asia) for professional activities like education, research, peace keeping, volunteers, etc. which put them at a risk of mosquito borne diseases.

Enormous efforts in controlling mosquito borne diseases through drug treatment, use of insecticides and other vector control measures are used. Even with this, mosquito borne diseases still remains a major risk to public health. This can be attributed to resistance of mosquitoes to the current synthetic repellents along with environmental, health and regulatory concerns [79]. Additionally, the efficacy of most repellents is limited only to a short period and must be re-applied after a given period of time. Moreover, these repellents are generally impregnated on the surface of textiles and their durability and resistance to abrasion is questionable.

Therefore, there is a scientific as well as a technological gap in developing safer mosquito repellent textiles with an efficacy that can last the lifetime of the product. This gap can only be filled by identifying effective and safe repellent to be permanently incorporated into textiles to produce novel functionalized mosquito repellent textile. Moreover, the produced novel textile should give a slow release mechanism to ensure a long protection time without re-treatment. This need has led to an increased interest in biological compounds and their derivatives in search for new repellent compounds to be incorporated in textiles at fiber spinning stage for protecting against mosquito-borne diseases.

1.6. Research objectives and outline of the dissertation

This research was in the framework of the NO BUG project which aimed at developing effective and long lasting mosquito-repellent textile Personal Protection Equipment (PPE). The project addressed two main problems: first, the efficacy and lifetime of the current bio-repellent textile is too short and second, the current mosquito repellents are harmful and resistance of mosquitoes to the conventional repellents is increasing. Thus, the project aimed at selecting innovative bio-repellents and developing a slow release mechanism based on multilayer coatings or by developing textile bio-aggregates. Finally, the repellency of the developed novel materials was to be tested in lab and in real world conditions.

The department of textiles at Ghent University was involved in the project to offer expertise in the field of functionalization of fibers, biotechnology, extrusion and electrospinning. As this research was carried out at this department, the studies main objective was aimed at functionalizing textile materials by incorporating biological mosquito repellent compounds in fibers during melt extrusion and electrospinning.

The study aimed at answering three key research questions which are:
- Can the biological repellents withstand the process conditions of melt extrusion and electrospinning?
- Can biological mosquito repellents be incorporated into textiles during the fiber production process and which fiber production technique is most feasible (melt extrusion or electrospinning)?

- Does incorporating repellents deteriorate the quality of the resulting textile fabric and are the resulting textiles effective in repelling mosquitoes?

To make the new functional textiles for repelling mosquitoes through melt extrusion and electrospinning and to answer the outlined research questions, the classical functional textile materials were first reviewed and classified. Afterwards, an in-depth discussion of current techniques for functionalizing textile materials is given in **Chapter 1**.

Chapter 2 reviews mosquitoes and mosquito repellent compounds. The mosquito taxonomy, life-cycle, mosquito-borne diseases and stimuli that attract mosquitoes are discussed and the different types of mosquito repellent compounds examined. This provided a base on which the repellents used in the thesis are selected. The *B. amyloliquefaciens* spores are selected for use as model repellent in melt extrusion because they are known to be resistant to extreme environmental conditions like high temperature and pressure used during extrusion. For electrospinning, Para-methane-3,8-diol(PMD), permethrin, chili and catnip oil were used because they are safe and are reported to be effective in repelling mosquitoes. However, these repellents cannot be extruded into fibers because they are volatile are not resistant to the extreme process conditions used during melt extrusion.

Chapter 3 investigates the resistance of *B. amyloliquefaciens* spores to melt extrusion process parameters and a model system for testing the resistance of different biological compounds to melt extrusion process parameters is developed. This is the first step in testing the feasibility of adding biological compound in fibers during melt extrusion.

Afterwards, **chapter 4** studies the morphology and mechanical properties of Poly(ethylene terephthalate) (PET) fibers with incorporated spores. This is done for examining if the quality of the resulting textile fabric is affected by incorporating the spores.

Later, microcapsulated biological mosquito repellent and mosquito repellent emulsions are electrospun in poly vinyl alcohol (PVA) nanofibers as discussed in **chapter 5** and **6** respectively. In these chapters, the possibility of getting a slow release mechanism for the repellent though electrospinning is investigated. This is important because the aim of this research was to develop a textile material with incorporated repellent that can give protection for the life time of the material without any need for re-treatment.

Finally, **chapter 7** gives the general discussion, conclusion and recommendation for future research.

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Chapter 2

Mosquito and Mosquito Repellent Compounds

Mosquitoes are annoying insects which can not only make outdoor activities unbearable but are also notorious in causing various diseases resulting in many deaths.

This chapter reviews these insects by explaining their taxonomy and life-cycle as well as the stimuli that attracts them and the types of mosquito repellent compounds available. This is aimed at giving an in-depth understanding of mechanisms through which mosquito borne diseases are transmitted so as to get an informed insight on how to prevent the transmission.

2. Mosquito and mosquito repellent compounds

2.1. The mosquito

Mosquitoes are slender, long-legged insects with distinct long proboscis and scales on most parts of their body. They breed in bushes, soft moist soil or in stagnant water sources like in birdbaths, wading pools, storm drains, etc. Male mosquitoes feed on plant nectar and sweet juices while female not only feed on plant nectar for energy but also need blood for developing their eggs. Their body is composed of three main parts namely head, thorax and abdomen (Figure 6).
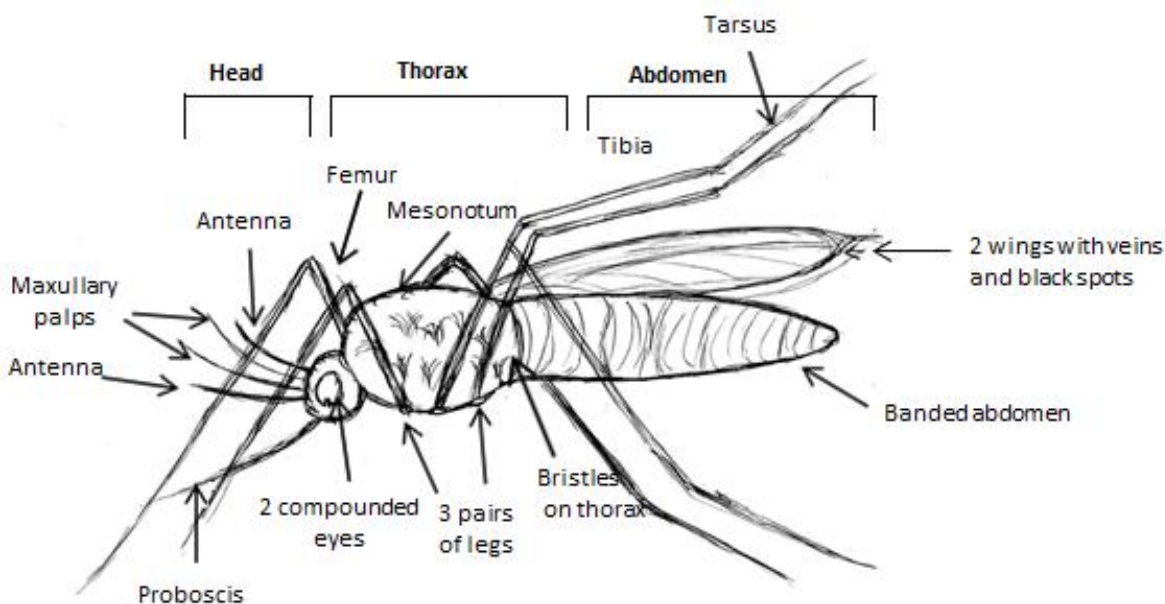
Figure 6. The anatomy of mosquito (© drawing James Ciera)

The head: Forms the sensory center of the mosquito and consists of the antennae, two compounded eyes and a proboscis. The long and feathery antennae have sensitive receptors which detect carbon dioxide present in human breath and a maxillary palp detecting chemicals released from human sweat. The compounded eyes are covered with small lenses (ommatidia) for detecting movements and a photosensitive eye (ocelli) at the top of the head for detecting variations in light [1]. The proboscis, a long serrated mouthpart is for piercing the skin and sucking up blood. The proboscis also has two tubes, one for injecting saliva with an anti-coagulant together with a mild painkiller while the second tube draws blood from the human/animal [2].

The thorax: Has three segments namely prothorax, mesothorax and metathorax which comprises of legs, wings and halters [3]. Each segment has a pair of legs with each leg having four segments namely coxa, trochanter, femur, tibia and tarsal. The tarsal segment has small claws that enables mosquito to

attach on surfaces [4]. The thorax also has a pair of flat, narrow and membranous wings in addition to small wing like structures (halters) which function as gyroscopes to inform the mosquito about the rotation of the body during flight [5].

The abdomen: Contains the digestive, respiratory and reproductive organs.

2.1.1   Taxonomy of mosquito

Mosquitoes are in the kingdom Animalia, phylum Arthropoda, class Insecta (Hexapoda), order Diptera and family Culicidae [6]. The Culicidae family is large with many groups and sub-groups that occur throughout the tropical and temperate regions of the world. There are over 3, 500 different species and sub-species of mosquitoes forming 42 genera with 140 sub-genera [1, 7].

2.1.2   Mosquito life cycle

The life cycle of the mosquito has four distinct and separate stages namely the egg stage, the larval stage, the pupal stage and the adult stage each having unique and distinction features (Figure 7). The time length of each stage is determined by factors like temperature, humidity, time of the year, species type and sex. Most male mosquitoes have a very short life span of about a week in contrast to females living up to a month [1].
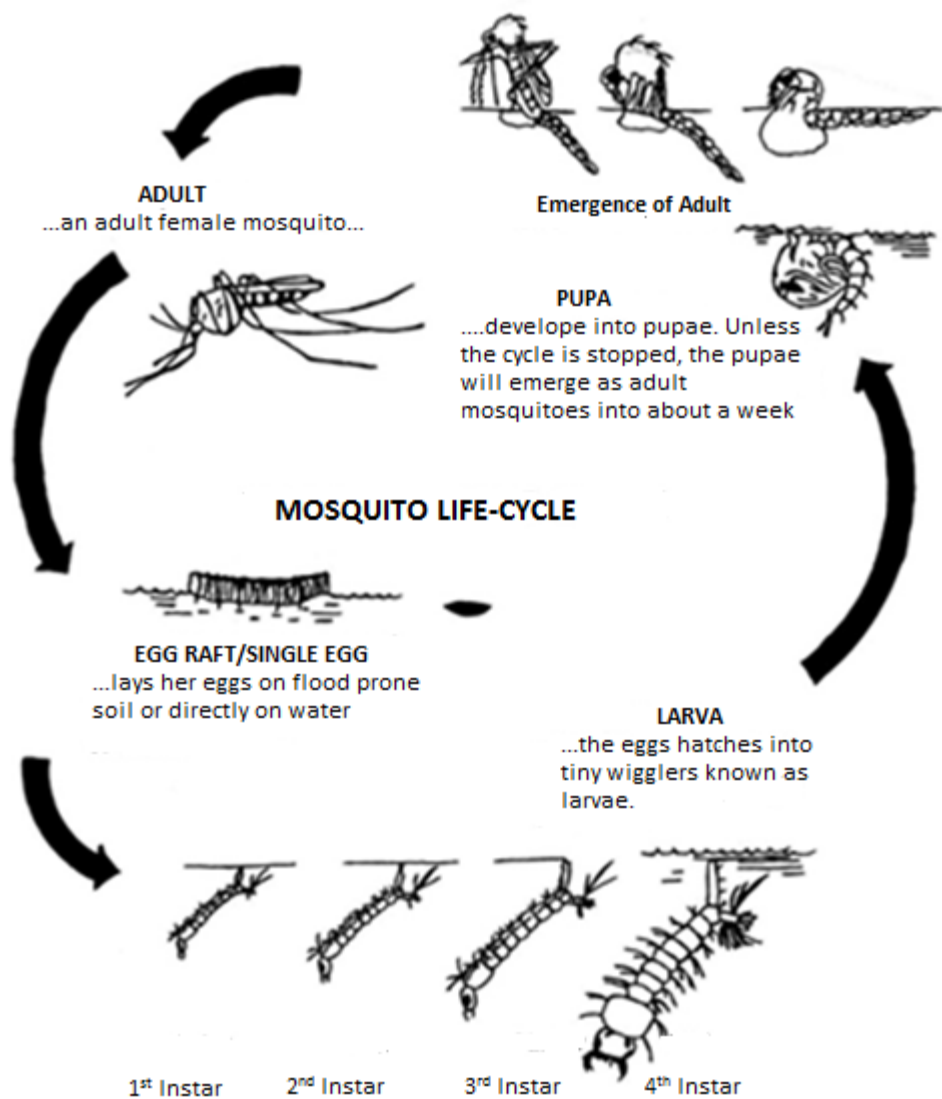
MOSQUITO LIFE-CYCLE

ADULT
...an adult female mosquito...

Emergence of Adult

PUPA
....develope into pupae. Unless the cycle is stopped, the pupae will emerge as adult mosquitoes into about a week

EGG RAFT/SINGLE EGG
...lays her eggs on flood prone soil or directly on water

LARVA
...the eggs hatches into tiny wigglers known as larvae.

1st Instar   2nd Instar   3rd Instar   4th Instar

Figure 7. A schematic diagram representing the life-cycle of a mosquito [8]

a) Egg

The female mosquitoes lay eggs on the surface of stagnant water or on soft moist soil. Eggs can be laid separately or stuck together in rafts of up to a hundred or more depending on the species e.g. the *Culiseta* and *Culex* species lay rafts, while *Aedes*, *Anopheles* and *Ochlerotatus* lay separate eggs [9]. Some female species lay between 1 and 3 batches of eggs in their life time while others lay up to 7 batches [9 -11]. Most eggs hatch into larva within 48 hours [2].

b) Larva

The eggs then hatches into larva which lives in water and occasionally surfaces to breathe. The larva sheds its skin four times, growing bigger after each molting. Larva of most species have siphon tubes hanging from the water surface for breathing while the *Anopheles* species lacks these siphon tubes and

breath by laying parallel to the water surface [2]. In the tropics, the larval stage lasts between 8 to 10 days while it may take longer in low temperate regions [12]. The larva transforms into the final pupa after the fourth molt (4th instar).

c) Pupa

The pupa is a stage of non-feeding and resting in which the mosquitoes transform into an adult. At this stage, the pupa is very mobile and active and are comma-shaped. This stage takes about 2 days within which an adult mosquito is fully developed.

d) Adult

Once the pupating is complete, the skin splits and an adult mosquito emerges. The mosquito temporary rests on the water surface to dry up, to allow body parts to harden and to spread wings before flying off. Mating starts after about 3 or 5 days [11].

Male mosquito uses their feathery antennae to trace the sound of the flying female. Each species has a distinct sound that enables the male mosquitoes to get to the female of same species [13]. Female mosquitoes mostly mate only once in their life time which gives them enough seeds for their entire hatching period. After that, the female takes a blood meal and rests for a few days to digest the blood and develop eggs [5].The female later lays the eggs and resumes host-seeking. This cycle repeats itself until the mosquito dies [13].

Generally, male mosquitoes live for about a week while the females can live longer up to a month. Their life-span is determined by the humidity, temperature and their ability to obtain a blood meal while avoiding predators and host defenses [2].

2.1.3   Mosquito-borne diseases

Mosquito-borne diseases are illness transmitted by mosquitoes. It involves spreading of diseases causing pathogens from mosquito to human. Generally, mosquito bites are known to transmit diseases like malaria, yellow fever, encephalitis, etc. Nevertheless, not all mosquitoes are disease vectors and even among the mosquitoes that transmit diseases, neither all species nor all strains of specific species transmit the same disease. Moreover, not all mosquitoes transmit diseases under the same environmental conditions: some species feeds at night (*Anopheles*-vector for malaria*)* while others feed during the day (*Aedes aegypti*-vector for dengue fever). A list summarizing some important vector borne mosquito species is presented in Table 1.

Table 1. Mosquito species and the diseases they transmit [1, 14]

MOSQUITO SPECIES	BITING TIME	DISEASE AGENT
Aedes aegypti	Crepuscular (dusk and dawn), Day	Dengue, Yellow Fever
Aedes albopictus	Crepuscular (dusk and dawn), Day	Dengue, Yellow Fever, California group encephalitis
Aedes vexans	Crepuscular (dusk and dawn), Night	California group encephalitis, Eastern equine encephalitis, West Nile virus
Anopheles punctipennis	Crepuscular (dusk and dawn), Night	Malaria
Anopheles quadrimaculatus	Crepuscular (dusk and dawn), Night	Malaria, West Nile virus
Anophelescrucians complex	Crepuscular (dusk and dawn), Night	Venezuelan equine encephalitis, Eastern equine encaphalitis
Anopheles freeborni	Crepuscular (dusk and dawn), Night	Malaria, Western equine encephalitis, St. Louis encephalitis
Culex tarsalis	Crepuscular (dusk and dawn), Night	Western equine encephalitis, West Nile virus, St. Louis encephalitis
Culex nigripalpus	Crepuscular (dusk and dawn)	St. Louis encephalitis, West Nile virus
Culex quinquefasciatus	Crepuscular (dusk and dawn), Night	West Nile virus, St. Louis encephalitis, Western equine encephalitis, Venezuelan equine encephalitis
Culex restuans	Crepuscular (dusk and dawn), Night	West Nile virus, Eastern equine encaphalitis, Western equine encaphalitis
Culex salinarius	Crepuscular (dusk and dawn), Night	West Nile virus, Eastern equine encaphalitis

2.1.4 Stimuli attracting mosquitoes

Mosquitoes have a complex sensory mechanism for detecting potential feeding hosts. Their attraction stimuli vary from species to species. Some of the known chemical and visual attraction stimuli are listed here:

a) Carbon dioxide
Carbon dioxide is generally known as the greatest attractant for mosquitoes. Human exhales carbon dioxide when breathing which is sensed by female mosquitoes. This results in an adaptation of the mosquitoes flight path in a zigzag pattern as it locates the source [15]. Fat people and pregnant women

tend to exhale more carbon dioxide and are expected to attract more mosquitoes than the average person [16]. However, even though heavy breathing attracts the mosquitoes to the potential host, the mosquitoes may attack a different person in the group if that person excretes more other competing stimuli.

b) Sweat
Because mosquitoes breed in areas with high humidity levels, the wetness of sweat may act as an attractant. Additionally, sweat may dilute applied mosquito repellents leaving the person unprotected to mosquito attacks [16].

c) Skin temperature
The exact body temperature that is attractive to mosquitoes depends on the type of mosquito under investigation. However, most species seem to be attracted to the slightly cooler temperatures of the arms and legs of humans [15].

d) Blood type
Each blood group has specific blood-type markers which may be more or less attractive to mosquitoes. For example, people with blood type O have been found to attract *Aedes* mosquitoes more than any other blood type [17].

e) Floral fragrance
Mosquito generally feeds on plant nectar hence floral fragrances tend to be attractants. As a result, use of perfumes, colognes, shampoos, detergents, soaps and fabric softeners with a floral smell attracts mosquitoes to the person.

2.2 Mosquito repellent compounds

Due to the increasing drug resistance of mosquito borne diseases coupled with the mosquito's increasing resistance to insecticides, means of preventing mosquito bites is one of the only ways in countering mosquito borne diseases transmission in the world. Conventional prevention measures for minimizing mosquito bites like limiting outdoor activities, wearing long pants and long sleeved protective clothing in woody areas in addition to disposing off stagnant water work well. However, these measures are not sufficient hence the need for specialty products like mosquito repellents [18].

2.2.1 Definition

Mosquito repellents are defined as substances applied to a surface to make the substrate and its surrounding unpleasant or unattractive for mosquitoes [18, 19]. Such products can be applied onto the skin or incorporated into textiles to minimize vector-host contact thus reducing the chances of disease transmission and skin discomfort resulting from mosquito bites or repellents. Most mosquito repellants on the market today are made-up of a composition of two compounds, namely primary compounds that

repel or kill mosquitoes and a secondary compound that either helps in the delivery of the repelling component or gives a cosmetic appeal [18, 20].

Generally, mosquito repellents are available in different brand names and formulations, including lotions, aerosols, powders, creams, clothing-impregnating laundry emulsions, grease sticks, pump sprays and suntan oils [21]. The selection of a repellent is mostly based on its efficacy against a specific mosquito species as well as the method of application.

2.2.2   Ideal characteristics of mosquito repellents

Ideally, a mosquito repellent should have the following characteristics [22-24]:

i   Odorless or a pleasant smell.
ii   Provide long protection against a variety of arthropods (for example, protection against day and night feeding mosquitoes).
iii   Nontoxic to human and environment.
iv   Should not affect the textile after application (example, an ideal mosquito repellent should not bleach, stain or weaken the fibers).
v   Repellents incorporated into textiles should withstand use and care conditions without losing their efficacy (washing, laundry auxiliaries ironing etc.).
vi   Should not irritate the skin upon application.
vii   Should be chemically stable and must show an optimal degree of volatility.
viii   Should be economically viable.
ix   Should not leave any oily residual on the skin and at the same time should resist removal by sweating and wiping.

Various factors determine the effectiveness of a repellant including: the number and type of mosquito species attempting to bite, the uniformity of application, frequency of application and the user's inherent attractiveness to mosquitoes [25, 26].

2.2.3   Classification of mosquito repellents

Mosquito repellents can be categorized in two main classes based on:

a) Source/origin
b) Physiological mode of action

a) *Mosquito repellents classified according to their source*
In this category, mosquito repellents can be categorized in two main groups namely synthetic repellents produced from chemicals and biological repellents offered by nature.

Synthetic repellent compounds

Synthetic repellents are the most commonly used and are said to have excellent repellency against mosquitoes. However, they are not environmentally friendly and some are associated with allergies, skin irritations and asthmatic reactions [23, 27, 28]. They include:

1) N,N-diethyl-3 methylbenzamide (DEET)

DEET is one of the most popular synthetic repellents used today. It has and still is a golden standard used for the further development of a diversity of mosquito repellents. It is considered to have a broad-spectrum of repellency on against almost all species of mosquitoes [23]. According to the U.S. Environmental Protection Agency (EPA) register, there are over 230 DEET-based products marketed under more than 70 different brand names [29]. DEET products come in different formulation of products including sprays, lotions, creams, liquids and impregnated fabrics. Studies have shown that a concentration of 10 to 35% DEET provides sufficient protection hence most products on the market contain less than 40% DEET [23, 30]. DEET can be used directly onto the skin or can be applied onto clothing where it has been found to be safe on wool, nylon and cotton. In contrast, it has been shown to damage rayon, pigmented leather, spandex and acetate and it dissolves plastics like vinyl [27].

DEET repels mosquitoes by giving a vapor barrier which produce a bad odor and taste and in this way inhibit mosquitoes from coming close to the treated surface. When applied correctly, DEET provides excellent repellency against mosquitoes and is safe to use. However, when improperly used and over a long period of time, it can be toxic causing allergic reactions and can harm the nervous system [21]. Other shortcomings associated with DEET include unpleasant smell, irritation of the skin, oily and requires frequent re-application [28]. Nevertheless, studies have reported that it's safe and can be used even by pregnant women and children [19, 22, 31, 32].

2) Permethrin

Permethrin ($C_{21}H_{20}Cl_2O_3$) is a synthetic repellent with a high insecticidal activity against different insects like mosquitoes, sand flies, fleas, head lice and ticks. This repellent only works when it comes in direct contact with the mosquito hence not suitable for skin application. It excites the mosquito's nervous system blocking the sodium movement in nerve cells through restricting adenosine triphosphate, acetylcho-linesterase and the gamma-Aminobutyric acid receptors resulting in paralysis /"knock down" (out of air) or death of the mosquito [33]. Permethrin can be applied on shoes, clothing, bed nets and camping clothing but it requires reapplication after every five washings [34]. However, Permethrin has been reported to be toxic when used in high dosages for a long period of time causing asthma-like reactions, hyperactivity, muscle weakness, increase in body temperature, swelling sensations, neurotoxic effects, mutagenicity, paralysis and can alter the immune system as well as cause eyes and skin irritations [23]. Additionally, due to permethrin`s contact mode of action, which means that it kill mosquitoes that come in direct contact with it, some researcher do not consider it as repellant because it does not provide a physical barrier protecting individuals from bite [35, 36].

3) Picaridin (Icaridin, KBR 3023 or Saltidin)

Picaridin (2-(2-hydroxyethyl)-1-piperidinecarboxylic acid 1-methylpropyl ester) is a new repellent which is reported to be as effective as DEET in repelling mosquitoes, ticks and biting flies [37]. Its mode of action is not well understood but it is assumed to provide a vapor barrier that inhibits the mosquito from coming near [38].

4) IR3535 (ethyl butyl acetyl aminoproprionate)

The IR3535 (Ethyl butyl acetyl aminopropionate) repellent compound is normally applied on human skin and clothing to protect against mosquitoes, flies and black legged ticks. IR3535 has been reported to have better repellency than DEET against *Aedes* and *Culex* mosquito species [39]. However, when it is used wrongly it can be irritating to the eyes.

Biological repellent compounds

In contrast to synthetic repellents, natural repellents are thought to be less toxic, environmental friendly and harmless to fabrics [23, 28]. However, most biological repellents are expensive, provide a short-term repellency and their efficacy spectrum cannot match that of some synthetic repellents [25, 26, 40]. Common biological repellent compounds include:

1) Repellents from micro-organisms

Toxins from various bacterial species such as *Bacillus thuringiensis* and *B. sphericus* are commonly used in developing bio-insecticides to control agricultural and forestry pests, as well as insects like mosquitoes and black flies [41]. The insecticidal properties of bacteria are mainly attributed to the proteins they produce during sporulation. *B. thuringiensis* and *B. subtilis* are known to repel different species of mosquitoes like the C*ulex* species [42, 43]. The responsible proteins for repelling mosquitoes in *B. thuringiensis* are Cry11A (72 kD), Cry4B (134 kD), Cry4A (128 kD) and Cyt1A (27 kD) while responsible proteins in *B. subtilis* includes 51.4-kD (BinB) and 41.9-kD (BinA) [42-45]. Re-engineering of the bacteria by combining these species to develop a single superior bacterium with improved repellency have been attempted but the resulting strains showed little to no improvement over the original strains [44]. Another study has reported *B. thuringiensis* to be effective in controlling the mosquito population by spraying the toxins they produce over the mosquito breeding sites [45]. The pathogenic effect of the microorganisms on the target insect and pests occurs by invasion through the gut or integument which is normally followed by multiplication of the pathogen leading to the death of the host [45].

The main advantage of using bacteria as a bio-insecticide is its specificity in action and the slow rate of development of resistance to the bacteria by insects [44, 45]. Some brand names of mosquito larvicides developed from bacteria include VectoBac® Teknar® and VectoLex® based on *B. thuringiensis* subsp. *israelensis (Bti)*. These products have had a fairly good commercial success in the developed countries but their high prices hinder their use in developing countries [43-46].

2) Capsicum (Chili pepper)

Red chili pepper from the Solanaceae family and *Capsicum* genus is a common shrub found in most places over the world. The fruits of chili pepper have a hot flavor due to seven closely related capsaicinoids compounds with capsaicin and dihydrocapsaicin compounds contributing to about 90% of the pungent smell [40].

Capsaicin (8-methyl-N-vanillyl-6-nonemide) has been reported to have significant anti-feedant and lethal effects on mosquitoes, dogs, rabbit, cats, birds, cotton pests, Alfalfa weevil larvae, rice grain pests and beetles like *Tribolium castaneum* (Herbst) and *Stophiluszeamais motschulsky* (Coleoptera: Curculinidae) [45, 47]. However, this repellent causes skin and eye irritation and must be used with caution.

3) Catnip

Catnip also known as *Nepeta* of the Lamiaceae family is famous for its attraction effect on cats engaging them to start licking, chewing the plant, sniffing, body rubbing, etc. The main active component in catnip essential oil is nepetalactone and two isomers Z,E cis, trans and E,Z trans, cis with Z,E-nepetalactone being the most dominant. It has a pungent, minty, distinctive smell that repels mosquitoes, spittlebugs, termites, ticks, spiders and cockroaches. From studies carried out by Iowa State University, it was reported that nepetalactone repels *Aedes aegypti* mosquito 10 times better than DEET [48]. Nevertheless, the basis for its effectiveness is unknown but it is believed that it acts as an irritant on the mosquitoes, hence discouraging them from biting. However, its high attractiveness to cats has prevented its wide spread use as a mosquito repellent.

4) Lemon Eucalyptus oil (PMD)

Lemon *Eucalyptus* oil is an extract from twigs and leaves of *Eucalyptus citriodora Hook* species of the Myrtaceae family. Among its components is p-menthane-3, 8-diol (PMD) that has repellent properties that masks the environmental cues used by mosquitoes to find its host. PMD from lemon eucalyptus essential oil was registered by the U.S. Environmental Protection Agency (EPA) and has been used as a repellent against mosquitoes, gnats and biting flies. It is available as sprays and lotions which are applied on clothing and human skin [49].

5) Ocimum Basilicum (Sweet Basil oil)

*Ocimum basilicum*is an aromatic, short growing herb commonly grown in tropical regions. The basil plant extracts and oils are known to have many medicinal and healing properties such as anti-anxiety and anti-depressant due to its ethanolic active compounds [50]. The composition of the volatile oil includes vitamins A and C, barnoel, geraniol, estragole, linalool, phenolic acid, cineol and eugenol. These oils keep flies and mosquitoes away. However, the estragole which is an important component of the volatile oil has been found to be carcinogenic and mutagenic [51, 52].

6) Garlic

Garlic, scientifically known as *Allium sativum* is a species in the onion genus *Allium.* It is reported to have some repellency against mosquitoes, fleas and ticks. It either kills mosquitoes on contact or the strong smell keeps them away [19].

7) Neem oil

Neem oil produced by fruits and seeds of the Neem plant (*Azadirachta indica*) member of the Meliaceae family is a versatile plant with the extracts being used for cooking, making cosmetics, traditional medicine and repelling arthropods like mosquitoes, sand fleas and ticks. It has also been used as a fungicide to control powdery mildew and as a pesticide against aphids, Japanese beetles, white flies, spider mites, scales, mealy bugs, locusts and thrips, etc. [19, 53]. Among its main components is Azadirachtin which when ingested by the insect works like a growth regulator preventing the larva from developing into an adult [54]. Neem oil has been reported to have low dermal toxicity and cause skin irritation such as dermatitis when used undiluted [19]. Due to the scarcity of reliable studies on its efficacy against mosquitoes, it is not recommended as an effective protection against mosquito bites. Nonetheless, it may offer some degree of protection.

8) Celery extract

Compounds found in the extracts of celery (*Apium graveolens*) seeds are reported to have mosquitocidal activity against some *Aedes and Culex* species, antifungal effects against *Candida albican, C. parapsilasis* and *C. kruseii*as as well as nematocidal effects against *Panagrellus redivivus* and *Caenorhabditis elegans.* Its mosquitocidal activity is comparable to a 25% DEET formulation [55, 56].

9) Clove

From 207 B.C. to 220 A.D., essential oil from clove (*Syzygium aromaticum*) was used to mask bad breath. However, the applications evolved over time using it as a traditional Chinese medicine for treating indigestion, fungal infection, diarrhea, athlete's foot and ringworms. An undiluted eugenol oil, which is the active compound in the cloves, keeps mosquitoes away and has previously been used as an aromatic, astringent an anesthetic and antiseptic [56, 57]. Nevertheless, clove oil is an irritant to the skin.

10) Fennel

Fennel scientifically referred to as *Foeniculum vulgare* Mill is an annual, biennial or perennial herb commonly found in India. This plant is reported to have some mosquito repellency, antibacterial, flavoring, antifungal and antioxidant properties. The fennel essential oil is composed of several chemicals with the main compounds being anethole (40-70%), fenchone (1-20%) and estragole (2-9%) [58]. However, the fennel essential oil irritates the skin.

11) Thyme oil

Thyme essential oil from the *Thymus* species plant has thymol as its main component repelling mosquitoes and some bacteria species. It has applications as an antiseptic, disinfectant and repellents [55]. However, it has a strong smell and it irritates the skin.

12) Citronella

Extracts and essential oils from the plants of the *Citronella* genus are used as mosquito repellents especially essential oils from *Cymbopogon nardus* and *C. winterianus* grass plants. These essential oils have a lemon scent and are widely used at 5-10% concentrations since higher

concentrations cause skin irritation [59]. Additionally, the citronella oil has a fast evaporation time losing efficacy within a short time after application hence leaving the user unprotected. However, mixing the citronella essential oils with compounds that have large molecular weight like 5% vanillin can reduce its volatility by reducing the release rate hence increasing protection time [60]. Other techniques that can be used to reduce the release rate include microencapsulation and nanotechnology. Citronella extracts and essential oil have been incorporated in candles, torches and are being used in sprays [61, 53].

13) Soybean oil

Soybean oil produced from the seeds of the soybean plant has been reported to exhibit repellency against mosquitoes, black flies and mites. Tests executed by the United States Department of Agriculture (USDA) reported that soybean oil can provide protection against mosquito bites up to 8 hours depending on the mosquito species while another study contradicts this by showing that it only protects for about one and a half hours [28, 39]. However, soybean oil has poor fastness to light and irritates the skin.

b) Mosquito repellents classified according to their physiological mode of action

Repellents manipulate the behavior of mosquitoes by sending signals that discourage them from landing on a possible host or by killing them directly. Most repellents operate along one of the following three main principles: they may cause pain, illness, or scare the mosquitoes. Some common classification of mosquito repellents based on their physiological mode of action includes:

i. Sodium ion channel blockers

This class of repellents accomplishes their tasks by interfering physiologically with the nervous system of the mosquito. Among such repellents is permethrin that interferes with the movement of the positively charged sodium ions restricting them from moving across the cells resulting in knockdown, paralysis or death of the mosquito [62].

ii. Discourage landing (Hot foot effect)

Some repellents discourage mosquitoes from landing on a treated surface by giving unpleasant odors to the mosquitoes which deters them from getting closer. Alternatively, the landing surface is made unpleasant making the mosquito avoid the treated surface (Hot foot effect) [63].

iii. Eyes, trachea and cuticle irritants

Repellents with strong unpleasant odors are frequently used as repellents for controlling mosquitoes by irritating its eyes, trachea and cuticle making it avoid the origin of the smell [64].

iv. Chemical receptors blockers

As discussed earlier, compounds like carbon dioxide and lactic acid released by humans serve as attractants to mosquitoes. Some repellents like DEET are designed in such a way that they block the chemical receptors present in the mosquitoes' antennae responsible for detecting lactic acid and carbon dioxide. By blocking these receptors, the mosquito is no longer able to locate a potential host [27, 62, 63].

v. *Growth regulators*

Growth regulators interfere with the mosquito's ability to grow normally by disrupting the molting process or by obstructing the hormones that govern normal development, making it impossible for the mosquito to reach the adult stage. However, growth regulators only affect immature mosquitoes and not the adults [64]. Therefore, they can only be used to control mosquitoes in their breeding sites and not as a repellent to avoid bites. Growth regulators are very specific to the target species hence they help in conserving beneficial species [27].

vi. *Antifeedant*

Antifeedant compounds make the mosquito stop feeding leading to starvation and eventually the insect die. An example of this is *Bacillus thuringiensis* (Bt) insecticides which produces toxins that paralyzes the target insect's gut and rupture stomach lining cells which makes the insect stop feeding and eventually die [27, 65-68].

2.2.4 Selection of mosquito repellents to use in the study

In this study, melt extrusion and electrospinning fiber production techniques are used in incorporating the mosquito repellents in the textile fibers. For the mosquito repellents to be successfully incorporated, they must survive the processing conditions which includes high temperature, pressure and residence time for melt extrusion while important process parameters in electrospinning is solvent used and high voltage. The selection of mosquito repellents was based on these processing conditions.

Micro-organisms from *Bacillus* species are reported to be effective in controlling the mosquito population and some are recorded to be have some mosquito repellency and are reported to be safe to non-target species, safe to human and have low persistence in the environment [44, 45]. For micro-organisms to be successfully incorporated in fibers during melt extrusion, they must be able to survive the high temperature and pressure in the extruder. However, micro-organisms in their living state are not resistant to extreme environmental conditions but some can produce spores which are tough non-productive structures that can endure extreme environmental conditions. For this reason, the *B. amyloliquefaciens* spores were selected to be incorporated in the PET fibers during melt extrusion. In this study however, the spores of *B. amyloliquefaciens* were used as a proof of concept and therefore did not aim at adding any functionality to the PET fibers at this point.

Permethrin, PMD, chili and catnip oil were selected for electrospinning because they are known to be safe and are reported to be effective in repelling mosquitoes [33-36, 40-49]. The PMD was microencapsulated in melamine formal resin to give it a slow release mechanism that can prolong its protection time. The permethrin, chili and catnip oils were used to form emulsions that were electrospun into the nanofibers. Formation of an emulsion is important as previous studies has reported that electrospinning emulsions gives a possibility of directly microencapsulating the oils in the core of the resulting electrospun fibers which can act as a slow release mechanism [69-74]. However, these repellents cannot be extruded into fibers because they are volatile and are not resistant to the extreme process conditions used in the extruder.

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Chapter 3

Resistance of *Bacillus amyloliquefaciens* Spores to Melt Extrusion Process Conditions

Bioactive compounds and their benefits have been and are still considered as possible source of novel mosquito repellents. For biological compounds to be successfully embedded into textile fibers, they must survive the extreme process parameters such as temperature, pressure, residence time, shear stress and polymer type used. Most biological compounds cannot survive such extreme conditions in their living state. However, some biological organisms produce spores which are dormant and tough non-reproductive structures that can resist extreme environmental conditions.

In this chapter, Bacillus amyloliquefaciens spores are used as a model bioactive mosquito repellent and their resistance tested against melt extrusion process parameters, like temperature, pressure and residence time. Consequently, the spores are successfully embedded in PET (polyethylene terephthalate) films and fibers through melt extrusion. Thereafter, the survival rate of the spores is determined after extrusion and the data used to develop a quadratic equation that relates the survival rate with spore concentration.

3. Resistance of *Bacillus amyloliquefaciens* spores to melt process conditions

3.1 Introduction

Biotechnology is becoming increasingly important in the textile industry leading to novel multifunctional textile products with unique values and functionalities in contrast to classical textile materials. Some of the new functional properties obtained in textiles and clothing materials via biotechnology include antimicrobial properties and enzymatic modification of textiles e.g. polyester, wool, cotton etc. [1, 2]. Due to the growing need of multifunctional, eco-friendly and efficient textile products, the use of biological compounds like micro-organisms and their benefits is being explored. Earlier studies have shown that additives can be introduced in textiles at different production stages like during fiber production which ensures a strong bond between the polymer and additives. Moreover, the added compound in the fiber can migrate slowly to the surface acting as a slow release mechanism during use [3, 4]. However, for any additive to be successfully incorporated into synthetic textiles during fiber production, they must survive the extreme processing parameters used. During extrusion, the polymer is subjected to high temperature, pressures and shear stress for a specific amount of time (residence time) which depend on several factors; type of polymer, screw profile, die diameter, etc. [5, 6].

Micro-organisms in their living state cannot withstand such extreme environmental conditions. However, some bacteria are able to produce spores which are tough non-productive structures that can endure extreme environmental conditions such as low and high temperature, pressure, enzymatic digestion, radiation, mechanical disruption, desiccation and extreme chemical environments which make them ideal candidates for embedding into fibers during melt extrusion [7-10]. Spores are normally formed through sporulation a process that is usually triggered by environmental stress like starvation [11, 12]. The initial step in the sporulation process is generally an unequal cell division which forms a larger mother cell and a smaller pre-spore or fore-spore. This is followed by engulfment of the fore-spore by the mother cell which results in a cell within a cell. Later, the spore matures as it goes through various morphological and biochemical changes after which the mother cell lyses, releasing the spore into the environmental [11].

Among the mechanisms that contribute to their high resistance is the spore's structure shown in Figure 8. The outermost layer is the exosporium, though not present in all species of spores, the exosporium is a loosely fitting balloon-like structure composed of carbohydrates, protein and water. Under the exosporium is spore coat which contain multiple protein coats that protect the spore from being destroyed by lytic enzymes and toxic chemicals. Under the spore coat is the outer membrane then the cortex that is made of peptidoglycan. The cortex is important in spore resistance properties as it causes reduction in the core water content which contributes to the spore's resistance to wet heat. Under the cortex is the germ cell wall which later grows to become the cell wall of the spore when it returns to life [11, 13]. Next after the germ cell is the inner membrane containing immobile lipids that are impermeable to small hydrophilic and hydrophobic molecules [13, 14]. The low permeability of the inner

membrane makes the spore resistant to toxic chemicals. The last part of the spore structure is the core that is located at the center of the spore. The core carries the spore's DNA, small molecules, enzymes and pyridine-2, 6-dicarboxylic acid (dipicolinic acid (DPA). The core has low water content which gives resistance to wet heat while the DPA helps in reducing the spore core water content and promotes and maintains the spore dormancy [11, 15].
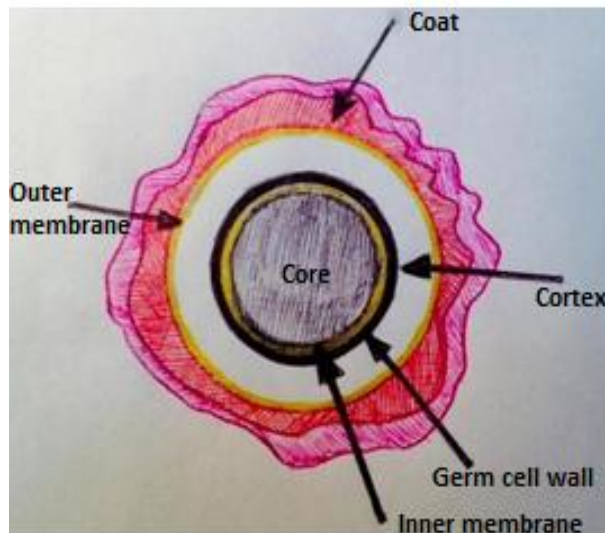

Figure 8. The structure of a bacteria spore (© drawing Yared Zekarias)

Due to superior mechanical properties, recyclability, low cost and easy processing, PET polymer is extensively used in various textile and technical applications [5, 6]. PET and other thermoplastic polymers are usually processed into fibers through melt extrusion. However, even if the resistance of *Bacillus amyloliquefaciens* spores to high temperature and pressure has been widely documented, the link between their resistance and the textile fiber extrusion process conditions is missing. Most of the documented studies focus on killing the microorganisms (e.g. infections, food contamination, etc.) whereas we want them to stay alive. Moreover, none of the reported conditions matched the extrusion process condition of PET which is normally a temperature of 210-320°C, pressure of 0.5-5 Mpa and a resident time of 1-15 minutes [17-23].

The characteristics of the extruded polymer type, like heat conductivity and shear stress, can influence the survival of spores during extrusion. Besides, the *Bacillus* species like *B. amyloliquefaciens* has received a lot of attention for their potential in producing variety of bioactive compounds for example biopesticides [20]. In this study however, the spores of *B. amyloliquefaciens* are used as a proof of concept and therefore do not aim at adding any functionality to the PET fibers at this point.

This study first tests the survival of *B. amyloliquefaciens* spores against the extrusion parameters used for PET after which a model system for testing the resistance of bioactive compounds to melt extrusion process parameters is developed. Finally, a quadratic equation is derived to relate survival rate with concentration of spores during extrusion as a function of temperature, pressure, residence time, shear stress and polymer used.

3.2. Experimental set-up

3.2.1. Microorganism (*Bacillus amyloliquefaciens* spores)

The thermophile bacterial strain *B. amyloliquefaciens was* identified from bio-mining in the Moroccan desert. A stock culture was cultivated at Ghent university (Biology department) and these spores were used in all experiments. Spores of *B. amyloliquefaciens* are known to be resistant to high temperatures and pressures [17, 18, 21] making them good candidates for extruding in polymers.

3.2.2. Resistance of *Bacillus amyloliquefaciens* spores to temperature, pressure and residence time

To test the resistance of *B. amyloliquefaciens* spores to melt extrusion process parameters like temperature, pressure and residence time, a special device (Figure 9) was designed and developed at Ghent University (Biology Department, Jürgen Verstraten).
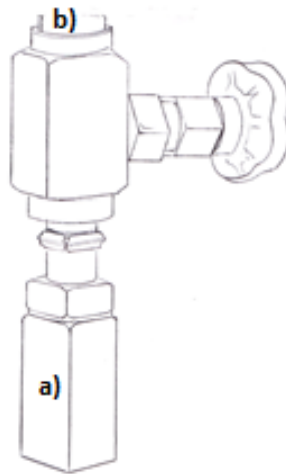
Figure 9. Device used to test the resistance of spores to high temperature, pressure and residence time: a) Sample holder, b) An opening to connect to pressure vessels (© drawing James Ciera)

Chapter 3 - Resistance of *Bacillus amyloliquefaciens* Spores to Melt Extrusion Process Conditions

Spores were exposed to different test parameters (Table 2) based on a pilot study of film extrusion.

Table 2. Test parameters under which the Bacillus amyloliquefaciens spores were tested

Parameters	Values
Temperature (° C)	21 (Control), 200, 250 and 300
Pressure (MPa)	0.1 (control), 0.6 and 1.0
Residence time (minutes)	0 (control), 1 and 10

* Control is 21°C at 0.1 MPa and residence 0 minutes

Three different conditions were used:
- Temperature and atmospheric pressure
- Temperature and pressure
- Room temperature and pressure

A compressor (HP3) was used to pressurize a known amount of dry spores in the sample holder (Figure 9a) and then placed in a preheated dry air oven at the needed testing temperature (Memmert UFE 500). A probe of thermocouple wire connected to a data logger (Testo 175-T3) was placed in direct contact to the sample in order to follow the actual temperature on the sample. When the required temperature was reached, the sample was left in that temperature for the specified amount of time after which, the sample was removed and left to cool down to room temperature.

The culture medium was prepared by dissolving 28g of Nutrient Agar (Oxoid CM0003) in 1 liter of distilled water. The solution was autoclaved at 125°C for 15 minutes, poured sterile into petri dishes and left overnight to ensure the culture medium was free from contamination.

Then, the treated spores were inoculated by dissolving in 1000 µl sterile physiological saline solution (0.8% NaCl) and diluted up to 10^{-15} fold. From this dilution, 50 µl was inoculated on the previously prepared culture medium. The plates were incubated for 24 h at 40°C. Survival of spores was determined by counting the colony forming units (CFU) after which a log calculation was made to obtain the results expressed in log CFU/g dry weight.

The Anova statistical test was used to determine the significance of each parameter, being temperature, pressure and residence time on the number of resistant spores. The null hypothesis states that the mean value of viable spores for the control and each of the experimental groups (i.e. temperature, pressure and residence time) is the same while the alternative hypothesis states that the mean value of viable spores between the control and each of the three experimental groups is different.

3.2.3. Extrusion of *Bacillus amyloliquefaciens* spores in PET films

Arnite thermoplastic PET pellets (Arnite A02 307) from Royal DSM N.V. (Netherlands) were used in extruding both films and fibers. This a long-chain semi-crystalline thermoplastic polymer with a density of 1.34 g/cm^3, water absorption (Equilibrium, 23°C, 50% RH) of 0.30%, viscosity number of 85.0 cm^3/g, a melt temperature of 255°C and processing temperature range between 265°C - 295°C.

Prior to extrusion, the PET pellets were dried in an oven for two days at 70°C after which pure *B. amyloliquefaciens* spores were homogeneously mixed with the pellets to obtain a 0.5 wt% concentration of spores to PET polymer. Finally, this mixture was extruded in a Haake polydrive extruder (Thermo Electronic Corporation, USA).

Haake polydrive extruder is a single screw extruder with a 19 mm diameter screw (barrel). The three different heating zones of the barrel were set to 265°C for the feed stock (transport the material), 270°C for plasticizing (compression) and 275°C for pumping (metering). The die was heated at 275°C while the screw speed and take-up velocity were set at 20 r.p.m. and 80 m/min respectively, resulting in a pressure of 0.5 MPa and a film thickness of 0.8 mm. The average residence time during the extrusion was approximately one minute.

The resistance of the extruded spores in the PET films was determined by the growth/germination biological assay [16]. This model test is ideal for the optimal germination of spores under favorable conditions. This means *B. amyloliquefaciens* spores that survived the extrusion processing conditions will form a colony and be detected as viable. The samples were sterilized by subsequently soaking first in a sodium hypochlorite (12% NaClO) solution, followed by a Dettol solution (5%) and finally in an ethanol (96%) solution for 10 minutes each, where after they were inoculated on Nutrient Agar plates and incubated at 40°C for 24 hours. Finally, the plates were visually checked for colony forming spots.

The distribution and abundance of spores in the films was determined by scanning electron microscopy (Joel Quanta 200 F FE-SEM). The samples for SEM were prepared by placing them on stub where after they were gold coated using a sputter coater (Balzers Union SKD 030).

3.2.4. Extrusion of *Bacillus amyloliquefaciens* spores in PET fibers

Prior to extrusion, the PET pellets (Polyethylene terephthalate (PET) - Arnite thermoplastic) were dried in an oven for two days at 70°C after which pure *B. amyloliquefaciens* spores were added by gravimetric dosing during spinning to obtain a 0 (control), 2, 4, 6, 8 and 10 wt% concentration of spores to PET polymer. This mixture was extruded into multi-filament fibers by a single screw extruder (General extrusion technology, China). The three different heating zones of the barrel were set to 280 ±10°C for the feed stock (transport of material), 290 ±5°C for plasticizing (compression) and 295 ±2°C for pumping (metering). The die was heated at 295°C, the pressure was around 6.0 ±0.2 MPa while the average residence time was approximately 5 ±0.5 minutes. The resulting fibers had an average linear density of about 7 ±0.5 dtex.

Chapter 3 - Resistance of *Bacillus amyloliquefaciens* Spores to Melt Extrusion Process Conditions

The tensile properties, like tensile strength, Young's modulus and elongation at break of resulting extruded spores/PET fibers were determined with the FAVIMAT-ROBOT (Textechno, Germany). All samples were kept in a conditioned room of 65 ±2% and a temperature of 20 ±2°C for at least 24 hours before testing. An average of 50 single filaments was tested for each property investigated.

Before inoculating a sample for testing the survival of the spores after fiber extrusion, it had to be sterilized to remove contamination due to extrusion. Several sterilization techniques like pasteurization and sterilization with chemicals can be used [22]. In order to test the most effective sterilization method to use, two techniques were tested; pasteurizing and sterilization with chemicals.

The dry PET fibers were cut into small pieces to end up with 48 samples each weighing about 85 mg. Half of the samples were then sterilized by soaking in Sodium hypochlorite (12% NaClO), in Dettol solution (5%) and in ethanol (96%) for 10 minutes each. The other half of the samples was sterilized by pasteurizing in a water bath (Grant JB aqua 12) at 80°C for 10 minutes. After each sterilization process, the samples were dissolved in 1000 µl sterile physiological saline solution, diluted and inoculated as described earlier.

To generate a statistical equation that explains the relationship between survival rate and the concentration of extruded *B. amyloliquefaciens* spores, the experimental results from the column 'sterilized' were used (Table 4).

3.3 Results and discussion

3.3.1. Resistance of *Bacillus amyloliquefaciens* spores to temperature, pressure and residence time

a) *Resistance to temperature*
The effect of temperature on the heat resistance of *B. amyloliquefaciens* spores is given in Figure 10, showing a decrease in number of viable spores with increasing temperature. The decrease in surviving spores means spores were killed during the heat treatment and the death increased with increase in temperature. Extreme dry heat can kill spores by damaging and mutating the DNA [23-25]. DNA damage is mostly due to loss of base through depurination [10, 26-28].
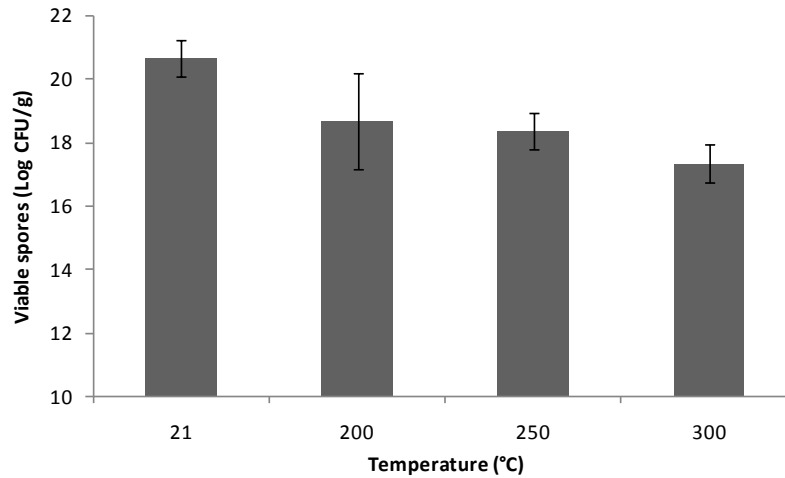
Figure 10. The effect of temperature on the viability of Bacillus amyloliquefaciens spores (four replicas each)

b) Resistance to pressure

The effect of pressure on the viability of *B. amyloliquefaciens* spores is shown in Figure 11. For the pressure range tested, from 0.1 to 1 MPa, pressure seemed to have no effect on the number of surviving spores. An explanation for the resistance of spores to pressure could be provided by DNA-binding proteins protecting the spore's DNA, repair of DNA damage during spore germination and impermeability of the spore's coat to high pressure [8, 9, 29]. Additionally, *B. amyloliquefaciens* spores were reported to resist pressures up to 1,700 MPa [30-32] which is much higher than the highest pressure tested (1 MPa). The pressure range tested was too low to kill the spores and therefore is no important parameter in the extrusion of spores/PET films and fibers.
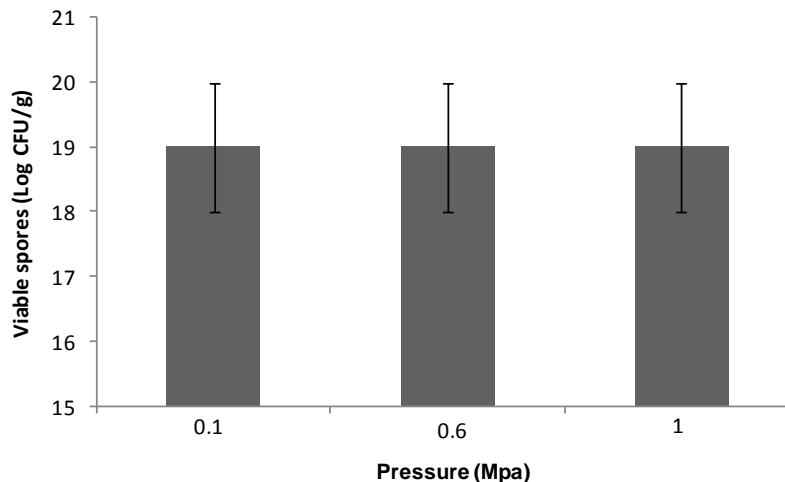
Figure 11.The effect of pressure on the viability of Bacillus amyloliquefaciens spores (four replicas each)

51

c) Resistance to residence time

The influence of the residence time on the viability of *B. amyloliquefaciens* spores under pressure (1 MPa) and at high temperature (300°C) was examined. Whereas residence time under pressure showed no effect (Figure 12a), residence time at high temperature showed a decrease in number of surviving spores after 10 minutes (Figure 12b). Since *B. amyloliquefaciens* spores are well known to be resistant to high pressures [31 - 34], a pressure of 1 MPa was too low to cause any decrease in survival of spores even after 10 minutes of exposure. In contrast, the effect of residence time at high temperatures resulted in a linear decreasing amount of surviving spores with increasing temperature (Figure 10). This is in accordance with previous results in that fewer spores of *B. amyloliquefaciens* survive with increasing temperature [18, 34-37]. This means that any change made in residence time or temperature will be reflected by a great effect in the number of spores that survives the process. The longer the spores are exposed to high temperatures, the more likely it becomes that the spores will not survive the processing.
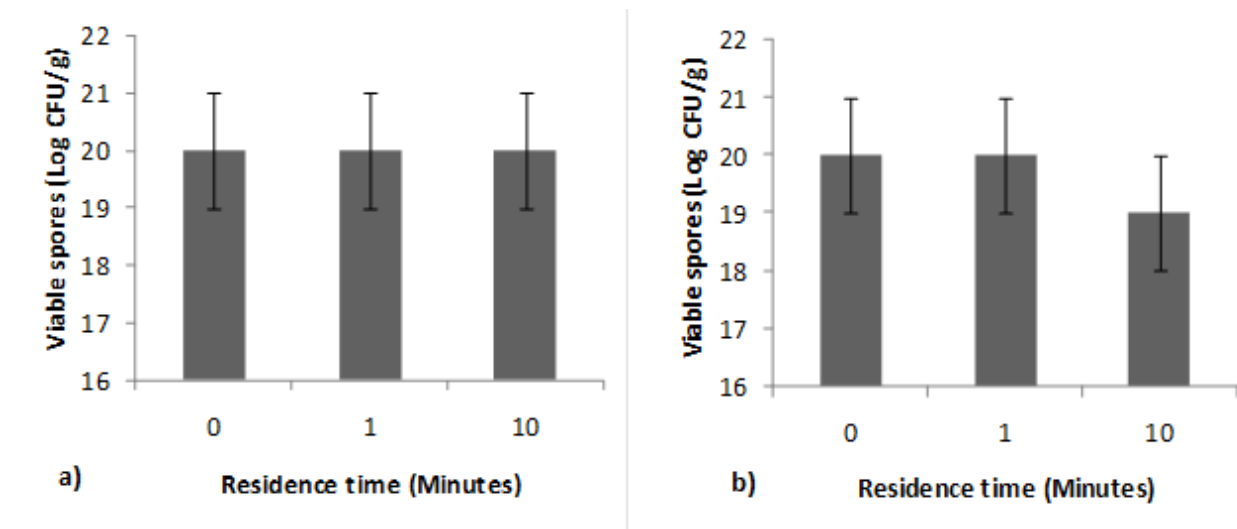
Figure 12. The effect of residence time on the viability of Bacillus amyloliquefaciens spores; a) under pressure (1 MPa) and b) under high temperature (300° C) (four replica each)

d) Statistical analysis to test significance of temperature, pressure and residence time on the number of viable spores

The statistical significance of temperature, pressure and residence time on the number of viable spores was determined with Anova statistical tests with the results tabulated in Table 3. This test compared the distribution of values for each parameter against the control values. The analysis shows that temperature of 200°C did not have a significant effect on the number of viable spores but at 250 and 300°C, a significant effect was observed ($p \geq 0.05$). On the other hand, the applied pressure did not show any significant effect even at 10 minutes residence time while temperature of 300°C showed no significant effect at 1 minute resident time ($p \leq 0.05$) but a significant difference was noted at 10 minutes with p-value of 0.025. These results shows that temperature is the most critical parameter with the greatest effect on the survival of spores during extrusion.

Table 3. *Results for two-sample Kolmogorov-Smirnov tests for temperature, pressure and residence time on number of viable spores (n: number of replica).*

Test parameters	Amount of samples (n)	p-value
Temperature (°C)		
200 vs. control[1]	3	0.519
250 vs. control[1]	3	0.047
300vs. control[1]	3	0.013
Pressure (MPa)		
0.6 vs. control[2]	3	0.288
1.0 vs. control[2]	3	0.274
Time (minutes) at 300° C		
1 vs. control[3]	3	0.670
10 vs. control[3]	3	0.025
Time (minutes) at Pressure 1 MPa		
1 vs. control[4]	3	0.643
10 vs. control[4]	3	0.643

1: Control[1] represents a control temperature of 21°C

2: Control[2] represents a control pressure of 0.1 MPa

3: Control[3] represents a control time of 0 minutes

4: Control[4] represents a control time of 0 minutes

3.3.2 Extrusion of *Bacillus amyloliquefaciens* spores in PET films

PET films of 0.8 mm thickness incorporated with *B. amyloliquefaciens* spores were successfully extruded in a stable process. The surface of the PET films was very characteristic in that many spots were visible due to the inclusion of the spores in the film as can be seen in Figure 13. This is a first indication of the feasibility to successfully incorporate bacteria spores in extruded polymer films.

Visible spots of spore

***Figure 13. A photograph of PET film embedded with spores showing visible
spots of spores on the film surface (indicated by arrows)***

The viability of the spores in the spores/PET films was tested by inoculation in nutrient medium and incubating for 24 h at 40°C. This tests the survival of spores through germination/growth under favorable conditions. When specific favorable conditions are provided, a spore germinates/grows through the following steps: i) spore activation, ii) first stage of germination whereby the spore core is partially rehydrated and dipicolinic acid (DPA) is released, iii) second stage of germination which involves cortex hydrolysis and metabolic activity resumes, and iv) outgrowth [22].

The viability test results of the PET films are shown in Figure 14. The control sample being pure PET film with no spores (Figure 14a) did not show any growth, whereas the sample extruded with 0.5 wt% spores showed bacterial growth all around the polymer film (Figure b). The growth observed in Figure 14b confirms *B. amyloliquefaciens* spores survived the extrusion parameters very well.
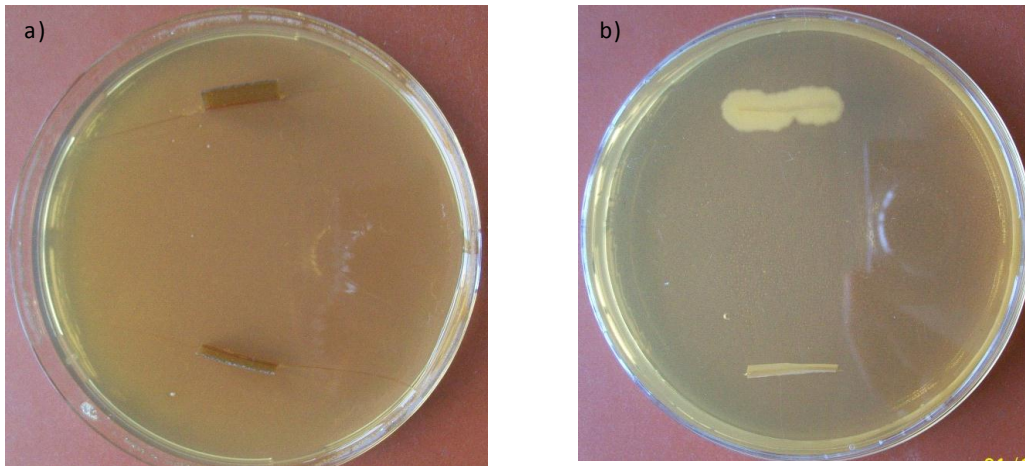
Figure 14. *Viability tests (PET Films inoculated in nutrient medium and incubated for 24 h at 40°C. a) Control PET film with no spores showing no growth, b) PET films extruded with 0.5 wt% spores showing bacterial growth.*

As discussed earlier, the resistance of *B. amyloliquefaciens* spores to PET film extrusion can be explained by three factors, namely; the low operating pressures, the short residence time and the formation of spore agglomerates during extrusion. Several factors can cause the spores to agglomerate in the extruder, including; the hydrophobic nature of the spores, heat treatment and shear stress [38, 39]. An example of spore aggregation in the PET film is given in the SEM picture shown in Figure 15.
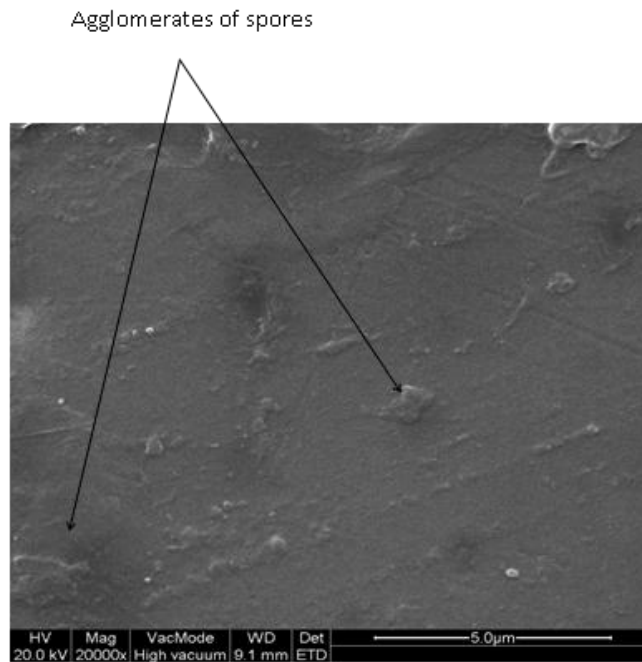
Figure 15. SEM micrograph of a PET film embedded with spores. The surface is characterized by spore aggregations (indicated by arrows)

3.3.3 Extrusion of *Bacillus amyloliquefaciens* spores in PET fibers

The concentration of spores successfully incorporated in PET fibers was from 0 to 10 wt%. Concentrations higher than 10 wt% blocked the spinnerets and therefore extrusion could not take place. The two sterilizing techniques studied proved to be equally efficient for fibers before inoculation (Table 4). PET fibers extruded with a 2 wt% spore concentration showed the smallest survival of spores while concentrations of 4, 6, 8 and 10 wt% of spores showed almost the same survival rate. These results further confirm the resistance of *B. amyloliquefaciens* spores to melt extrusion process parameters.

Table 4. *Surviving spores in fibers after sterilizing in soaking in a sodium hypochlorite (NaClO) solution, a Dettol solution (5 %) and in an ethanol solution (96%) for 10 minutes each or by pasteurizing at 80° C for 10 minutes (n: number of replica)*

Concentration of spores (wt%)	Amount of samples (n)	Sterilized (Mean Log CFU/g dry fibers)	Pasteurized (Mean Log CFU/g dry fibers)
0		0	0
2		14 ±0	14 ±1
4		20 ±1	20 ±1
6	4	20 ±1	19 ±1
8		20 ±1	19 ±1
10		20 ±1	19 ±1

3.3.4 Exponential function equation to relate the survival rate of spores with concentration of extruded spores

In order to be able to relate the survival rate of spores with concentration of extruded spores, and exponential function equation was used (Figure 16). The exponential curve has a fitted equation of Y= -18,855 exp(-x) + 19,133, where Y is the number of viable spores while X represents the concentration of spores incorporated in the PET fibers. The R-squared of 1 shows a very strong linear relationship between the number of viable spores and concentration of spores incorporated in the PET fibers. This means that an increase in spore concentration incorporated in PET fibers results in increase in the number of viable spores. However, there seems to be leveling on the number of viable spores at 4-10 wt% spore concentration.

$y = -18,855 \exp(-x) + 19,133$
$R^2 = 1,00$
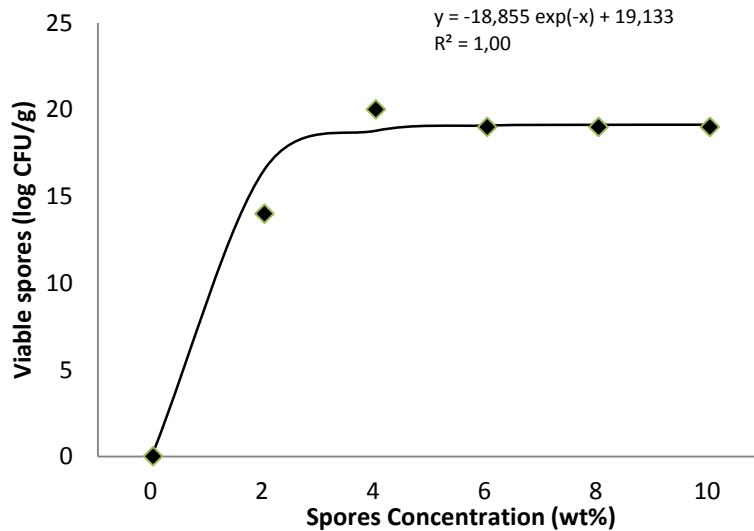
Figure 16. An exponential curve to relate survival rate of spores with concentration of extruded spores.

The observed leveling on the number of viable spores is probably the yield point showing the maximum concentration of spores that can be added in the PET fibers without negatively affecting the number of spores that survives the processing. The yield point is probably as a result of poor blending of PET/spore at higher spores concentration. As the concentration exceeds the optimal level, some spores may have been left in the extruder walls. Additionally, as can been seen in Figure 17, at lower spore concentration (Figure 17a), less spores are observed on the fiber surface as compared to fibers extruded with higher concentration of spores (Figure 17b). This means that higher spore concentration of spores could have forced more spores on the surface of the fibers, probably leaving some spores hanging loosely and may have been dropped in the processing hence lowering the number of spores that survives. However, more tests are required to verify this trend.
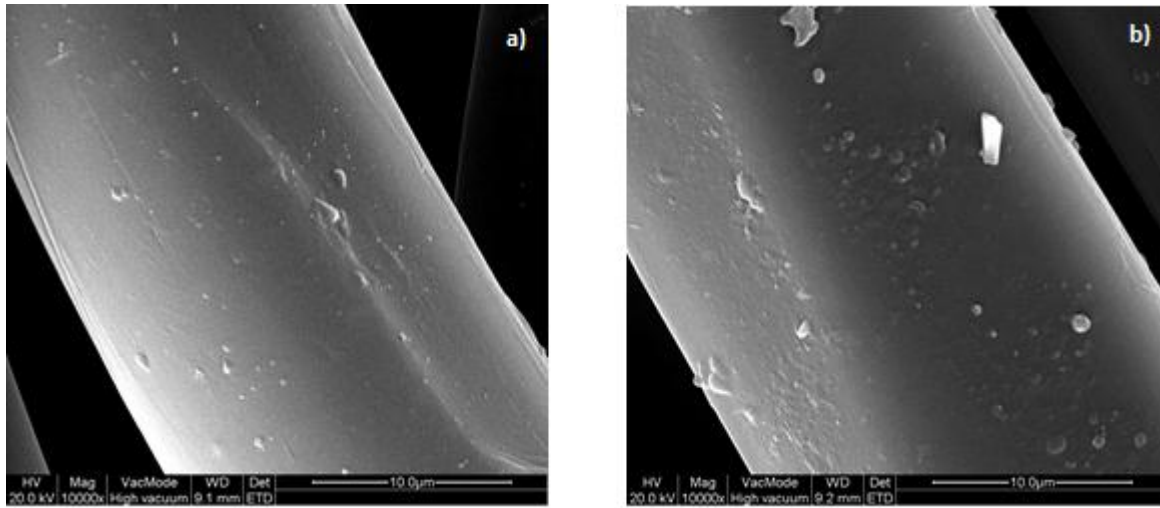
Figure 17. SEM micrographs showing the distribution of spores in the PET fibers at different spores concentration: a) 6 wt% spores, b) 10 wt% spores.

3.4. Conclusion

We have demonstrated a suitable technique for testing resistance of spores to extrusion process parameters (temperature, pressure and residence time). Presented results showed that pressure did not have a significant effect on the number of viable spores. Increase in temperature resulted in decrease in the number of viable spores showing a significant effect at 250 and 300°C. Residence time did not have any significant effect at 1 Mpa while 300°C temperature showered a significant effect at 10 minutes residence time but no significant effect was noted at 1 minute. These results shows that temperature is the most critical parameter with the greatest effect on the survival of spores during extrusion.

Therefore, the demonstrated technique proves to be cheap, easy and fast hence can be used as a model system to study the biological response of spores in relation to high temperature, pressure and residence time. It was also shown that spores can be successfully incorporated directly into PET polymer matrix during melt extrusion to develop textile bio-aggregates.

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Chapter 4

Morphological and material properties of Poly Ethylene Terephthalate (PET) fibers with incorporated spores

Following the current demand for textiles with new functionalities and improved properties, there has been a continuous effort to modify Poly(ethylene terephthalate) (PET) materials. In the preceding chapter, it was demonstrated that Bacillus amyloliquefaciens spores can be incorporated in PET fibers during extrusion. However, the extent to which they can be incorporated without fundamentally changing the properties of the fibers is not known.

In this chapter, scanning electron microscopy (SEM), transmission electron microscopy (TEM), optical microscopy (OM), differential scanning calorimetry (DSC), tensile tester, Raman and Fourier transform infrared spectroscopy (FT-IR) are used to study the properties of PET/spores fibers.

4. Morphological and material properties of Poly Ethylene Terephthalate (PET) fibers with incorporated spores

4.1 Introduction

Poly Ethylene Terephthalate (PET) popularly known as polyester is a long-chain semi-crystalline thermoplastic polymer that is widely produced with a total production of 31.929 million tons in 2009 [1]. Due to its superior mechanical properties, ability to be recycled, low cost and ease of processing, PET fibers are extensively used in various textile and technical applications [2-4].

In order to meet the current need for multifunctional textiles with improved and specific properties, a continuous effort is been made in modifying PET fibers and materials. To date, various additives such as nucleating, antimicrobial, enzymes, colorants, cross-linking and matting agents have been incorporated in PET for applications in various fields [5-8]. Techniques for incorporating additives in PET fibers include addition of compounds during the fiber production process or applying compounds on the material surface as a post-treatment process (Figure 18). The choice of technique depends on functionality that is required as well as the characteristics of the additives, like its migration to the surface during use [2, 9, 10].
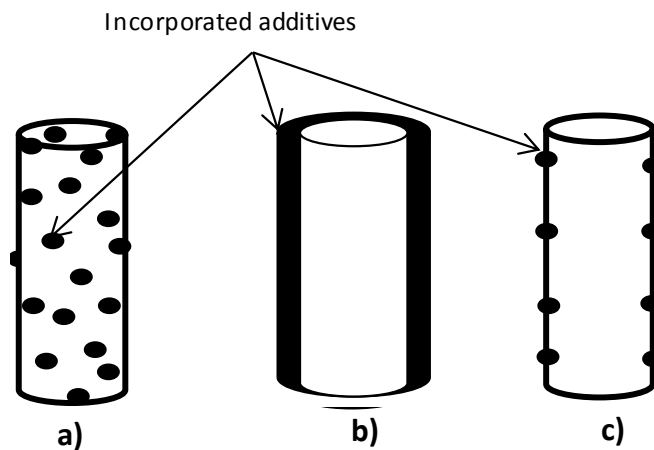
Figure 18. Additives incorporated in textiles: a) during fiber production, b) as a post-treatment (Padded, Sprayed, etc.), c) Chemically bonded on the surface

When additives are incorporated at the finishing stage, they are physically or chemically bond to the surface of the material and their resistance to abrasion and washing is never guaranteed. Furthermore, finishing techniques like spraying can cause spills which may be detrimental to the environment. Moreover, badly applied coatings may lead to partial loss during use, care and storage [11]. In contrast,

incorporating additives during fiber production entraps the additives in the polymer matrix during solidification of the fiber forming polymer and may take part in crystallization process resulting to a strong bond between the polymer and additives as well as providing high resistance to abrasion [2, 10]. In addition, the additive can migrate slowly to the surface acting as a slow release mechanism which guarantees an extended period of additive activity [12]. The release rate is influenced by the physical and chemical characteristics of the polymer in relation to the characteristics of the additive [13, 14].

Earlier studies have shown that any additive incorporated in semi-crystalline polymers like PET during melt extrusion can greatly influence the overall properties of the end product. The data available reports effects on tensile properties like tensile strength, Young's modulus, flexural strength and elongation at break [3, 15, 16]. Onuegbu et al., [17] investigated the effects of extruding 0.5 ±0.2μm particulates in polypropylene during melt extrusion. Their results revealed an increase in tensile strength, Young's modulus and flexural strength with increase in particulate loading until a given level where increase of particulates resulted to decrease in the mentioned properties. Similar results have been reported by Leskovšek et al., [9] who incorporated 2 μm diameter microcapsules in polypropylene and Tavman, [18] who extruded PET fibers with incorporated particulates of 4-6 μm diameter which are the same size with spores used in this study. This means that it is important to investigate the optimal concentration of additives that will give a good compromise on material properties and the required functionality.

Several other studies have investigated factors that influence the material properties of additive-polymer products. Zeng et al. [4] who extruded Phosphonium Vermiculite fillers in PET fibers and Arencón [19] who investigated the fracture toughness of polypropylene-based particulate composites found out that interfacial bonding between the additive and polymer matrix and the dispersion of the additive in the matrix are the main determinant of properties of the end products. These factors depend on the characteristics of the additives like size distribution, shape, loading and the chemical nature. Generally, smaller particles and good dispersion produce better tensile properties while larger particles give poor material properties [15, 20]. This trend is mostly as a result of stress transfer between the additives and the polymer. Large particles can block the stress transfer between the additives and the polymer which may leads uneven fibers as well as formation of voids and cracks in the polymer matrix that can weakens the fibers (Figure 19) [2, 21]. On the other hand, small sized particles allow efficient stress transfer resulting in improved properties of the fibers [21, 22, 23]. High loading and poor dispersion of small sized particles can result in formation of agglomerates which can have the same effect as large sized particles. Additionally, chemical compatibility between the polymer and the additives can also influence the morphology and the material properties of the resulting materials. Therefore, understanding the interfacial bonding and dispersion of additives as well as the chemical compatibility of the additives and the polymer is important while investigating the material properties of the additive-polymer products.

Chapter 4 - Morphological and Material Properties of poly Ethylene Terephthalate (PET) Fibers with Incorporated Spores
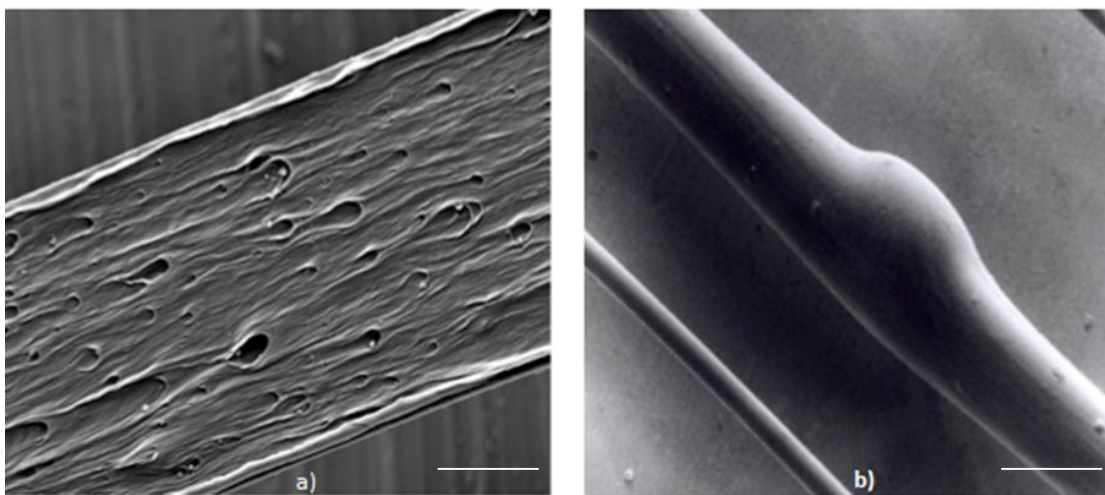

Figure 19. Polypropylene fibers with incorporated particulates of average diameter of 2μm: a) SEM micrograph showing voids in the fiber, b) Electronic micrograph showing uneven polypropylene fiber (63x) (The scale bar is 2 μm) [9]

In chapter 3, it was reported that bacteria spores are resistance to melt extrusion process parameters and can be successfully incorporated in the polymer matrix during fiber formation [24]. However, the extent to which the spores can be incorporated without fundamentally changing the properties of PET fibers is not known. The outer coats of the spore is mainly made of proteins and at high temperature these proteins are denatured causing spore agglomeration [24]. These agglomerates of spores may possibly have an effect on the properties of the resulting fibers. Therefore, the current study aims at giving an in-depth understanding of the morphological and material properties of PET fibers incorporated with spores. PET fibers incorporated with 0 to 10 wt% spores were extruded in a single screw extruder. The spores-PET interaction and dispersion was studied with Scanning electron microscopy (SEM), Transmission electron microscopy (TEM) and optical microscopy (OM). The material properties like tensile strength, Young's modulus and elongation at break were studied on Favimat tensile fiber testing machine, while thermal properties and crystallinity behavior were tested on Differential scanning calorimetry (DSC), Raman and Fourier transform infrared spectroscopy (FT-IR).

Results from this study are expected to give an insight in how incorporated micro-organisms affect the fiber properties. This knowledge will give new inspirations on how and where to improve the extrusion process in order to develop better quality fibers. This is valuable as the integrated micro-organisms could add a multitude of functions into textiles creating novel and niche products.

4.2 Experimental set- up

4.2.1 Extrusion

Spores of the thermophile bacterial strain *Bacillus amyloliquefaciens,* were extruded in Arnite thermoplastic PET pellets (Arnite A02 307) from Royal DSM N.V. (Netherlands).

Prior to extrusion, the PET pellets were dried in an oven for two days at 70°C after which pure *B. amyloliquefaciens* spores were added by gravimetric dosing during spinning to obtain a 0 (control), 2, 4, 6, 8 and 10 wt% concentration of spores to PET polymer. This mixture was extruded into multi-filament fibers by a single screw extruder (General extrusion technology, China). The three different heating zones of the barrel were set to 280 ±10°C for the feed stock (transport of material), 290 ±5°C for plasticizing (compression) and 295 ±2°C for pumping (metering). The die was heated at 295°C, the pressure was around 6.0 ±0.2 MPa while the average residence time was approximately 5 ±0.5 minutes. The resulting fibers had an average linear density of about 7 ±0.5 dtex.

4.2.2 Characterization methods

a) Tensile testing
The tensile properties, like tensile strength, modulus and elongation at break of resulting extruded spores/PET fibers were determined with the FAVIMAT-ROBOT (Textechno, Germany). The tensile properties were tested using settings similar to ISO 5079 using 10 mm/min speed and a gauge length of 50 mm. All samples were kept in a conditioned room of 65 ±2% humidity and a temperature of 20 ±2°C for 24 hours before testing. Average of 50 single filaments was tested for each property investigated.

The two-sample Kolmogorov-Smirnov statistical test was used to determine how significantly the spores affected the tensile properties at each spore concentration level (0-10 wt%).

b) Microscopy characterization
The morphological properties of the fibers were studied using an optical microscope (Olympus BX51), Olympus ocular and plan 100x/1.25 lens with oil, a scanning electron microscope (Joel Quanta 200 F FE-SEM) and a transmission electron microscope (Joel JEM 2200 FS-TEM).

To avoid electron loading on the SEM sample, the samples were placed on a stub and gold coated using a sputter coater (Balzers Union SKD 030). The SEM micrographs were made under high vacuum voltage of 20.0 kV with a spot size of 4 to 6 nm, a dwell of 3000 μs and a working distance of 10 mm.

c) Differential Scanning Calorimetry (DSC)
Differential Scanning Calorimetry was used to study the thermal properties of PET/spores fibers were investigated using a DSC (Q 2000, TA-Instruments, DA, USA) with a RCS cooling accessory and nitrogen as a purge gas flowing at a rate of 50 ml/min. Calibrations were performed based on the Tzero™ approach and in T4P mode. The temperature calibration was performed using two certified standards

66

(indium and tin) at 20°C/min. Samples weighing 5 ±1 mg were loaded in an aluminum DSC pan (Tzero Pan) and sealed with an aluminum lid (Tzero lid) using the TA crimping tool. The samples were first equilibrated at 50°C for 10 minutes, the temperature was then increased to 290°C at a ramp of 10°C/min after which they were isothermal conditioned for 5 minutes at 290°C and then cooled down to 0°C at 10 °C/min. Two heating were recorded: the first heating investigated the effects of spores on melting temperature and heat fusion which is defined as the area under the melting curve, while the second heating was used as an intrinsic material reference.

The modulated mode of the DSC instrument was used to study the glass transition and onset temperatures of the PET samples. Heat flow of the materials was monitored over the range of 0°C to 290°C with a temperature modulation of ±0.2°C/min superimposed on a 2°C/min run. Universal Analysis 2000 software was used for the interpretation of the thermographs.

The degree of crystallinity was calculated from the obtained enthalpies using the following equation (1):

$$Crystallinity\ (\%) = \frac{\Delta Hx1 - \Delta Hy1}{\Delta Hx0\ .\ (1 - \frac{wt\ \%}{100})} * 100 \qquad (1)$$

Where $\Delta Hx1$ is the melting enthalpy of the sample, $\Delta Hy1$ is the crystallization enthalpy while $\Delta Hx0$ is the melting enthalpy of perfect crystalline PET sample and wt% is the total weight percentage of spores. An enthalpy of 140 J/g was used for perfect crystalline PET.

d) Spectroscopic analysis

To investigate the crystallinity and bonding quality of PET fibers incorporated with spores, Raman and FT-IR spectroscopy was used.

Raman spectra were obtained by using the Perkin-Elmer Spectrum GX 2000 spectrometer with a Raman beam splitter, a diode-pumped YAG infrared laser (1064 nm and laser power of 800Mw) and InGaAs detector. The measurements were recorded in the back-scattering mode (180° excitation optics) with 64 scans, a resolution of 4 cm^{-1}, a data-interval of 1 cm^{-1} from 200 to 3200 cm^{-1}. The samples were aligned on a sample holder and spectra taken in a random orientation.

For the FT-IR studies, a Perkin-Elmer Spectrum GX 2000 with a Peltier-cooled DTGS Mid-IR detector, a Mid-IR source an extended KBr beam splitter and a diffuse reflection (DRIFTS) accessory was used. All samples were studied with 64 scans, a resolution of 4 cm^{-1}, a data-interval of 1 cm^{-1} from 400 to 4000 cm^{-1} in the DRIFT accessory. Before recording the DRIFT spectrum, a background spectrum to correct the atmospheric H_2O and CO_2 bands was recorded using the provided sandpaper standard samples after which the sample was focused and the energy analyzed.

4.3. Results and Discussion

The selection of materials for textile and industrial applications strongly depends on their thermal, chemical and tensile properties. Because fibers are the basic units from which complicated material structures are developed, their intrinsic characteristics influence the overall properties of the materials. For this reason, engineers and designers use fiber properties to develop materials of specific quality for a given application. Fiber properties are influenced by their morphology which is determined by polymer type, additives used, process technique and processing conditions. Among the important fiber properties are the mechanical characteristics which are determined by the tensile strength, Young's modulus and elongation at break.

4.3.1 Fiber morphology

a) *Optical microscopy*

The optical micrograph of blank PET-fibers shows a smooth appearance (Figure 20a) whereas the samples extruded with spores are very characteristic in that they exhibit cracks parallel to the fiber axis (Figure 20b). These cracks can be associated with a decrease in macromolecule arrangement of PET along the fiber axis possibly caused by adding the spores in the structure [25, 26]. This suggests poor dispersion of spores in the polymer matrix and poor spore-matrix interface adhesion bonding [25-28].
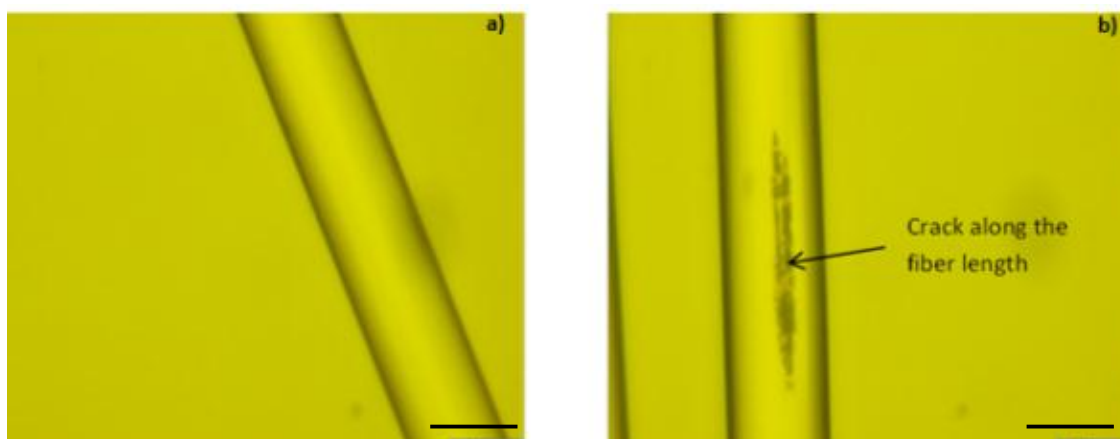
Figure 20. Two representative optical micrographs of PET fibers. A) Blank PET fiber (control), B) PET fiber incorporated with 2 wt% spores exhibit cracks parallel to the fiber axis (arrow showing) (The scale bar is 20 µm)

b) *Scanning Electron microscopy*

The SEM micrograph of the blank PET fibers show a smooth fiber surface (Figure 21a) whereas the fibers incorporated with spores have visible spore agglomerates on the surface (Figure 21b). Two main factors that may have contributed to the agglomeration of spores in the polymer matrix. First to consider is the

extrusion process conditions specifically high temperature and shear stress. Spores are known to form agglomerates in suspensions during heat treatment due to increased surface hydrophobicity caused by denaturing of the protein coats [18, 29, 30]. On the other hand, force originating from shear stress possibly impacted a higher energy on the spores which overcame the spores' electrostatic repulsive force leading to formation of agglomerates. The second factor is poor dispersion of spores in the polymer matrix influenced by spore size and loading. Normally, small sized particles tend to agglomerate more than larger particles while chances of forming agglomerates increases with loading [4, 15, 16, 31, 32].
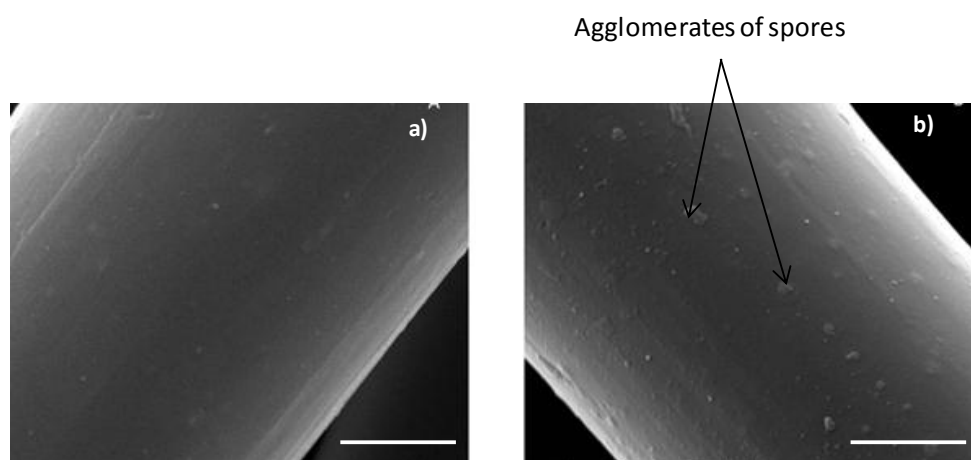
Figure 21. SEM micrographs for PET fibers. A) Blank PET fiber (control), B) PET fiber incorporated with 2 wt% spores exhibiting spores agglomerates on the fiber surface (arrow showing) (The scale bar is 10 μm)

c) *Transmission electron microscopy*

The transmission electron microscopy was used to look at the induced roughness of the surface of the fibers by the inclusion of spores noticed by optical and SEM microscopy. The electron dense spots (black) are most likely the spores embedded into the PET-matrix (Figure 22b) as they were not found on the blank fibers (Figure 22a). No preferential orientation was seen of the spores in the PET-fibers suggesting a random distribution.
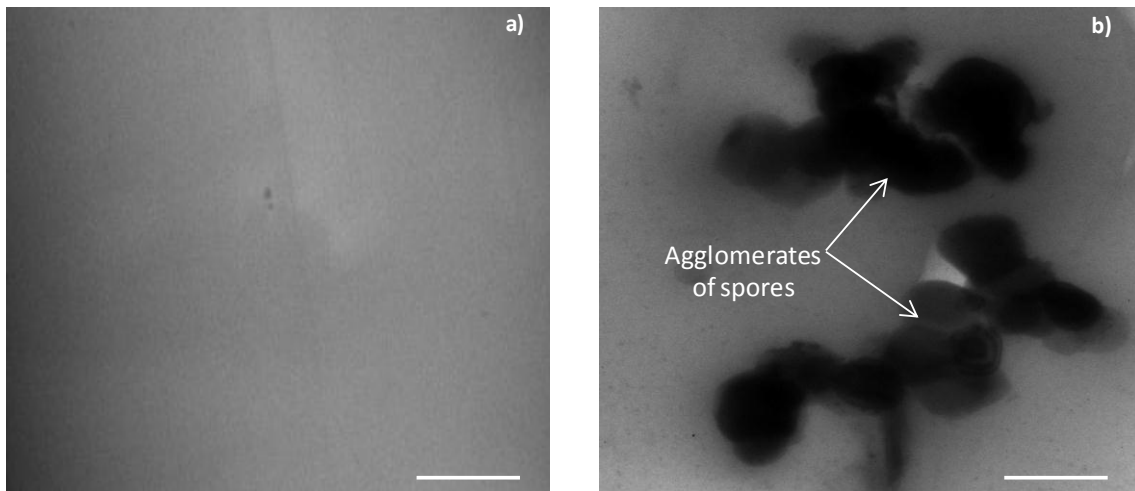
Figure 22. *TEM micrographs of: a) Blank PET fiber, b) PET fibers incorporated with 6 wt% spores showing spores agglomerates in the polymer matrix (The scale bar is 20 μm)*

4.3.1. Mechanical properties

A negative trend can be noted whereby an increase in spore's concentration results to a decrease in tensile strength, Young's modulus and elongation at break as shown in Figure 23a-c. Though the statistical tests shows a significant decrease in the tested mechanical properties ($p \leq 0.005$), the R squared shows a very low linear relationship which means that the decrease may be negligible from a practical point of view.

This decreasing trend in the mechanical properties with increase in spores concentration can be attributed to the observed spore agglomerates in the polymer matrix. Spore agglomerates may have induced cracks on the fiber surface, obstructing stress transfer between the spores and the polymer matrix creating weak points in the fibers which resulted in earlier failure [21, 26-28]. Additionally, poor dispersion of spores that can be influenced by spore size and loading may have also led to formation of agglomerates which possibly caused de-bonding between the spores and the PET polymer that resulted to decreased material properties [32-36].
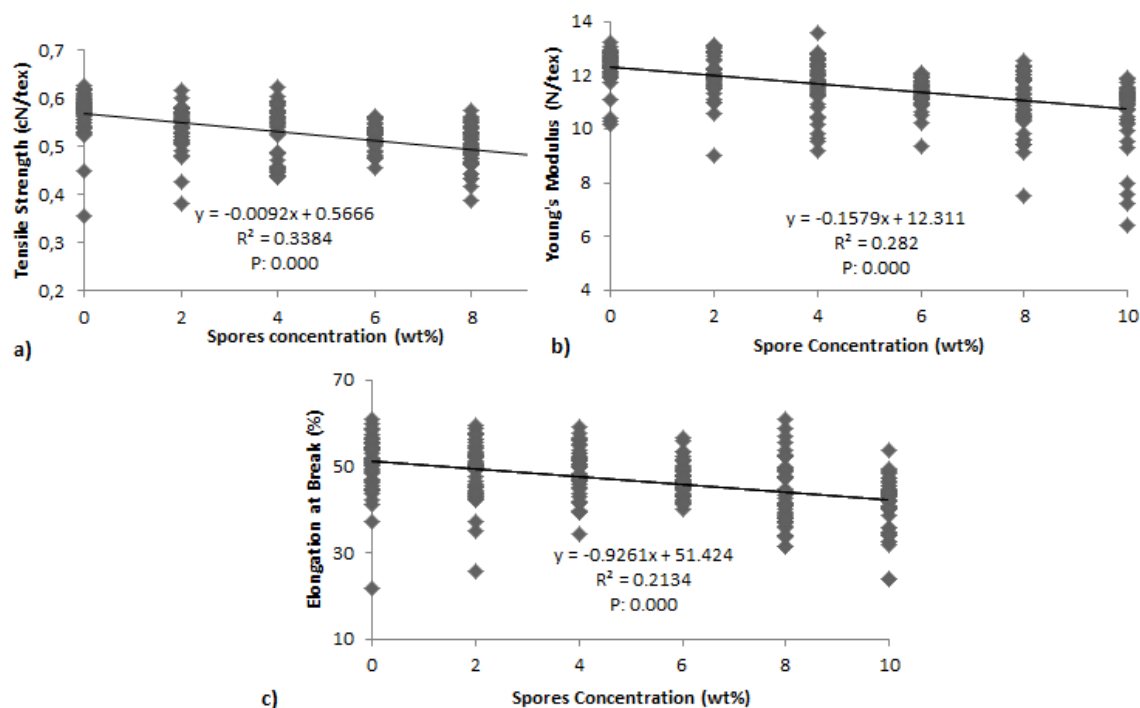
Figure 23. Tensile properties of extruded PET fibers with different concentration of spores. a) Tensile strength, b) Young's modulus, c) Elongation at break (50 replicates each)

4.3.3. Thermal properties

The thermal properties of a material determine how it will react to heat applications during care and use [39]. The Differential Scanning Calorimetry (DSC) results for thermal properties of fibers incorporated with spores are given in Figure 24a-c. The addition of spores leads to a slight increase in the endothermic melting peak (melt temperature) and the exothermic peak of cold crystallization (crystallization temperature). However, the crystallization percentage increases with the concentration of spores which shows that the spores may have induced PET crystallization upon cooling by reducing the amorphous regions and increasing the crystalline regions.
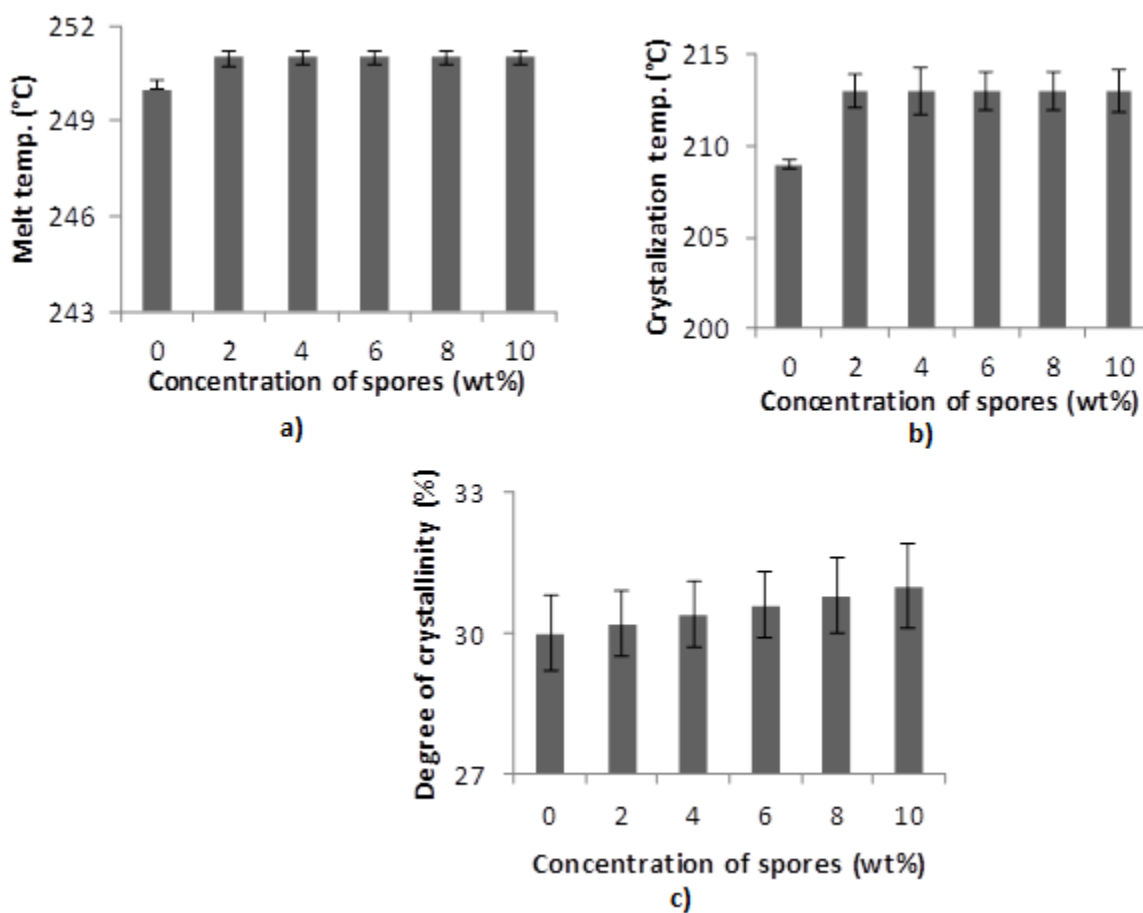
Figure 24.The DSC results for thermal properties of fibers embedded with different concentration of spores. a) Melt temperature, b) Crystallization temperature, c) Degree of crystallinity (five replicates each)

These results further suggest that spores may have worked as crosslinking point of PET molecular chains making them more complete hence restricting the motions of the molecular segments resulting to a brittle fiber that higher strength, lower elongation at break and high Young's modulus. This implies that because PET is a semi-crystalline polymer, any foreign material interacts with the polymer at the amorphous phase resulting to increased crystallinity, a phenomenon previously reported by [3] in their work on Polyethylene Terephthalate/Clay nanocomposites. Due to the fact that crystallinity can influence the thermal of a material [40, 41], the slight increase in crystallinity could be the reason behind the observed increase in melt and crystallinity temperatures.

4.3.4. FTIR and Raman spectroscopy

Figure 25 and Figure 26 respectively shows FT-IR and Raman spectra for blank PET and PET fibers extruded with spores. All the samples regardless of their spores' content have identical spectrums.
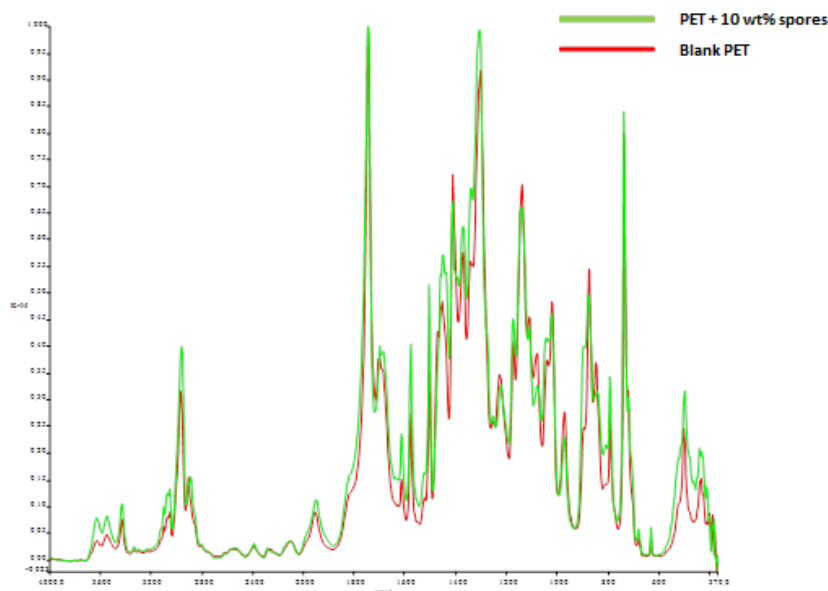
Figure 25. FT-IR spectrum for blank PET fibers (shown in green color) and PET fibers incorporated with 10 wt% spores (shown in red color)
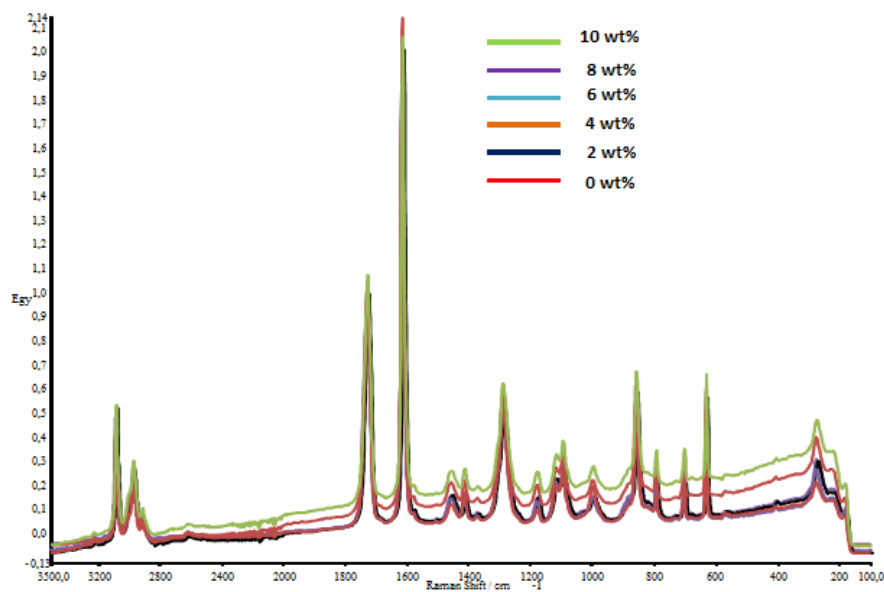

Figure 26. Raman spectrum for PET fibers embedded with different concentrations of spores

The crystalline content of semi-crystalline PET can be determined from the Raman spectra in two ways [14]. The first technique determines the peak ratio of band at approximately 1,120 cm^{-1} (Ester CO–O or C–C stretching) and the Raman shift at approximately 1,100 cm^{-1} band (C–O and C–C stretching or C–O–

73

C bending). Highly crystalline samples show a large peak at 1,095 cm^{-1} while amorphous samples show a shoulder at the 1,120 cm^{-1} peak. Figure 27(a) shows that sample at all concentration levels had the same Raman shift at 1,095 cm^{-1} band and an identical shoulder at 1,120 cm^{1}.

 The second technique determines the peak width of Raman emission at 1,730 cm^{-1}. Highly crystalline samples give a narrow peak while the amorphous bandwidth is evidently broader. Observing the Raman spectrum in Figure 27(b), all samples regardless of spore's concentration had the same peak width which was neither too broad nor too narrow.
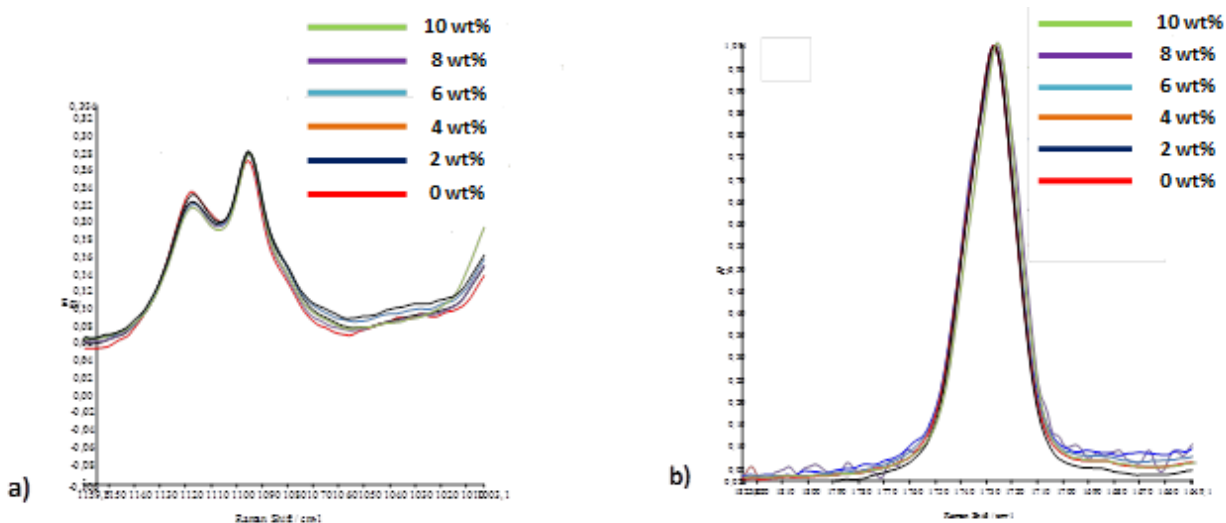
Figure 27. Raman spectrums of PET fibers extruded with different concentrations of spores. a) At 1,095 cm^{-1} and 1,120 cm^{-1} band, b) At 1,730 cm^{-1} band

The difference between the DSC and spectroscopy results may be explained by the fact that spectroscopy tests analyze behavior changes at a specific point on the surface of the sample while DSC analyzes the bulk of a relatively bigger sample. Therefore, the FT-IR and Raman results suggest absence of spores on the surface of the fibers which may be attributed to poor dispersion and inhomogeneous distribution of spores in the polymer matrix.

Optimal process conditions

Generally, the most optimal conditions for producing spores/PET fibers would be to have the highest concentration of spores integrated in the PET fibers as this would result in a higher functionality. However, many papers have shown that incorporating foreign additives in polymer matrix can influence the fiber's tensile properties resulting in poor quality fibers with high load of additives [3, 4, 9, 15-23]. So the optimal conditions in this study follows a fair compromise between the concentration of spores and the corresponding tensile properties.

Chapter 4 - Morphological and Material Properties of poly Ethylene Terephthalate (PET) Fibers with Incorporated Spores

The concentration of spores successfully incorporated in PET fibers ranged from 0 to 10 wt%. Concentrations higher than 10 wt% blocked the spinnerets and therefore could not be extruded. Increase in spores concentration resulted to a decrease in tensile properties with 10 wt% spores giving the lowest properties (Figure 3). In order to select an optimal spore concentration, it important to have a fair trade-off between the tensile properties and concentration of spores. Therefore, 6 wt% spores was selected as the optimal concentration because its tensile properties fall within the acceptable range and has the narrowest standard deviation on the tested properties.

Hence based on this study, the optimal process parameters for extruding *B. amyloliquefaciens* spores in PET fibers are:
- Spore concentration of 6 wt% of *B. amyloliquefaciens* spores
- Barrel section 1 heated at 280°C for the feed stock
- Barrel section 2 heated 290°C for plasticizing
- Barrel section 3 heated 295°C for pumping
- Die heated at 295°C
- Pressure of 6.0 ±0.2 MPa
- Residence time of 5 ±0.5 minutes

Chapter 3 on resistance of *B. amyloliquefaciens* spores to melt extrusion process parameters [3] clearly showed that spores can be successfully incorporated in PET fibers during extrusion. However, the present study shows incorporating spores in PET fibers can result in decreased in tensile strength (12 ±4.5%) and elongation at break (11 ±5.8%) and an increase in Young's modulus (8 ±4.9%). Nevertheless, PET fibers normally have a tensile strength of 0.3-0.7 N/tex, strain of 20-50%, modulus of 7.7-8.7 N/tex and a melting temperature around 250 °C [33]. By comparing the results of this study, it can be seen that the reported properties falls within the acceptable range and therefore the produced fibers are as good as normal PET fibers only that the fibers produced in this work have an additional advantage that they can have an additional function.

4.4. Conclusion

From the study on morphological and material properties of PET fibers incorporated with *Bacillus amyloliquefaciens* spores**,** the following can be concluded:

The tensile strength, Young's modulus and elongation at break were dependent on the spore's concentration. Additionally, a slight increase in degree of crystallinity, melting and crystallization temperatures was observed and it remained constant at all spore's concentration levels. This indicates that incorporating spores possibly changed the polymer's structure due to poor dispersion of spores and formation of agglomerates in the polymer matrix. However, the properties of the produced fibers fall within the acceptable range and are therefore as good as normal PET fibers and can be used in textile materials similarly.

In case of a need to further optimize the properties of these fibers, the problem of spores dispersion and formation of agglomerates should be solved. Hence, the critical challenge would be to identify the best technique to promote homogeneous dispersion of spores and find ways to prevent spores from forming agglomerates in the polymer matrix.

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Chapter 5

Electrospinning Microencapsulated Biological Mosquito Repellents in Polyvinyl Alcohol Nanofibers

There is an increasing interest for use of repellents to prevent not only the bites of nuisance mosquitoes, but also the transmission of mosquito-borne diseases. A mosquito repellent from a biological source, which is commercially available and has been reported to have DEET-like efficacy, is oil of lemon eucalyptus, which has p-menthane-3,8-diol (PMD) as its active ingredient.

In this chapter, process parameters for electrospinning blank PVA are first optimized after which 0-16 wt% microencapsulated PMD are incorporated. The properties of the resulting nanofibrous structure are studied using scanning electron microscopy (SEM), differential scanning calorimetry (DSC), tensile tester, Raman spectroscopy and vertical landing bioassay.

5. Electrospinning microencapsulated biological mosquito repellents in polyvinyl alcohol nanofibers

5.1. Introduction

Mosquitoes are vectors for many infectious diseases like malaria, yellow fever, dengue, Nile fever, and encephalitis, which are transmitted by infected females as they blood-feed on their human hosts [1, 2]. Each year, there are around 200 million cases of malaria alone, causing over a million deaths worldwide with the majority being reported in sub-Saharan Africa [3-6]. The lack of an effective vaccine against malaria makes vector control the most important strategy in countering the transmission of this disease. Repellents are an alternative or additional tool to existing vector-control methods such as insecticide treated nets (ITNs) or indoor residual spraying (IRS), against which mosquitoes are increasingly developing resistance [7-9]. Over the last years, the use of repellents has received increasing interest from the scientific community [10-12].

One of the most effective biological mosquito repellents from biological origin is p-menthane-3,8-diol (PMD), commonly known as oil of lemon eucalyptus oil, although it is actually a by-product of the hydro-distillation of the essential oil [13-16]. It is extracted from twigs and leaves of *Corymbiacitriodora* (formerly *Eucalyptus citriodoraHook*) of the Myrtaceae family. PMD has been shown to be effective against mosquitoes of several genera including vectors of human disease like malaria mosquito *Anopheles gambie s.s* [17-19]. The U.S. Environmental protection agency (EPA) considers PMD as safe and not posing a risk to human health [20]. PMD products on the market today are only available in sprays, creams and lotions that are greasy, can irritate the skin and are not comfortable to the user [21]. Additionally, PMD has a fast evaporation rate making it only effective for a limited amount of time, hence frequent re-applying is needed. This chapter describes how these disadvantages were overcomed by incorporating encapsulated PMD microcapsules in PVA nanofibers by electrospinning.

Micro-encapsulation is a common technology used to improve and sustain a controlled release of active compounds, protecting the compounds from environmental damage as well as facilitating easy handling of the compounds [22]. Hence, by using encapsulated PMD, a slow release is achieved providing a longer protection time against mosquitoes by breaking the capsules over time. Moreover, the electrospun nanofibers create a large surface area which, when incorporated with PMD microcapsules will lead to a higher functionality textile because a larger surface results in more evaporation of the repellent when freed therefore maximizing protection. Also, microencapsulation prevents PMD from being in direct contact with the skin thus reducing skin irritation. Consequently, the fact that electrospinning is a cheap and fast technique of spinning fibers with diameters ranging from 10nm to several hundred nanometers from polymer solutions using an electric field, a cheap and fast production method for mosquito repellent textiles is achieved by incorporating the encapsulated PMD in nanofibers during electrospinning [23].

Chapter 5 - Electrospinning Microencapsulated Biological Mosquito Repellents in Polyvinyl Alcohol Nanofibers

Generally, polymers like polyamides, polyester, polyvinyl alcohol (PVA), etc. can successfully be electrospun into nanofibers [24]. However, most polymers like polyester can only be dissolved in toxic solvents which are not environmental friendly and can be harmful to workers. Additionally, some polymers are not biodegradable which further harms the environment. Unlike most of these polymers, PVA is water soluble hence doesn't require toxic solvents to dissolve it, is easy to process and is biodegradable [24, 25]. These attributes have made PVA cover a wide range of applications like the medical sector, cosmetics as well as in textiles and therefore PVA polymer was chosen for this study.

In the recent past, many studies have shown that it is possible to add additives like inorganic particles, cellulose nano-crystals, polymer resin, multi-walled carbon nanotubes, etc., in nanofibers during electrospinning [23, 26, 27]. Incorporating particulate additives during electrospinning can have some effects on the properties of the spinning solution, on the stability of the process, on the morphology and material properties of the resulting nanofibrous structure. These effects depend on the characteristics of the particles like the size distribution, chemical properties of the materials used, interfacial bonding, agglomeration, dispersion and amount incorporated [28, 29]. Ideally, incorporation of particles into nanofibers potentially provide nonrandom structure of microcrystalline in the resulting nanofibrous structure which would lead to improved mechanical properties like tensile strength and modulus. However, in most cases, adding particulates affects the solution conductivity and or viscosity which can then have an influence on the spinning process resulting in defects such as beads on the fiber surface like what was observed by Xiao-Jian et al., [27]. Such defects affect the cohesive force between the fibers of the nanofibrous structure resulting in poor mechanical properties.

The electrospinning process parameters were first optimized with blank PVA nanofibers, where after 0-16 wt% microencapsulated PMD was incorporated. The morphology of the resulting nanowebs was determined with Scanning Electron Microscopy (SEM) and the tensile strength analyzed on the Favimat tensile testing machine. Furthermore, the thermal properties and crystallinity behavior were determined with the Differential Scanning Calorimetry (DSC). Finally, the mosquito repellency of the nanowebs was tested using the malaria mosquito *Anopheles gambiae s.s.* in a vertical landing bioassay.

Therefore, this chapter presents slow release capabilities for mosquito repellents not present in other textiles. This leads to an increase in efficiency of the repellant therefore providing an improved protection against mosquito bites. Additionally, the possibility to incorporate other volatile biological repellents is provided as well as the produced nanowebs can be integrated into existing textiles to develop a new generation of mosquito repellent textiles.

5.2. Experimental set-up

5.2.1. Materials

Polyvinyl alcohol (Mowiol 40-88, MW 205 000) was bought from Sigma Aldrich. Microcapsules with a mean diameter of 5 ±1 μm made from melamine formal resin encapsulating p-menthane-3, 8-diol (PMD) were supplied by Devan chemicals (Belgium).

5.2.2. Method

a) Optimizing electrospinning parameters for pure PVA

First we investigated the process parameters for electrospinning blank PVA nanofibers, namely concentration, tip-to-collector distance, flow-rate and applied voltage on the fiber morphology and stability of the process. The influence of PVA concentration was determined at 4, 8, 12, 16 wt% PVA concentrations electrospun at 14 cm tip-to-collector distance, 1 ml h^{-1} flow rate and voltage of 16-22 kV. Afterwards, the influence of applied voltage was determined at 8, 12, 16, 20 kV, using 8 wt% PVA solutions at 14 cm tip-to-collector distance and 1 ml h^{-1} flow rate. Later, the influence of distance was investigated at 8, 10,12,14,16 cm using 8 wt% PVA solution, 1 ml h^{-1} flow rate and 16-22 kV applied voltage. While the influence of flow rate was tested at 0.5, 1, 1.5, 2 ml h^{-1} using 8 wt% PVA solutions at 14 cm tip-to-collector distance and 16-22 kV applied voltage. The optimal process conditions were selected based on the stability of the spinning process and the morphology of the resulting nanofibers.

b) Preparation and characterization of the spinning solutions

The spinning solutions were prepared by dissolving the required amount of PVA in 20 ml distilled water to come to the above mentioned concentrations (wt%). This solution was then gently stirred with a magnetic stirrer for 6 hours at room temperature (21±1°C) and used for the optimization tests mentioned above. To incorporate the PMD-microcapsules, the wet microcapsules were well shaken and a predetermined amount pipetted into the prepared PVA solution to obtain 0 (control), 4, 8, 12 and 16 wt% concentration of PMD capsules. The solution was then sonicated for 30 minutes at a frequency of 30 kHz using a Branson 1510 sonicator (USA).

Prior to the electrospinning process, the viscosity of the solutions was measured with a Brookfield viscometer LVDV-II with an adapter spindle. About 7 ml PVA solution was held in a sample holder and stirred at 15±5 r.p.m. Each measurement was taken as an average value of five recorded readings. Thereafter, the conductivity of the solutions was determined using a CDM210 conductivity meter with a combined electrode holder and magnetic stirrer (Radiometer Analytical). The electrode was immersed in 6 ml PVA solution held in a sample beaker and stirred at 0.01 to 20 r.p.m. Each measurement was taken as an average value of five recorded readings.

c) Electrospinning of PVA and PVA/PMD microcapsules nanofibrous structure

The prepared solutions were electrospun into nanofibers using a mononozzle electrospinning setup presented in Figure 5. The polymer solution was pumped from a 20 ml plastic disposable syringe into a 15 cm long stainless steel needle with an inner diameter of 1.024 mm (Sigma-Aldrich) connected to the positive terminal. The flow rate of the spinning solution was controlled with a syringe pump (KD Scientific, U.S.A.), while the distance between the needle tip and the collector was adjusted with a laboratory jack. The high voltage was sourced from a Glassman High Voltage Series EH30P3 supply unit having a power range of 8-22 DC kV. An iron plate connected to the negative electrode of the power supply was covered with an aluminum foil and was used as the collector. The flow rate of the spinning

solution was set at 1 ml h^{-1} and the distance between the needle tip and the collector was 14 cm. The nanofibers were electrospun at room temperature of 21±1°C and a relative humidity of 40±5%.

d) Characterization of the electrospun samples

Scanning Electron Microscopy (SEM): The morphology of the resulting nanofibers was studied using a Scanning Electron Microscope (Joel Quanta 200 F FE-SEM). The samples were placed on a SEM stub and gold coated using a sputter coater (Balzers Union SKD 030) for 1 minute prior to imaging. Later, the SEM micrographs were taken at a 20 kV with a spot size of 4 to 6 nm, a dwell of 3000 µs and a working distance of 10 mm. To determine the average fiber diameter, fifty diameter measurements of each sample at a magnification of 20 000x were taken using Cell D software (Olympus).

Raman spectroscopy (RS): Raman spectroscopy was used to spectrally confirm the presence of the microcapsulated repellents in the resulting nanofibrous structures. The tests were run in a Perkin-Elmer Spectrum GX 2000 spectrometer with a Raman beam splitter, a diode-pumped YAG infrared laser (1064 nm and laser power of 800Mw) and InGaAs detector. The measurements were recorded in the back-scattering mode (180° excitation optics) with 128 scans, a resolution of 4 cm^{-1}, a data-interval of 1 cm^{-1} from 3500 to 100 cm^{-1}. The samples were first peeled off from the aluminum foil and aligned on a sample holder prior to taking spectra.

Differential Scanning Calorimetry (DSC): Differential Scanning Calorimetry was used to study the thermal properties of PVA/repellent nanofibrous structure (DSC- Q 2000, TA-Instruments, DA, USA) with a RCS cooling accessory and nitrogen as a purge gas flowing at a rate of 50 ml/min. Calibrations were performed based on the Tzero^{TM} approach and in T4P mode. The temperature calibration was performed using two certified standards (indium and tin) at 20°C/min. Samples weighing 2.5 ±0.5 mg were loaded in an aluminum DSC pan (Tzero Pan) and sealed with an aluminum lid (Tzero lid) using the TA crimping tool. The samples were first equilibrated at 50°C for 10 minutes, the temperature was then increased to 250°C at a ramp of 10°C/min then cooled down to 0°C using a cooling rate of 10 °C/min.

The degree of crystallinity was calculated from the obtained enthalpies using equation (2).

$$Crystallinity\ (\%) = \frac{\Delta Hx1 - \Delta Hy1}{\Delta Hx0 \,.\, (1 - \frac{wt\%}{100})} * 100 \qquad (2)$$

Where $\Delta Hx1$ is the melting enthalpy of the sample, $\Delta Hy1$ is the crystallization enthalpy, wt% is the total weight percentage of repellent and $\Delta Hx0$ is the melting enthalpy of perfect crystalline PVA sample taken as 155 J/g

Favimat tensile testing: To evaluate the mechanical properties of the resulting nanofibrous structures, a longer electrospinning collection time of about 1 hour was used in order to produce thicker nanofibers mats which were suitable for analysis. The mechanical analyses were performed with a FAVIMAT-ROBOT (Textechno, Germany). The tensile properties were tested using settings similar to ISO 5079. The measurements were carried out with rectangular sample of 3 x 30 mm cut out of the corresponding

electrospun material. Before testing, all samples were weighed to ensure that they were of the same thickness having an average weight of 0.00771±0.001 g. The gauge length and test speed were set at 20 mm and 20 mm/min, respectively. An average of 20 strips was tested per sample.

e) Characterizing the repellency of the functionalized materials

Mosquitoes: The mosquitoes (*Anopheles gambiae s.s.*) used in the experiments were reared in climate chambers at the laboratory of entomology of Wageningen University (The Netherlands) while the original population was collected in Suakoko (Liberia). The mosquitoes were kept under phase photo: scotophase of 12:12 hours at a temperature of 27±1°C and relative humidity of 80±5%. Adult mosquitoes were kept in 30 x 30 x 30 cm gauze wire cages and had access to human blood on a Parafilm® membrane every other day and a 6% glucose solution in water was provided (*ad libitum)*. Eggs were laid on a wet filter paper and then placed on a plastic tray with tap water for emergence. Larvae were fed on Liquifry® No 1 (Interpet, UK) for the first three days and then with TetraMin® baby fish food (Tetra, Germany) until they reached the adult stadium. Pupae were collected from the trays using a vacuum system and placed into a plastic cup filled with tap water for emergence.

The mosquitoes intended for these experiments were placed in separate cages as pupae, where they had access to a 6% glucose solution but received no blood meals. The day before the experiment, five to eight day old female mosquitoes were placed in release cages with access to tap water in cotton wool until the experiment. Both laboratory experiments took place during the last four hours of the scotophase, a period during which *A. gambiae s.s.* females are highly responsive to host odours [31].

Bioassay: A vertical landing bioassay was used to assess the effect of the incorporated repellents. It was set up in a climate-controlled room of constant air temperature (24±1°C) and humidity (60 and 75% RH) to mimic tropical dawn conditions. The bioassay was a modified version of the one described by Menger et al., [28]. In this version, the flight chamber consisted of a cubic-cage with steel gauze on three sides and Perspex on the other three sides. Underneath the gauze at the bottom of the cubic cage, a warmed circular plateau (Ø 15 cm) was positioned on top of which moist filter paper was applied and an attractive odour blend was released from nylon strips [32, 33]. The temperature at the gauze was kept at 35 ±2°C, comparable to the temperature of human skin. This resulted in a high attraction of female mosquitoes to the warm area on the bottom of the cubic cage where they would land and probe with their proboscis through the gauze in search of a blood-host.

The resulting PVA-nanofibers mats (9 X 9 cm sized) incorporated with different concentration of PMD microcapsules were stored in sealed plastic packing under the same conditions as the nylon strips until they were used. In the first day of the experiments, a circular hole of 5 cm Ø was cut in the middle of the sample to allow warm, moist air with attractive volatiles to rise up through the sample. The sample was then placed at the bottom of the cage over the warm attractive area. Five samples were tested: a control (Blank PVA nanofibers) and PVA nanofibers incorporated with 4, 8, 12 and 16 wt% PMD microcapsules. Number of replications was ten for each sample and the tests were randomised within each repetition and were spread over five different days.

The repellency of the samples was measured by releasing a group of ten, seven days old female mosquitoes into the cage. After two minutes of acclimatization time, landings made on the nanofibers or within the circular area that was cut at the middle of the sample were counted for a period of eight minutes. A landing was defined as the total period for which a mosquito maintained contact with the landing platform. Walking/hopping around on the landing plateau as well as short take offs (< 1 sec.) immediately followed by landing again were included in one landing. A new landing was recorded when a mosquito had left the plateau for more than one second before landing again. Landings shorter than one second during which no probing took place were ignored.

f) *Statistical tests*

The two-sample Kolmogorov-Smirnov statistical test was used to determine the significance of adding PMD microcapsules on the properties of the resulting nanofibrous structure. A significant difference is here assigned if the p-value is smaller than 0.05 and highly significant when the p-value is smaller than 0.01.

5.3. Results and discussion

5.3.1 Optimizing process parameters for electrospinning pure PVA

Successful production of nanofibers mainly depends on the solution properties like concentration and viscosity, the process parameters like applied voltage, tip-to-collector distance and flow rate and finally the ambient conditions like humidity and temperature [28, 34]. These parameters determine the morphology of the resulting nanofibers as well as the stability of the electrospinning jet.

Generally, a stable electrospinning jet has four regions, namely: the base, the Taylor cone, the stable segment and the bending instability segment [35, 36]. In the base segment, the jet emerges from the needle forming the Taylor cone while in the stable region, radial charge repulsions cause the jet to splay into fibers. After the fibers have traveled a short distance of about 10 mm at high electric field, the jet begins to whip and get unstable as it undergoes bending and stretching [35, 37, 38]. Achieving and maintaining a stable electrospinning jet depends on solution, process and ambient parameters. Failure to match these parameters can result in a spinning jet characterized by droplets and over-sprays producing non-uniform fibers.

The influence of independent parameter on the stability of the electrospinning process and fiber morphology was investigated aimed at optimizing the process. The parameters considered were the polymer concentration, applied voltage, tip-to-collector distance and solution flow rate.

a) *Polymer concentration*

Only concentrations ranging from 4 to 12 wt% were successfully electrospun as concentrations outside this range resulted in unstable fiber formations. At concentrations lower than 4 wt%, wet fibers with beads were produced (Figure 28). This suggests that the low viscosity and higher surface tensions of the solution could have caused electro-spraying rather than spinning resulting in beaded fibers [39, 40]. In

contrast, concentrations higher than 12 wt% lead to no spinning of fibers as the process was never stable. Generally, high polymer concentrations have an increased viscosity which restricts the free flow of the solution through the needle resulting in a blockage of the needle tip and no fiber spinning [40].
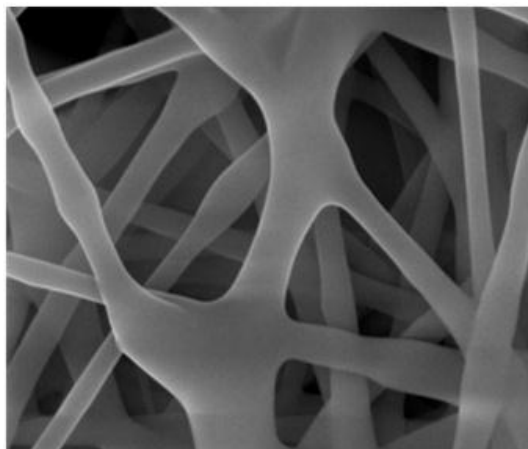

Figure 28. SEM micrographs showing PVA nanofibers characterized with beads electrospun using 2 wt% PVA concentration (Scale bar 5µm)

The data presented in Figure 29a shows a significant increase in average fiber diameter with increasing polymer concentration (p-value: 0.000). PVA concentration of 8 wt% produced better uniformed fibers (concentration with the smallest standard deviation) and was therefore picked as the optimal concentration to use in the subsequent tests.

b) *Applied voltage*

The applied voltage charges the spinning solution and provides an electric field for creating a polymer jet. Both the applied voltage and electric field affects the fiber morphology by influencing the acceleration and stretching of the polymer jet [41]. The influences of an applied voltage ranging from 8 to 20 kV on fiber diameter was investigated using 8 wt% PVA solutions at 14 cm tip-to-collector distance and 1 ml h^{-1} flow rate. Applied voltage lower than 8 kV could not form a stable Taylor cone possibly due to low electric charge that couldn't overcome the solution surface tension. On the other hand, applied voltage higher than 20 kV produced secondary jets or made the Taylor cone to disappear.

Results presented in Figure 29b show applied voltage had an effect on the morphology of the fibers whereby an increasing applied voltage lead to a significant decrease in the average fiber diameter (p-value: 0.000). This could be explained by large Coulombic and electrostatic forces introduced by the increased voltage which fully stretch the jet resulting into thinner, uniform and smooth fibers [42, 43]. However, further increase in applied voltage can reduce the volume of the drop on the needle tip making the Taylor cone to recede leading to spinning of fibers with beads [40].

Nevertheless, an applied voltage of 12 kV seemed to produce more uniformed fibers with the lowest standard deviation (414 ±40 nm).

c) *Tip-to-collector distance*

The tip-to-collector distance influences the time of flight of the jet and the electric field strength which directly affects the solvent evaporation rate and the stretching of the jet [36, 41]. Here, the influence of tip-to-collector distance was investigated by varying it from 8 to 16 cm using 8 wt% PVA solution, 1 ml h^{-1} flow rate and 16-22 kV applied voltage. Distance lower than 8 cm resulted in wet fibers with beads due to short time of flight and hence not enough time to evaporate the solvent, while distance higher than 16 cm resulted in an unstable jet.

In Figure 29c we see that an increase in distance lead to a significant decrease in the average fiber diameter (p-value: 0.000). The decrease in the fiber diameter with increase in distance can be explained by the fact that a short distance gives a short flight time and a high electric field that accelerates the jet to the collector leading to production of wet fibers with large diameters [44]. On the other hand, long distances produce dry and thinner fibers because of the long flight time that ensures complete evaporation of the solvent and full stretching of the jet [45]. A tip-to-collector distance of 14 cm was picked as the optimal distance because it gave a stable jet and relatively uniform fibers with the lowest standard deviation (348 ±43 nm).

d) *Flow rate*

The flow rate determines the stability of the spinning process and the quality of the fibers by controlling the amount of spinning solution available for electrospinning. The influence of flow rate was tested at 0.5, 1, 1.5, 2 ml h^{-1} using 8 wt% PVA solutions at 14 cm tip-to-collector distance and 16-22 kV applied voltage. Flow rates lower than 0.5 ml h^{-1} resulted in an unstable jet while those higher than 2 ml h^{-1} produced fibers with beads. Results presented in Figure 29d show the tested flow rates did not have a significant effect on the average fiber diameter (p> 0.05). Generally, very high flow rates usually result in fibers with beads because the large jet does not get enough time to evaporate all the solvent before they hit the collector [46]. A flow rate of 1.5 ml h^{-1} was picked as the optimal flow rate because it gave a good compromise of spinning speed and quality nanofibers.
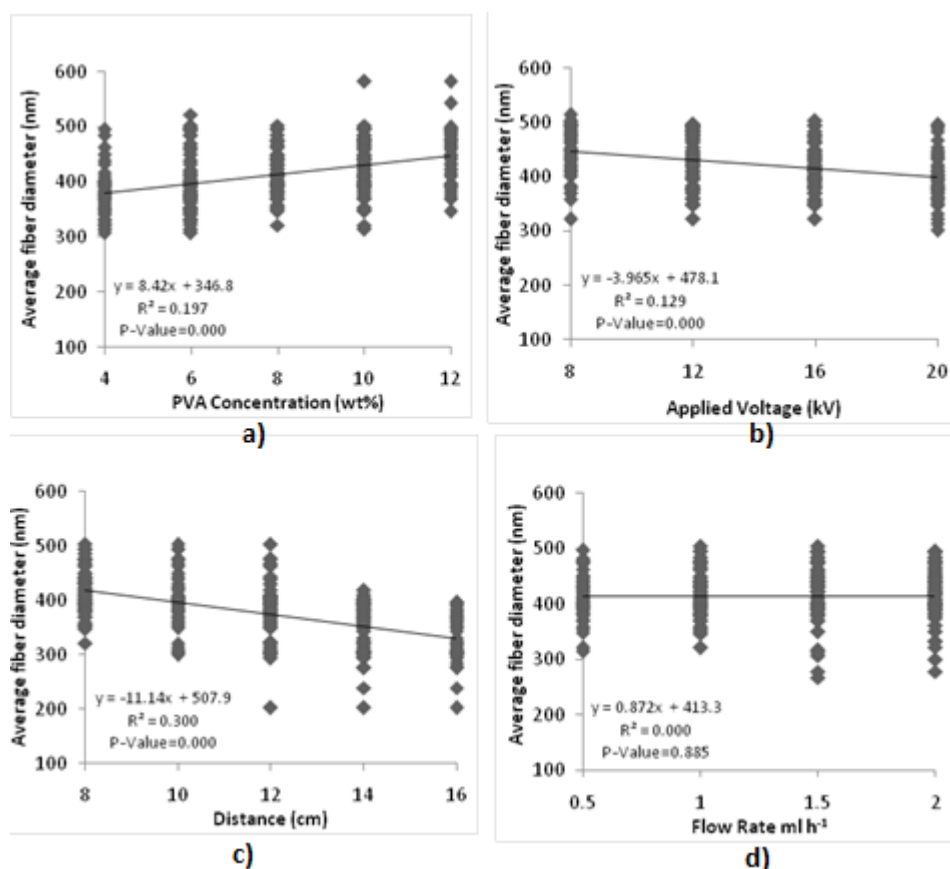
Figure 29. Linear regression model showing the influence of electrospinning process parameters on PVA fiber diameter: a) Effect of PVA concentration, b) Effect of Applied voltage, c) Effect of tip-to-collector distance, d) Effect of flow rate diameter of nanofibers

5.3.2 Electrospinning PVA/PMD microcapsules nanofibrous structure

a) Solution viscosity and conductivity

After optimizing the spinning conditions for pure PVA, adding the microcapsules could affect the solution viscosity and conductivity as well as the morphology of the resulting nanofibers. As previous studies have shown, continuous and smooth fibers cannot be obtained in very low solution viscosity and conductivity, while extremely high viscous solutions restrict the free flow of the solution through the syringe needle [42, 43]. Therefore, solution viscosity and conductivity was determined after adding different amounts of microcapsule. Adding PMD microcapsules lead to minimal changes in the solution viscosity and conductivity which could not have had any effect on the process and the fiber morphology. The Viscosity ranged between 797.8 - 793.8 cP while the conductivity was 0.313 ±0.002 mS/cm for all the solutions.

b) Fiber morphology

The fiber morphology of the electrospun samples with added PMD microcapsules (Figure 30a) appeared similar to that of the reference blank PVA nanofibrous structure (Figure 31b). The microcapsules seem to have been covered by the nanofibers encapsulating the microcapsules.
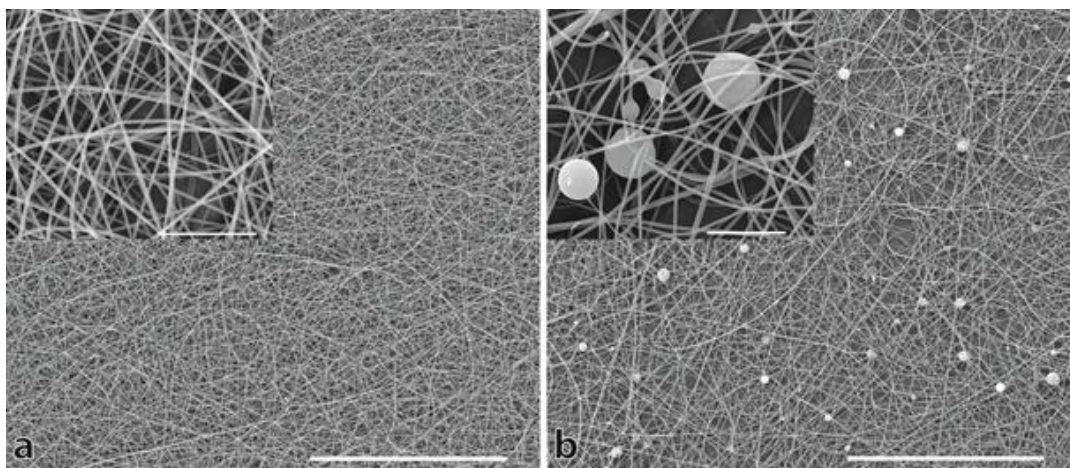

Figure 30. SEM micrographs of PVA nanofibers: a) Blank PVA nanofibers, b) PVA nanofibers incorporated with PMD microcapsules. (The scale bar bars a, b: 100um, The scale bars inset pictures: 10um)

c) Fiber diameter

Incorporating PMD microcapsules had a significant effect on the average diameter of the nanofibers (p-value: 0.001) (Figure 31). Nanofibers incorporated with 16 wt% PMD microcapsules show the lowest average diameter and a wide size distribution as compared to lower concentrations. This trend can be explained by the fact that adding additives in the electrospinning solution can lead to an unstable Taylor con which can result in secondary jets producing thinner, beaded and/or non-uniformed fibers [36, 39, 47, 48].
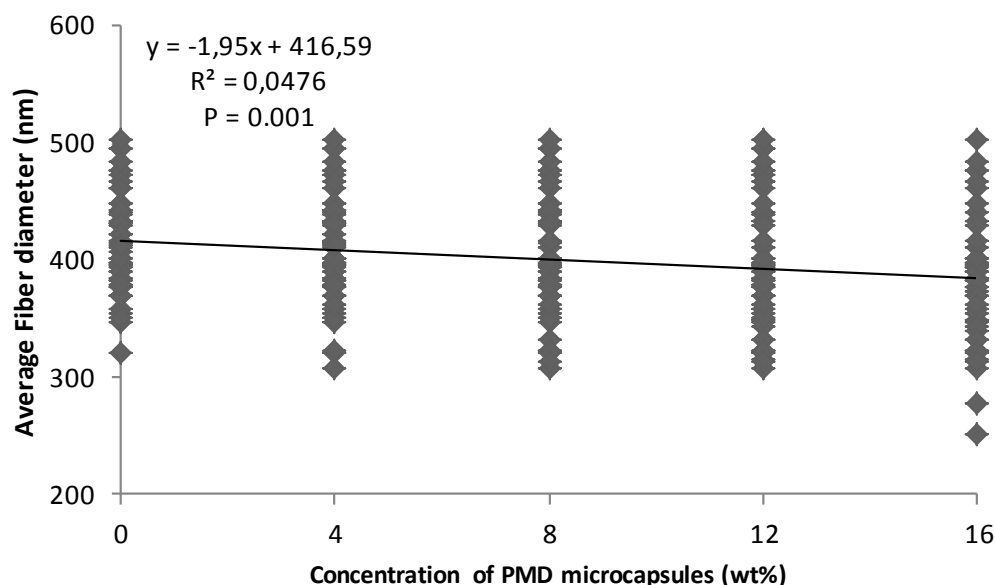
Figure 31. Fiber diameters of nanofibers incorporated with different concentrations of PMD microcapsules

d) Mechanical properties

The mechanical properties of the microcapsule-nanofibrous structures were investigated to determine the quality of the resulting nanofibrous structure as compared to the blank PVA nanofibrous structure in terms of tensile strength (Figure 32a), Young's modulus (Figure 32b) and elongation at break (Figure 32c). This is important because the selection of a textile material for various applications depends on their properties, like the mechanical properties, which determine the ability of the material to resist external damage. As shown in Figure 32, introducing microcapsules into PVA nanofibrous structures did not lead to any significant effect on all tested tensile properties (p>0.05). This shows that the microcapsules did not offer any reinforcement toward the PVA nanofibers matrix; neither did the capsules introduce early stress failure which could have deteriorated the properties. This implies that there was almost no interaction between the PVA nanofibres and the microcapsules and that these microcapsules were located in the spaces between the fibers in the nanofibrous structure which can be seen in Figure 32b. However, the standard deviations increased with increasing microcapsule concentration meaning that more microcapsules in the nanoweb filled up more available spaces thus influencing more and more the material properties of the nanofibrous structures.
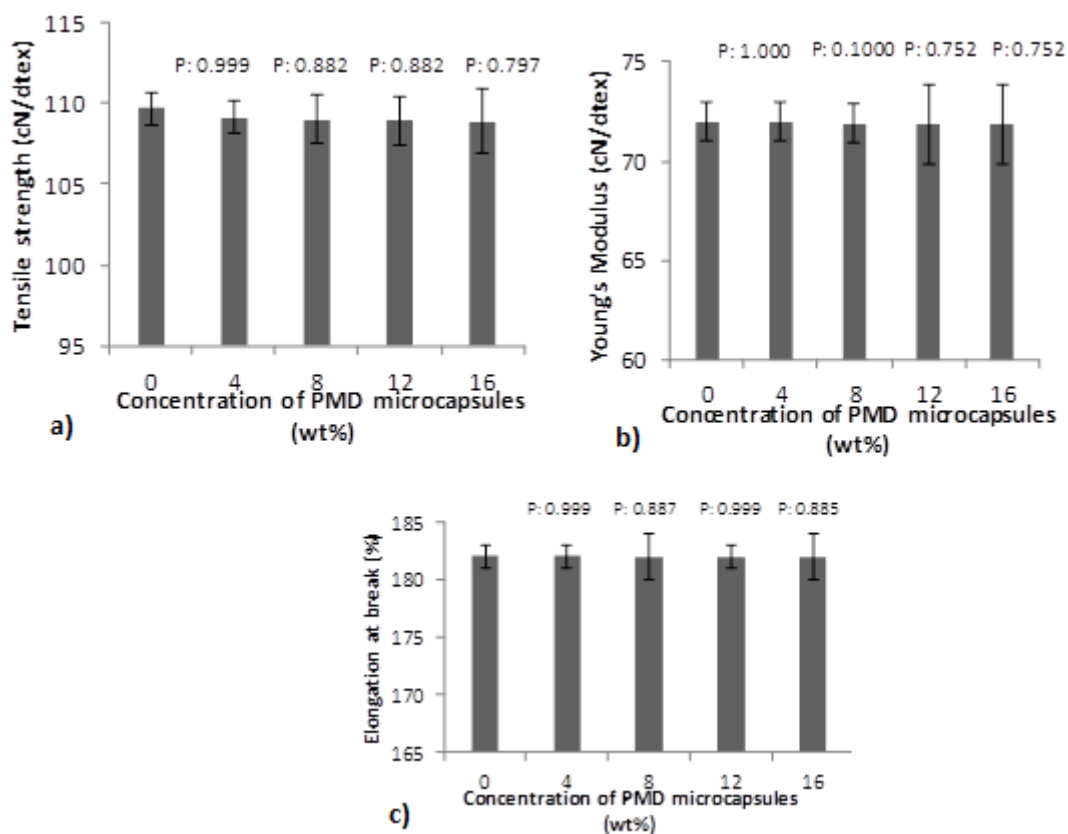
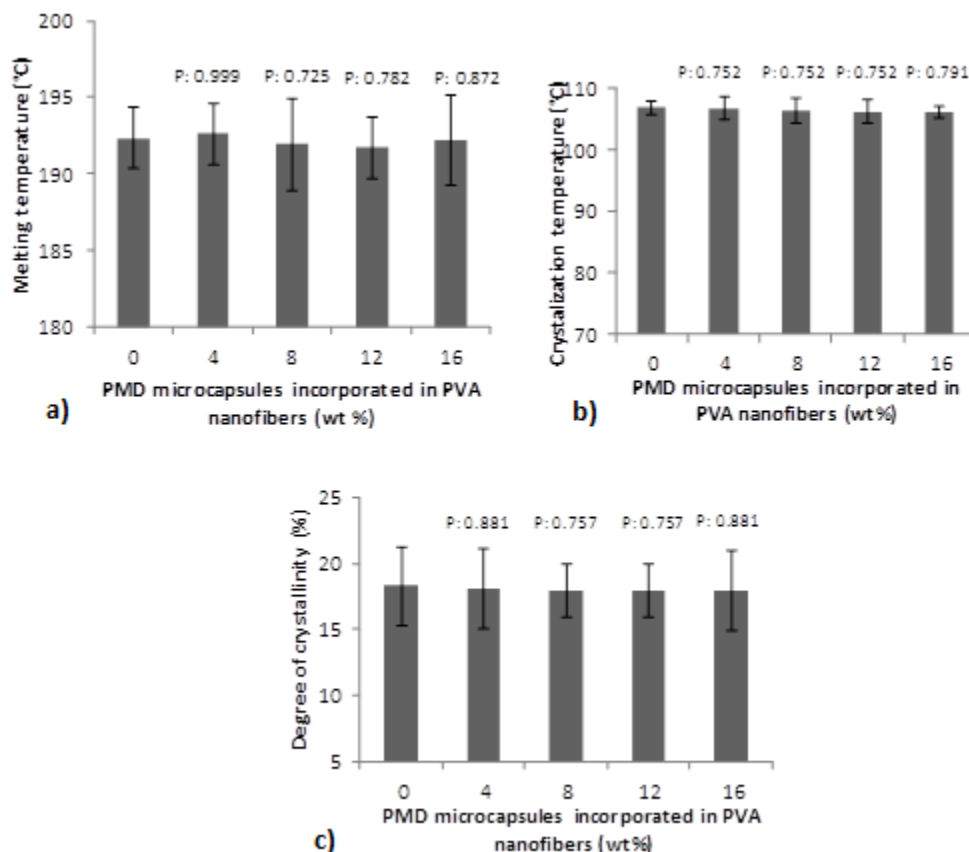*Figure 32. Mechanical properties and two-sample Kolmogorov-Smirnov tests showing the statistical significance of PVA nanofibrous structure incorporated with PMD microcapsules: a) Tensile strength, b) Young's modulus, c) Elongation at break*

e) Thermal properties

DSC was used to study the effect on the thermal properties by adding microcapsules to the PVA nanofibrous structure. Considering that PVA is a semi-crystalline polymer and the melamine formaldehyde resin used for making the microcapsules has a low thermal conductivity, it was expected that the addition of microcapsules would affect the polymer's thermal behavior. However, Figure 33 shows no significant effect on the melting ($p>0.05$) and crystallization temperatures ($p>0.05$) as well as in the degree of crystallinity ($p>0.05$).

Figure 33. DSC results for thermal properties and two-sample Kolmogorov-Smirnov tests showing the statistical significance of PVA nanofibrous structure incorporated with different concentrations of PMD microcapsules against the blank PVA nanofibrous structures: a) Melt temperature, b) Crystallization temperature, c) Degree of crystallinity

f) Spectroscopy

The presence of PMD microcapsules in the PVA nanofibrous structure was spectrally confirmed with Raman spectroscopy. Raman is a vibrational spectroscopy that can give information about the polymer structure, the structure of guest molecules incorporated in the polymer matrix and even evaluates qualitatively the interaction between guest molecules and the polymer matrix [49-51]. The Raman vibration spectra of pure PVA and PMD microcapsules were initially recorded separately and a subtraction spectrum was made, after which the spectra of samples of microcapsules incorporated PVA mats were taken (Figure 34).

For the microcapsules spectra, sharp peak are observed at 759 and 1438 cm^{-1}which can be attributed to melamine and formaldehyde derivatives. The peak at 759 cm^{-1} is assigned to aromatic ring breathing while the peak at 1438 cm^{-1} is associated with bending mode in the CH2, CH3 structures [52-55]. The spectra for blank PVA also shows a peak at 1438 cm^{-1}but there is no peak at 759 cm^{-1} while the

subtraction spectra and the spectra for PVA with incorporated microcapsules show both the 759 and 1438 cm^{-1}peak. This implies that the peak at 759 cm^{-1}is the most important for detecting the presence of microcapsules in the resulting nanofibrous structure, confirming the presence of micro capsules in the PVA structures. This peak is not present in blank PVA spectra. The peak at 1438 cm^{-1}is difficult to use because both the microcapsules and PVA have peaks in this region hence the interference. The absence of PVA caused an offset for peak 1438 cm^{-1} observed in the microcapsules and subtraction spectra.
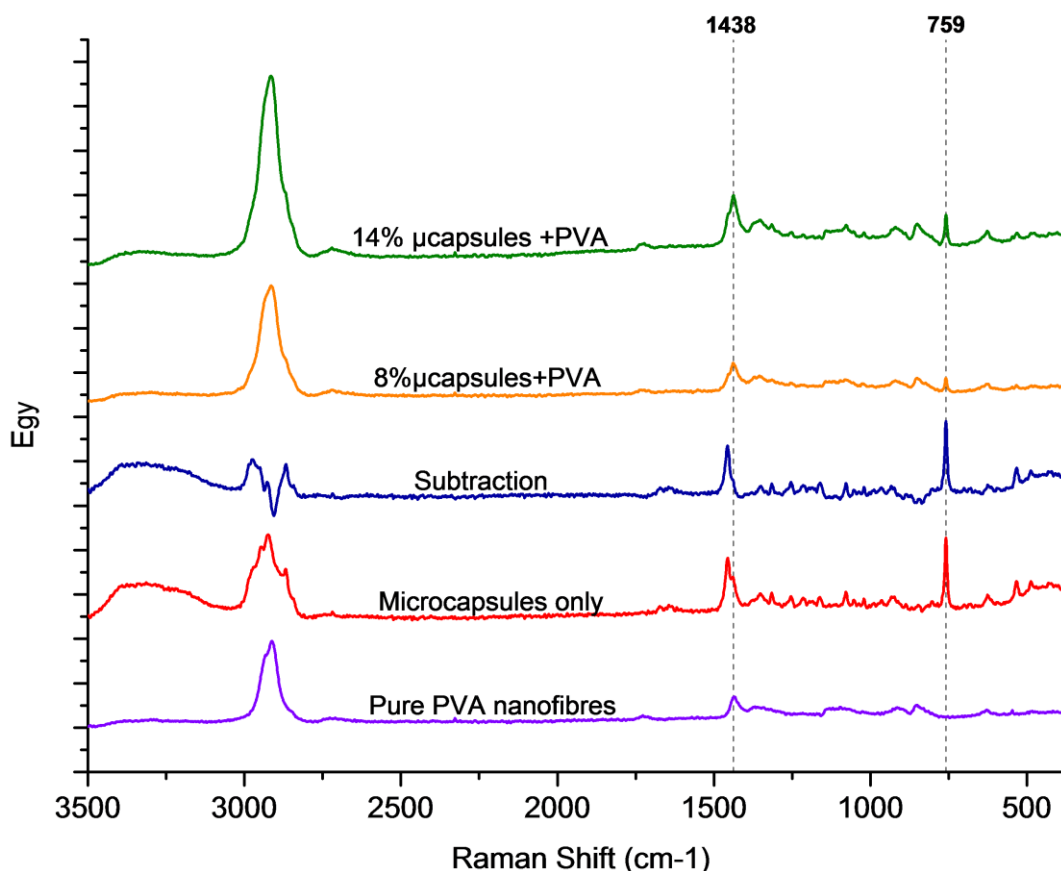
Figure 35. Raman spectra of: Blank PVA nanofibers (Black); PVA + 8 wt% PMD microcapsules (Blue); PVA + 16 wt% PMD microcapsules (Red); Pure PMD microcapsules (Green). The band indicated at 759 cm^{-1} is known for aromatic ring breathing, whereas the band at 1438 cm^{-1} is known for CH2, CH3 deformation.

g) Repellency

The repellency results of the different incorporated concentrations of PMD capsules in the PVA nanofibers mats showed a decrease in number of landings and therefore an increase in repellency against mosquitoes with increasing incorporation of PMD microcapsules (Figure 35). The data shows an inverse exponential relationship between PMD concentration and the number of mosquito landings with a fitted equation of $y = 24.49e^{-0.153x}$ and R^2 = 0.85. The results show that as the

concentration increases, the number of mosquito attacks reduces significantly ($p \leq 0.05$). Additionally, the variability of mosquito landings at 0 wt% PMD was high but as the PMD concentration increases, this variability in mosquito landings decreased (decreasing standard deviation).However, the greatest decrease in mosquito landings occurs when the PMD concentration increases from 0 to 8 wt% with higher concentrations only leading to a marginal decrease in the number of mosquito landing. Therefore, 8 wt% concentration of PMD microcapsules seems to be the optimal concentration that can be incorporated in the nanofibrous structure as it gives a good compromise between reducing the number of landing mosquitoes and the amount of microcapsules used. To halve the number of mosquito landings (value x = (27.6/2) = 13.8), a concentration of 3.75 wt% microcapsules is needed. This half value can be used to compare the effectiveness of different micro encapsulated repellents in nanofibrous PVA mats.

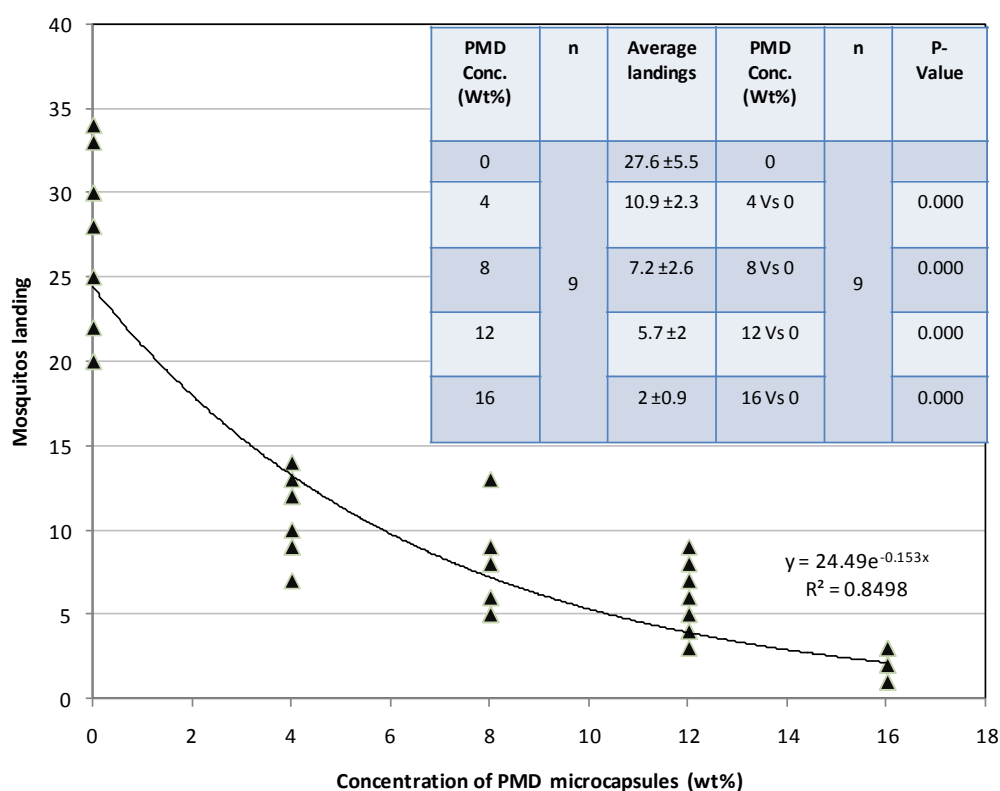| PMD Conc. (Wt%)	n	Average landings	PMD Conc. (Wt%)	n	P-Value
0		27.6 ±5.5	0		
4		10.9 ±2.3	4 Vs 0		0.000
8	9	7.2 ±2.6	8 Vs 0	9	0.000
12		5.7 ±2	12 Vs 0		0.000
16		2 ±0.9	16 Vs 0		0.000

$$y = 24.49e^{-0.153x}$$
$$R^2 = 0.8498$$

Figure 36. Number of landings by 10 female mosquitoes on PVA nanofibrous structures incorporated with PMD microcapsules during eight minutes observation time

Textile materials with incorporated novel repellents can be used for protection against mosquitoes and other nuisance insects and arthropods. These materials may offer an alternative to the current clothing with treated insecticides such as permethrin or other pyrethroids which are associated with some health concerns [56]. This is because repellents are increasingly considered as a tool in vector control with evidence showing that they can also be used in lowering house entry of mosquitoes [10-12]. This gives

an additional potential application for the textiles with incorporated repellents as they can be used in the push-pull system, which can be applied around a house to prevent the mosquitoes from getting in [56]. In the light of these potential applications, future studies should focus on the durability characteristics of the material like looking into their washing durability and the longevity of the repellent efficacy.

5.4. Conclusion

The present study investigated the electrospinning of microencapsulated PMD in polyvinyl alcohol nanofibers. The process parameters for electrospinning pure PVA were first optimized. It was found out that 8 wt% PVA concentration, 12 kV applied voltage, 14 cm tip-to-collector distance and 1.5 ml h^{-1} flow rate gave a stable spinning process and produced uniform, beadless fibers and were therefore picked as the optimal process conditions for spinning PVA nanofibers.

Thereafter, different concentrations of PMD microcapsules were electrospun in PVA nanofibers. Presented results indicate that adding PMD microcapsules did not change the solution viscosity and conductivity significantly. Further studies on morphology of the resulting nanofibers showed that the reference sample of blank PVA and that of PVA incorporated with PMD microcapsules had similar morphology and the microcapsules seem to have been covered by the nanofibers encapsulating the microcapsules.

Additionally, introducing microcapsules into PVA nanofibrous structures did not lead to any significant effect on all tested tensile and thermal properties (p>0.05) while Raman spectroscopy confirmed the presence of the microcapsules in the PVA nanofibrous structure. The repellency test results showed an inverse exponential relationship between PMD concentration and the number of mosquito landing. The greatest decrease in mosquito landing occurred when the PMD concentration was between 0 to 8 wt% with higher concentrations only leading to a marginal decrease in the number of mosquito landing. Therefore, this study proves electrospinning compounds into nanofibers as an effective technique for giving novel functionalities to nanofibrous structures.

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Chapter 6

Electrospinning Mosquito Repellent Emulsions in Nanofibers for Control Against Mosquito Bites

Significant progress has been made in electrospinning technique intended for producing high-tech nanofibers to use in various fields of applications. Among such progress is the electrospinning of emulsions that micro-encapsulate active compounds in the core of nanofibers during electrospinning. This technology can be useful in the fight against mosquito borne diseases by encapsulating mosquito repellents in the nanofibers giving them a slow release mechanism that can provide longer protection against mosquito bites.

In this chapter, emulsions of permethrin, chili oil and catnip oil in PVA solution are first prepared, after which, different concentrations of the repellents are electrospun into nanofibrous structures. Later, the PVA/repellents nanofibrous structures are studied using scanning electron microscopy (SEM), differential scanning calorimetry (DSC), tensile tester, Raman spectroscopy and vertical landing bioassay.

6. Electrospinning mosquito repellent emulsions in nanofibers for control against mosquito bites

6.1 Introduction

Recently, the basic electrospinning process of producing nanoscaled fibers from polymers through an electric field has been modified in efforts to improve the quality and enhance the functionality of the electrospun nanofibrous structures. One of such modifications that have gained importance is electrospinning of emulsions. Among the main applications of emulsion electrospinning is the direct micro-encapsulation of active compounds in the core of the resulting electrospun fibers. Unlike conventional microcapsules that require breaking them before the encapsulated compound can work, encapsulation through emulsion electrospinning continuously releases the incorporated compound hence providing continuous activity. Emulsion electrospinning technique has been successfully applied in the in situ encapsulation of proteins like lysozyme and bovine serum albumin (BSA) as well as a controlled delivery vehicle of active compounds. This helps to maintain an effective local concentration of active compounds which reduce runoffs hence prolonging and maximizing its activity [1-6].

The controlled delivery mechanism of active compounds is not only important in drug delivery but can also be applied in maximizing protection time of mosquito repellents for controlling against mosquito borne diseases such as malaria, dengue, yellow fever, etc. Mosquito repellents can either be from a biological or from a synthetic source and they include Permethrin, chili oil, catnip, etc. Permethrin ($C_{21}H_{20}Cl_2O_3$) is a synthetic pyrethroid with a high insecticidal activity against mosquitoes, flies, fleas, head lice and ticks. It excites mosquito's nervous system blocking the sodium movement into nerve cells through restricting adenosine triphosphate, acetylcho-linesterase and the gamma-Aminobutyric acid receptors resulting inparalysis/"knock down" (out of air) or death of the mosquito [7]. On the other hand, chili essential oil has capsaicin and dihydrocapsaicin as the active compounds. Chili oil gives a hot flavor which has a significant antifeedant and lethal effects on mosquitoes, dogs, rabbit, cats, birds, cotton pests, etc. [8-10]. Likewise, nepetalactone which is the main active compound in catnip essential oil has a pungent, minty distinctive smell that repels mosquitoes, spittlebugs, termites, ticks, spiders and cockroaches [11].

Generally, most repellents are applied on the skin or impregnated on the fabric surface. However, due to their volatile nature, their efficacy is only limited for a short period of time and must be re-applied frequently. Additionally, their durability and resistance to abrasion is questionable if the repellents are coated on the fabric surface. Moreover, some repellents like Permethrin and chili oil cause skin and eye irritation when in direct contact and must be used with caution [12]. Therefore, by possibly micro-encapsulating these repellents in nanofibers during electrospinning, a controlled delivery mechanism is achieved which gives a long protection time. Additionally, nanofibers generally have large surface area which may give higher functionality hence maximizing protection against mosquito bites.

Chapter 6 - Electrospinning Mosquito Repellent Emulsions in Nanofibers for Control Against Mosquito Bites

Polyvinyl alcohol (PVA) nanofibers with different concentration of repellents were electrospun and the process optimized. The morphology of the produced nanofibers was studied with Scanning electron microscopy (SEM), the material properties like tensile strength, Young's modulus and elongation at break were studied with Favimat tensile fiber testing machine, while thermal properties and crystallinity behavior were tested on Differential Scanning Calorimetry (DSC). Finally, the repellency of produced nanowebs against mosquitoes was tested using a vertical landing bioassay.

As the fight against mosquito borne diseases intensifies, there is a need for new functionalized textile materials to protect against mosquito bites. This work aimed at presenting a novel functionalized mosquito repellent textile by directly incorporating repellents in nanofibers during electrospinning. Such materials are important owing to the millions of deaths caused by mosquito borne diseases in tropical and sub-tropical regions. Therefore, the generated knowledge will be an eye opener to a new possibility if integrating a range of mosquito repellents into textiles.

6.2 Experimental set-up

6.2.1 Materials

Polyvinyl alcohol (Mowiol 40-88) with a molecular weight of 205, 000 was supplied by Sigma Aldrich. The Permethrin mosquito repellent was supplied by Utexbel (Belgium), while chili oil and catnip essential oil were supplied by Devan chemicals (Belgium).

6.2.2 Method

a) *Preparation and characterization of the spinning solutions*

The spinning solution was prepared by dissolving the required amount of PVA in 20 ml distilled water to obtain an 8 wt% solution. This solution was gently stirred with a magnetic stirrer for 6 hours at room temperature (21 ±1°C). Thereafter, repellents were pipetted into the prepared PVA solution to obtain a 0 (control), 2, 4, 6, 8, 10, 12, 14 and 16 wt% concentration of repellents to PVA solution. The solutions were then sonicated for 30 minutes using a Branson 1510 Sonicator (U.S.A.) and then electrospun immediately.

Prior to the electrospinning process, the viscosity of the solutions was measured with a Brookfield viscometer LVDV-II with an adapter spindle. About 7 ml PVA solution was held in a sample holder and stirred at 15±5 r.p.m. Each measurement was taken as an average value of five recorded readings. Thereafter, the conductivity of the solutions was determined using a CDM210 conductivity meter with a combined electrode holder and magnetic stirrer (Radiometer Analytical). The electrode was immersed in 6 ml PVA solution held in a sample beaker and stirred at 0.01 to 20 r.p.m. Each measurement was taken as an average value of five recorded readings.

b) *Electrospinning of PVA and PVA/mosquito repellent nanofibrous structure*

The prepared solutions were electrospun into nanofibers using a mono-nozzle electrospinning setup presented in Figure 5. The polymer solution was pumped from a 20 ml plastic disposable syringe into a 15 cm long stainless steel needle with an inner diameter of 1.024 mm (Sigma-Aldrich) connected to the positive terminal. The flow rate of the spinning solution was controlled with a syringe pump (KD Scientific, U.S.A.), while the distance between the needle tip and the collector was adjusted with a laboratory jack. The high voltage was sourced from a Glassman High Voltage Series EH30P3 supply unit and the nanofibrous structures were produced with a power range of 12-23 DC kV. A plate connected to the negative electrode of the power supply was covered with an aluminum foil and was used as the collector. The flow rate of the spinning solution was set at 1 ml/h and the distance between the needle tip and the collector was 14 cm. The nanofibers were electrospun at room temperature of 21 ±1°C and a relative humidity of 40 ±5%.

c) *Characterization of electrospun samples*

Because only up to 8 wt% catnip could be electrospun successfully, samples with 8 wt% of permethrin, chili and catnip oil were used in characterization for comparison.

Scanning Electron Microscopy (SEM): The morphology of the resulting nanofibers was studied using a Scanning Electron Microscope (Joel Quanta 200 F FE-SEM). The samples were placed on a SEM stub and gold coated using a sputter coater (Balzers Union SKD 030). The SEM micrographs were made at a 20.0 kV with a spot size of 4 to 6 nm, a dwell of 3000 µs and a working distance of 10 mm. To determine the average fiber diameter, fifty diameter measurements of each sample (20 000x magnification) were taken using Cell D software (Olympus).

Raman spectroscopy: Raman spectroscopy was used to spectrally confirm the presence of the repellents in the resulting nanofibrous structure. The tests were run in a Perkin-Elmer Spectrum GX 2000 spectrometer with a Raman beam splitter, a diode-pumped YAG infrared laser (1064 nm and laser power of 800Mw) and InGaAs detector. The measurements were recorded in the back-scattering mode (180° excitation optics) with 128 scans, a resolution of 4 cm^{-1}, a data-interval of 1 cm^{-1} from 3500 to 100 cm^{-1}. The samples were first peeled off from the aluminum foil and aligned on a sample holder and spectra taken.

Differential Scanning Calorimetry (DSC): Differential Scanning Calorimetry was used to study the thermal properties of PVA/repellent nanofibrous structure were investigated using a DSC (Q 2000, TA-Instruments, DA, USA) with a RCS cooling accessory and nitrogen as a purge gas flowing at a rate of 50 ml/min. Calibrations were performed based on the Tzero^{TM} approach and in T4P mode. The temperature calibration was performed using two certified standards (indium and tin) at 20°C/min. Samples weighing 2.5 ±0.5 mg were loaded in an aluminum DSC pan (Tzero Pan) and sealed with an aluminum lid (Tzero lid) using the TA crimping tool. The samples were first equilibrated at 50°C for 10 minutes, the temperature was then increased to 250°C at a ramp of 10°C/min then cooled down to 0°C using a cooling rate of 10 °C/min.

The degree of crystallinity was calculated from the obtained enthalpies using equation (2).

Favimat tensile testing: To evaluate the mechanical properties of the resulting nanofibrous structures, a longer electrospinning collection time of about 1 hour was used in order to produce thicker nanofibers mat suitable for analysis. The tensile properties were tested using settings similar to ISO 5079. The mechanical analyses were performed with a FAVIMAT-ROBOT (Textechno, Germany). The measurements were carried out with 3 x 30 mm sample strips cut out of the corresponding electrospun material. Before the testing, all samples were weighed to ensure that they were of the same thickness having an average weight of 0.00771 ±0.001 g. The gauge length and test speed were set at 20 mm and 20 mm/min, respectively. An average of 20 strips was tested per sample.

d) *Characterizing the repellency of the integrated mosquito repellents*

Mosquitoes: The mosquitoes (*Anopheles gambiae s.s.*) used in the experiments were reared in climate chambers at the laboratory of entomology of Wageningen University (The Netherlands) while the original population was collected in Suakoko (Liberia). The mosquitoes were kept under phase photo: scotophase of 12:12 hours at a temperature of 27±1°C and relative humidity of 80±5%. Adult mosquitoes were kept in 30 x 30 x 30 cm gauze wire cages and had access to human blood on a Parafilm® membrane every other day and a 6% glucose solution in water was provided *ad libitum*. Eggs were laid on a wet filter paper and then placed on a plastic tray with tap water for emergence. Larvae were fed on Liquifry® No 1 (Interpet, UK) for the first three days and then with TetraMin® baby fish food (Tetra, Germany) until they reached the adult stadium. Pupae were collected from the trays using a vacuum system and placed into a plastic cup filled with tap water for emergence.

The mosquitoes intended for these experiments were placed in separate cages as pupae, where they had access to a 6% glucose solution but received no blood meals. The day before the experiment, five to eight day old female mosquitoes were placed in release cages with access to tap water in cotton wool until the experiment. Both laboratory experiments took place during the last four hours of the scotophase, a period during which *A. gambiae s.s.* females are highly responsive to host odours [14].

Bioassay: A vertical landing bioassay was used to assess the effect of the incorporated repellents. It was set up in a climate-controlled room of constant air temperature (24±1°C) and humidity (60 and 75% RH) to mimic tropical dawn conditions. The bioassay was a modified version of the one described by Menger et al. [15]. In this version, the flight chamber consisted of a cubic-cage with steel gauze on three sides and Perspex on the other three sides. Underneath the gauze at the bottom of the cubic cage, a warmed circular plateau (Ø 15 cm) was positioned on top of which moist filter paper was applied and an attractive odour blend was released from nylon strips [15, 16]. The temperature at the gauze was kept at 35 ±2°C, comparable to the temperature of human skin. This resulted in a high attraction of female mosquitoes to the warm area on the bottom of the cubic cage where they would land and probe with their proboscis through the gauze in search of a blood-host.

The resulting PVA-nanofibers mats (9 X 9 cm sized) incorporated with different concentration of PMD microcapsules were stored in sealed plastic packing under the same conditions as the nylon strips until

they were used. In the first day of the experiments, a circular hole of 5 cm $\emptyset$ was cut in the middle of the sample to allow warm, moist air with attractive volatiles to rise up through the sample. The sample was then placed at the bottom of the cage over the warm attractive area. Five samples were tested: a control (Blank PVA nanofibers) and PVA nanofibers incorporated with 4, 8, 12 and 16 wt% PMD microcapsules. Number of replications was ten for each sample and the tests were randomised within each repetition and were spread over five different days.

The repellency of the samples was measured by releasing a group of ten, seven days old female mosquitoes into the cage. After two minutes of acclimatization time, landings made on the nanofibers or within the circular area that was cut at the middle of the sample were counted for a period of eight minutes. A landing was defined as the total period for which a mosquito maintained contact with the landing platform. Walking/hopping around on the landing plateau as well as short take offs (< 1 s) immediately followed by landing again were included in one landing. A new landing was recorded when a mosquito had left the plateau for more than one second before landing again. Landings shorter than one second during which no probing (trying to bite) took place were ignored.

e) *Statistical tests*

The two-sample Kolmogorov-Smirnov statistical test was used to determine the significance of adding PMD microcapsules on the properties of the resulting nanofibrous structure. A significant difference is here assigned if the p-value is smaller than 0.05 and highly significant when the p-value is smaller than 0.01.

6.3 Results and Discussion

6.3.1 Electrospinning PVA/repellent nanofibrous structures

Emulsion electrospinning involves dispersing an immiscible liquid phase into a polymer solution. However, mixing additives in the electrospinning solution can greatly affect the solution parameters such as viscosity and conductivity that determines the stability of the electrospinning process and the morphology of the resulting nanofibers [24-26]. Studies have proved that very low solution viscosity and conductivity cannot produce smooth and continuous nanofibers while very high viscous solutions can restrict the free flow of the solution through the syringe needle [27, 28]. Therefore, a moderate solution viscosity and conductivity must be maintained in order to produce good quality nanofibrous structure.

The electric conductivity and solution viscosity of the prepared emulsions was determined. Adding permethrin, chili and catnip oil lead to minimal effect on the solution viscosity and conductivity which could not have affected the process neither the fiber morphology. The Viscosity ranged between 825-797.8 cP while the conductivity was 0.313 ±0.002 mS/cm for all solutions.

Afterwards, the stability of the resulting emulsions was optically determined after 0, 6, 12, 18 and 24 hours. As shown in Figure 37a, pure PVA resulted in a homogeneous solution whereas permethrin, chili

oil and catnip oil gave heterogeneous solutions of stable emulsion in PVA immediately after making the emulsions (inset pictures) and even after 24 hours (Figure 36b-d).
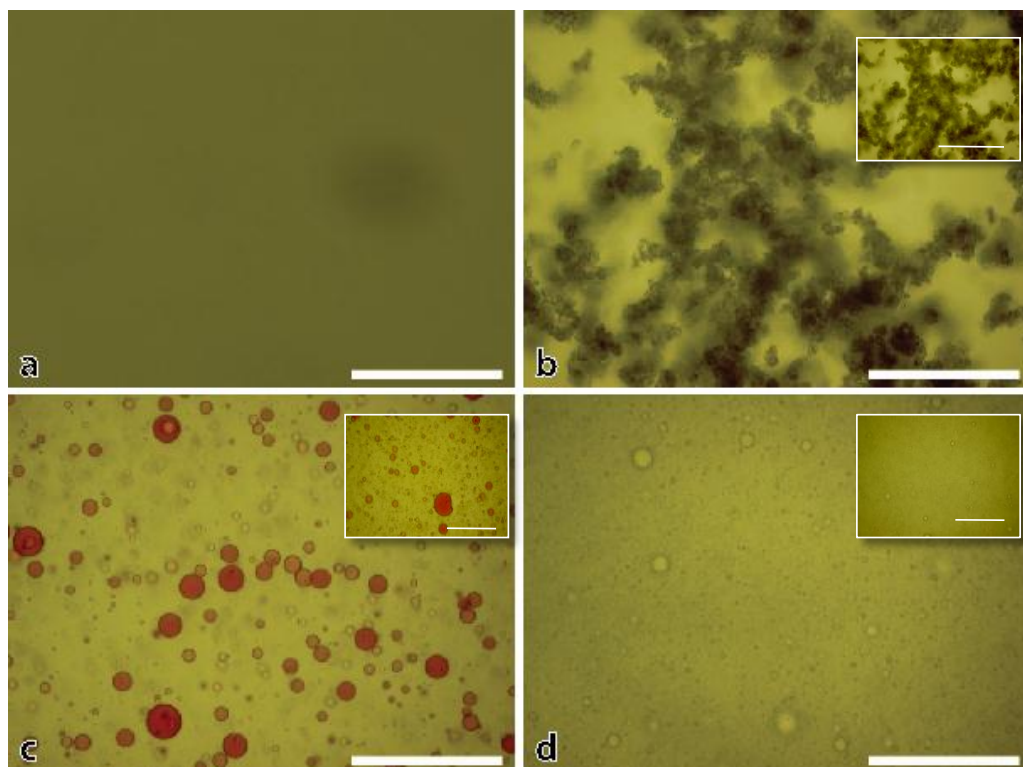

Figure 36. Optical micrographs showing the stability of emulsions after 24 hours: a) Pure PVA solution, b) PVA/Permethrin solution, c) PVA/Chili oil solution, d) PVA/Catnip oil solution (scale bars: 100um)

6.3.2   Characterization of electrospun samples

a) Morphology of the fibers

A smooth and uniform fiber surface can be observed for blank PVA nanofibers (Figure 37a). However, PVA nanofibers loaded with permethrin and catnip oil show bead-like structures along the fiber axis (Figure 37b, c (arrows)) while fibers loaded with chili oil show fibers with pores (Figure 37d (arrows)). The observed beads may suggest that repellents were encapsulated in the nanofibers during spinning as previously described by Elahi et al., [25] and Xiuling et al., [2]. During emulsion electrospinning, jets are generated from the emulsion liquid and stretched into ultrafine fibers. The dispersed drop in the emulsion makes the core of the electrospun fibers while the continuous matrix forms the shell here PVA [2, 27, 31-33]. An explanation for this phenomenon is that because water evaporates faster than oil, the polymer that forms the shell (PVA) solidifies faster than the oil drop (repellent). Additionally, the viscosity difference between the oil drop and the matrix can lead to an inward movement of the drop resulting to a core-sheath fiber [27, 31-37].
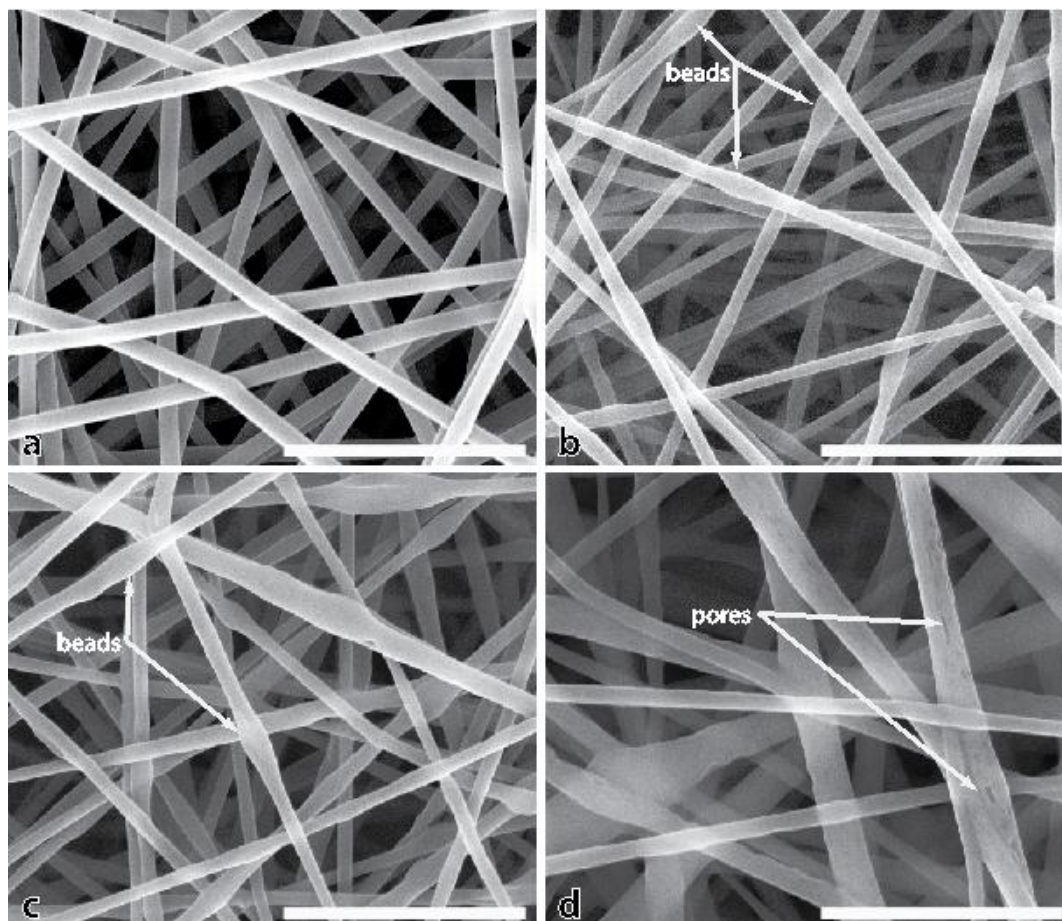
Figure 37. SEM micrographs showing pure PVA nanofibers (a), and emulsions of incorporated 8 wt% Permethrin (b), 8 wt% Catnip oil (c) and 8 wt% Chili oil (d) (Scale bar: 5um)

h) Influence of added repellents to the fiber diameters

The fiber diameters of PVA nanofibers loaded with 8 wt% permethrin, chili oil and catnip oil were compared with fiber diameters of blank PVA nanofibers using a linear regression statistical model. The results presented in Figure 38(a-c) show that loading the nanofibers with permethrin, chili oil and catnip oil had a highly significant effect on the average diameter of the resulting nanofibers with all having a p-value of 0.001. An increasing concentration of permethrin resulted in a decreasing fiber diameter (Figure 38a) while the diameter of fibers loaded with chili oil and catnip oil increased with increasing concentration of the repellents (Figure 38b, c). An explanation for this trend could be incorporating the repellents introduced beads, voids and pores inside the fibers that reduced the density of the nanofibers resulting in the observed increase in the fiber diameter. However, further microscopy analysis is required to confirm this.
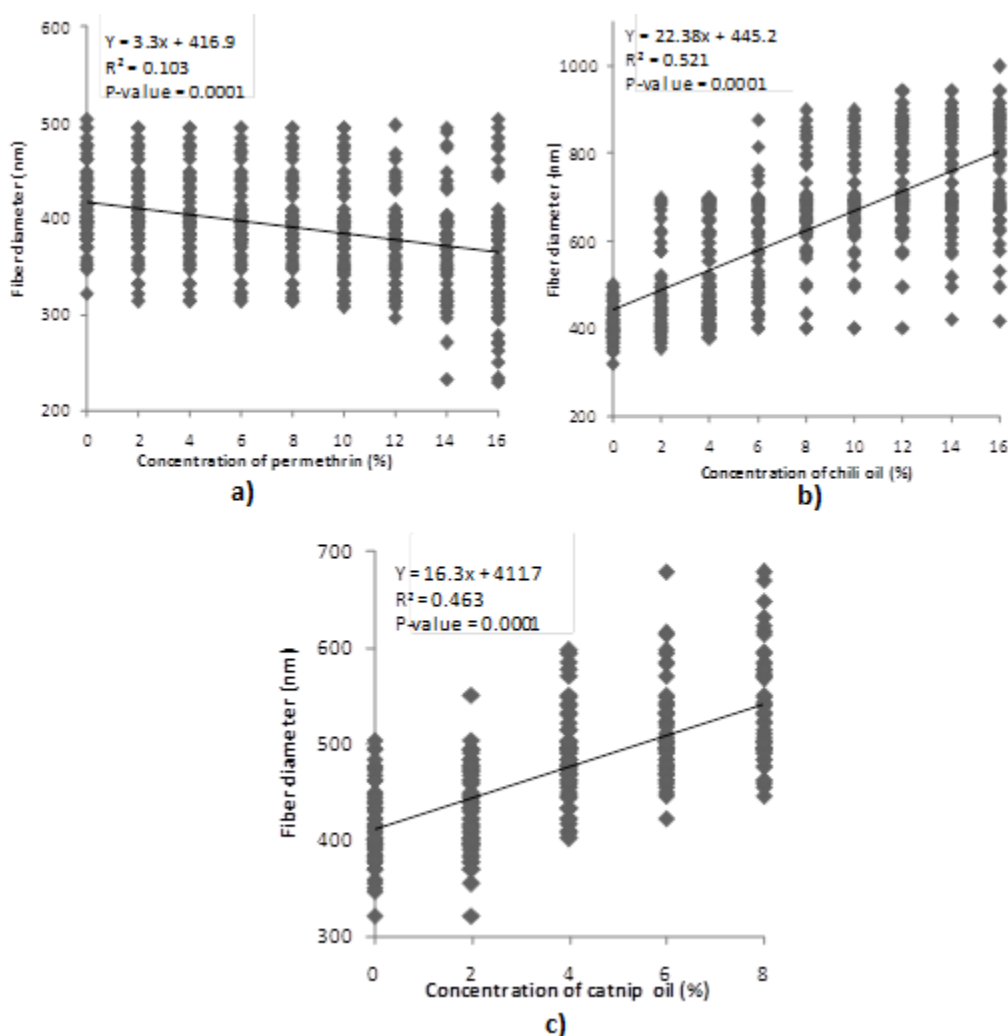
Figure 38. Linear regression model showing the diameter of nanofibers incorporated with: a) Permethrin, b) Chili oil, c) Catnip oil

i) Mechanical properties

Studying the mechanical and thermal properties of the resulting nanofibrous structure was important for knowing the quality of the produced structure which can determine their use and care in textile and industrial applications. The mechanical properties of the electrospun PVA nanofibrous structure with permethrin, chili and catnip oil were compared with the tensile properties of pure PVA nanofibrous structure using the two-sample Kolmogorov-Smirnov statistical tests. Results show that adding permethrin, chili and catnip oil did not significantly change the tensile strength ($p > 0.05$) Young`s modulus ($p > 0.05$) and elongation at break ($p > 0.05$) of the resulting nanofibrous structure (Figure 39a-c). However, samples with incorporated catnip showed a slight decrease in mechanical properties which may be attributed to a bad emulsion which may be resulted to some beads in the nanofibrous structure that lead to the decrease in the mechanical properties.
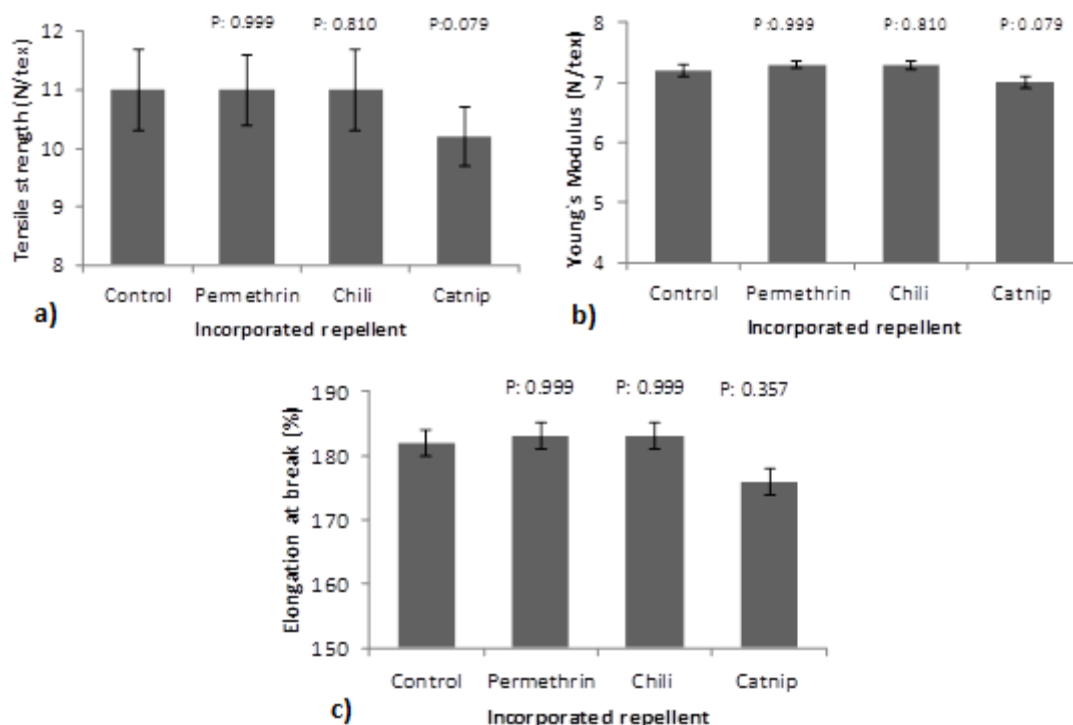
109

Figure 39. Mechanical properties and two-sample Kolmogorov-Smirnov tests showing the statistical significance of incorporating permethrin, chili oil and catnip oil on mechanical properties of the PVA nanofibrous structure : a) Tensile strength, b) Young's modulus and c) Elongation at break. (Control: Pure PVA nanofibers, Concentration of incorporated repellents: 8 wt%)

j) Thermal analysis

The DSC results for the thermal properties of PVA nanofibrous structure incorporated with permethrin, chili oil and catnip oil are shown in Figure 40a-c. The results show that the repellents did not have a significant effect on the melting temperature, crystallization temperature and the degree of crystallinity of the nanofibrous structures incorporated with permethrin and chili oil (Figure 40a, b). However, incorporating catnip oil had a significant effect on all thermal properties (p: 0.029, Figure 40c). It seems like adding catnip oil lowers the crystallinity of the PVA polymer hence the decrease in the thermal and mechanical properties of the resulting fibers. This low crystallinity caused by catnip oil explains why the tensile properties mainly the Young`s modulus and tensile strength had a lower p-value (p: 0.079) than the other tested materials (p: 0.8-0.9).
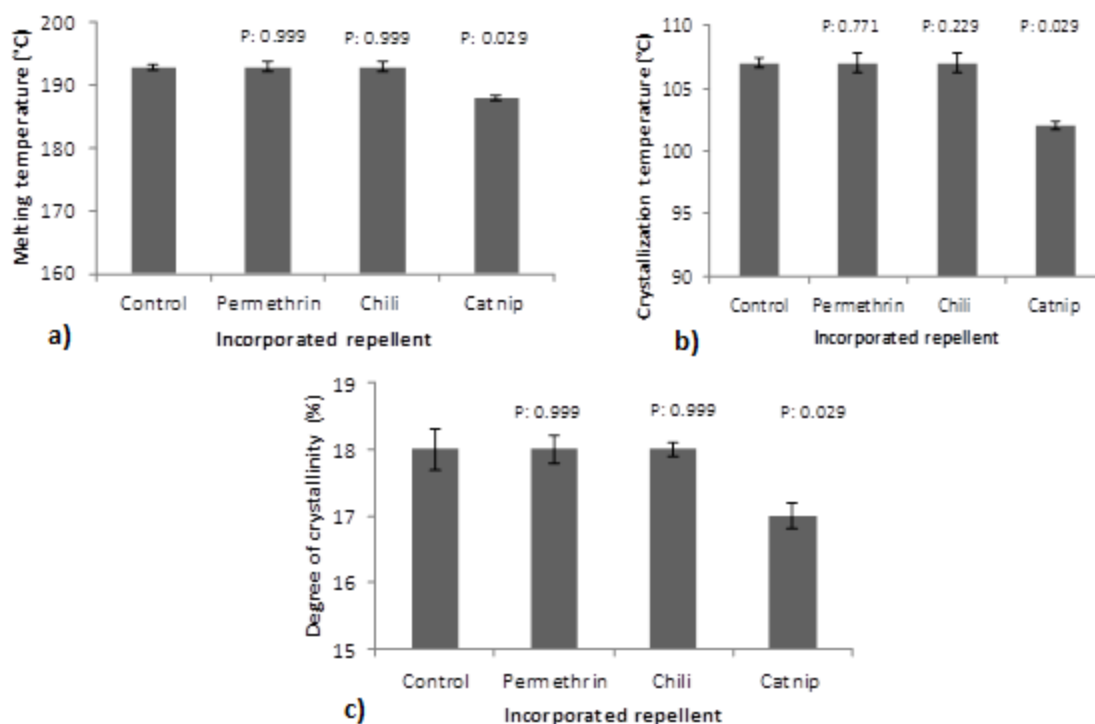
Figure 40. Thermal properties and two-sample Kolmogorov-Smirnov tests showing the statistical significance of incorporating permethrin, chili oil and catnip oil on mechanical properties of the PVA nanofibrous structure like: a) Melting temperature, b) Crystallization temperature c) Degree of crystallinity. (Control: Pure PVA nanofibers, Concentration of incorporated repellents: 8 wt%)

k) *Raman spectroscopy*

To determine the presence of incorporated repellents, like Permethrin, chili and catnip oil, in the PVA nanofibrous structure,Raman vibration spectra (Figure 41a-c) of pure PVA and each repellent were initially recorded separately, where after spectra of PVA nanofibrous structure incorporated with each repellent were taken. From these spectra a subtraction spectra was distilled by subtracting the pure PVA spectra from the spectra of the PVA structure with the repellent.

Figure 41a shows the Raman spectra for the PVA samples incorporated with permethrin. Pure permethrin spectra shows four characteristic peaks at 3064 (C-H stretch), 1614 (C=C stretch), 1437 (CH3 nonsymmetrical deformation) and 1000 cm^{-1} (N-C-N stretch) [17, 18]. The spectra for blank PVA only shows two main peaks at 2906 (CH, CH2 stretch) and 1437 cm^{-1} (CH3 nonsymmetrical deformation). It can be noticed that the peak at 1437 cm^{-1} appears both in PVA and in permethrin and therefore this peak is not useful in the determination of permethrin in the PVA nanofibers. However, the three other peaks found in permethrin, namely at 3064, 1614 and 1000 cm^{-1}, are not present in the pure PVA spectra and so can be used for identification of permethrin in PVA Nanofiber structures. When subtracting the spectra of PVA with permethrin and pure PVA, we find that this subtraction spectra

shows the same characteristic peaks like the ones found in pure permethrin (3064, 1614 and 1000 cm^{-1}). The spectra of the sample of PVA incorporated with permethrin shows the peaks for both PVA and permethrin. Thus, it can be concluded that permethrin was present in the PVA nanofibrous structures.

For the sample with incorporated chili oil, the Raman spectra are presented in Figure 41b. The spectra for pure chili show two peaks at 1520 (C-C stretch mode) and 1157 cm^{-1} (CCH in-plane band) while the blank PVA has three peaks at 2911 (C-H stretching), 1439 (CH2/CH3 deformation) and 848 cm^{-1} (C - C stretch) [19]. In contrast to permethrin, chili oil has no common characteristic peaks with pure PVA and so the peaks of pure chili oil can be used as indicators for the presence of chili oil in the PVA structure. The subtraction spectra shows the same peaks as pure chili and the sample of PVA with incorporated chili oil has a spectra with peaks associated with both chili oil and permethrin. Thus it can be confirmed that chili oil was successfully spun and is present in the PVA nanofibrous structures.

The spectra presented in Figure 41c are for PVA with incorporated catnip. The spectra for pure catnip shows four characteristic peaks at 2911 (C-H stretching), 1660 (C = C stretch), 1437 (C = C stretch) and 814 cm^{-1} (C - C stretch) [19, 20]. The blank PVA sample shows two sharp peaks at 2911 and 1437 cm^{-1}. Like with permethrin, catnip and PVA have common bands at 1437cm^{-1} and at 2911 cm^{-1}. This means that these two common bands cannot be used in the identification of catnip in PVA structures. The spectra of the PVA with incorporated catnip oil were expected to show peaks for both PVA and catnip but surprisingly only peaks for PVA (2911 and 1418 cm^{-1}) were seen. The subtraction spectrum however, shows sharp peaks at 1660 and 814 cm^{-1} as well as offset peaks at 2911 and 1437 cm^{-1} which clearly match the peaks in the pure catnip oil spectra. This means that catnip and PVA together result in spectra that resembles more PVA. However, the subtraction spectra clearly show the presence of catnip in the PVA nanofibrous structure. Thus, it can be concluded that catnip was successfully incorporated in nanofibrous PVA structures.

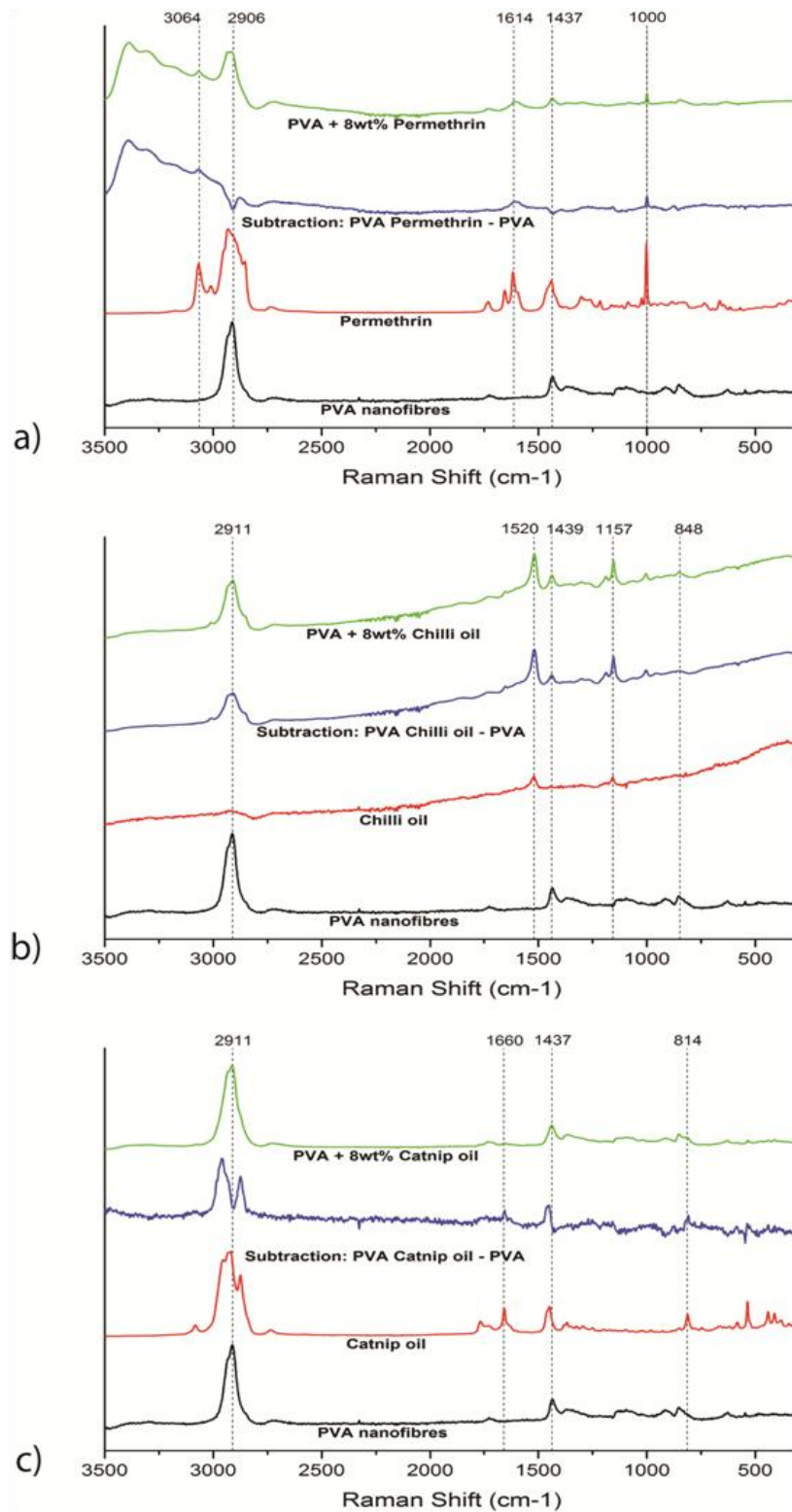*Figure 42. Raman spectra of PVA nanofibrous structure incorporated with: a) Permethrin, b) Chili oil, c) Catnip oil*

l) *Characterizing the repellency of the integrated mosquito repellents*

The repellency of permethrin, chilli and catnip to mosquitoes had been documented in various studies [7-11]. These repellents keep off mosquitoes from the treated surfaces hence protecting against mosquito bites. To test the repellency of the resulting nanofibrous structure with incorporated repellent and to compare the repellency of the incorporated compounds, samples with 8 wt% of permethrin, chilli and catnip oil were tested against malaria mosquitoes (*Anopheles gambiae s.s.*). The results presented in Figure 42 show that all the incorporated repellents significantly reduced the number of mosquito landings compared to the control. The sample incorporated with permethrin did significantly better than samples with chili oil and catnip oil. Incorporating chili and catnip oil in PVA nanofibers reduced 51% of mosquito landings while permethrin reduced the landing with 89% compared to the control. The repellency of permethrin is within the acceptable range of 82-100% that has been reported by other previous studies though the reported methods of incorporating the repellent in the textile is different [38-43]. Permethrin performed better possibly because synthetic repellents are known to give better repellency than biological repellents [40-43]. Therefore, these results further confirm the repellency of permethrin, chili and catnip oil.

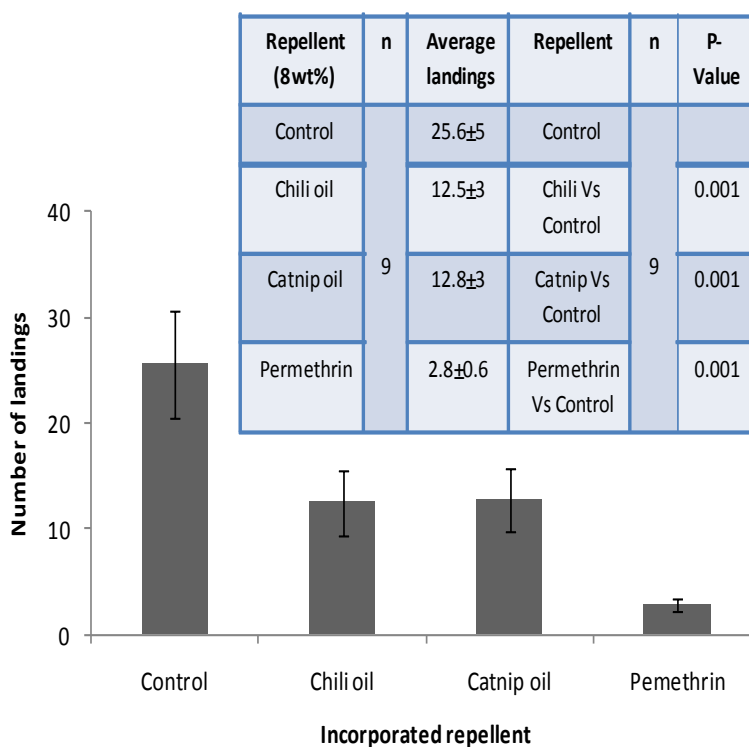| Repellent (8wt%)	n	Average landings	Repellent	n	P-Value
Control		25.6±5	Control		
Chili oil		12.5±3	Chili Vs Control		0.001
Catnip oil	9	12.8±3	Catnip Vs Control	9	0.001
Permethrin		2.8±0.6	Permethrin Vs Control		0.001

Figure 42. Number of landings by 10 female mosquitoes and two-sample Kolmogorov-Smirnov tests showing the statistical significance on repellency of chili oil, catnip oil and permethrin incorporated in PVA nanofibrous structures during eight minutes observation time

6.4 Conclusion

In this chapter, loading mosquito repellents in PVA nanofibers during electrospinning was investigated as a possible technique for developing slow release mosquito repellent textiles for control against mosquito bites. Permethrin, chili oil and catnip oil formed stable emulsion in PVA solution and adding the repellents did not affect the conductivity of the solution. Bead-like structures were observed along the fibers axis which were suspected to be repellents encapsulated by the nanofibers. The tensile and thermal properties of the samples incorporated with permethrin and chili were not affected while catnip caused a slight decrease in the material properties. The presence of each repellent in the nanofibers was proved with Raman spectroscopy which further indicated that the repellents did not affect the crystallinity of the nanofibers. Finally, the repellency tests showed that all incorporated repellents significantly reduced the number of mosquito landings compared to the control.

Electrospinning proved to be a suitable technique for producing functionalized nanofibrous structures. Therefore, the results presented here show a great potential in the use of electrospinning technique in functionalizing textiles materials which will contribute not only to the laboratory research but also in large-scale production of nanofibrous structures and industrialization of the electrospinning.

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Chapter 7

General Discussion and Conclusion

7. General Discussion and Conclusion

As global warming is anticipated to have significant effect on the earth in the coming years, one of its direct impacts will be an increase in temperatures in most countries as winter get shorter [1, 2]. This will create more mosquito-friendly habitats even in countries that previously were mosquito unfriendly thus increasing the danger of mosquito borne diseases worldwide [1].

Mosquito borne-diseases like malaria, dengue, yellow fever, etc. continues to be a major public health threat causing millions of deaths each year [3-7]. Though several preventive and control measures like use of repellents is being followed, the threat posed by mosquitoes is still high. In most mosquito repellent textile materials for personal protective use, two major problems arise. Firstly, most of the currently used repellents are harmful and are associated with negative reactions like allergies, skin irritation, asthma like reaction, muscle weakness, hyperactivity, increase body temperature, environmental pollution, etc. [8-10]. Moreover, mosquitoes are developing resistant towards the conventional repellents making them ineffective. Secondly, most repellents has a fast evaporation rate making them effective only for a limited period of time, hence frequent re-applying is needed as well as their durability and resistance to abrasion when impregnated on textiles is questionable. Solving these problems was the main goals of this dissertation.

Biorepellents were considered and evaluated as well as an in situ slow release mechanism of the repellent in order to maximize protection against mosquito bites. This work studied the best conditions of use of biorepellents and how they can be integrated in the textile products. The *B. amyloliquefaciens* spores were selected for use as a model repellent because they are known to be resistant to extreme environmental conditions like high temperature and pressure mostly used in textile processing. Other biorepellents considered in this research were PMD, permethrin, chili and catnip oil which were selected because they are known to be safe and are reported to be effective in repelling mosquitoes.

Two fiber production techniques were considered as possible processes to give an in situ slow release mechanism of repellents in textiles. The two fiber production techniques studied were melt extrusion and electrospinning. Melt extrusion was selected because it is a common technique for producing staple and multifilament fibers in micro-scale diameters from thermoplastic polymers. Therefore, success in using this technique would mean a possibility of producing different types of mosquito repellent textiles from various polymers. On the other hand, electrospinning was chosen because it is a simple and fast technique for spinning nano-scaled fibers from various polymers. Moreover, nanofibers generally have large surface area which may give high functionality hence maximizing protection against mosquito bites.

To the best of our knowledge, there is no easy, reliable and fast method that can be used for testing the resistance of repellents to melt extrusion process parameters. The only option available is to go through the actual extrusion process which is expensive, time consuming and labor intensive. Hence, a fast, reliable and economically viable technique for testing the resistance of biological compounds is therefore important for the industry as the textile industry continuously search for novel properties to

be incorporated into textile materials. This research developed a model system to study the resistance of biological compounds to melt extrusion process parameters like high temperature, pressure and residence time. The model system was used to test the resistance of *B. amyloliquefaciens* spores, a model biorepellents. The test parameters used with the model system were based on literature review and a pilot study that was conducted in the initial stages of this research.

Results of this study showed that the tested pressure did not have a significant effect on the number of viable spores while increase in temperature resulted in decrease in number of viable spores showing a significant effect at 250 and 300°C. Residence time did not have a significant effect at 1 Mpa pressure but a residence time of 10 minutes showed a significant effect at 300°C temperature. However, even though the statistics shows a significant effect on the number of viable spores at high temperature, the difference is not significant from a practical point of view because the resistance of spores to very extreme environmental conditions has already been proven and documented by many authors [11-15]. Therefore, these results showed the efficiency of the proposed model system in testing the resistance of biological compounds to high temperature, pressure and residence time. This model system makes it possible to know the resistance of a given biological compound before actual extrusion process, making the process economical in terms of time, use of polymer, labor, power as well as the wear and tear of the extruder.

Additionally, the research further proved the possibility of incorporating biological compounds in the textile materials during fiber production with the *B. amyloliquefaciens* spores being successfully extruded in PET films and fibers. The survival test on the extruded samples showed bacterial growth all around the sample which further confirmed the resistance and survival of spores to melt extrusion process parameter. This resistance was attributed to low operating pressure levels, short residence time and the formation of spore agglomerates during extrusion already discussed by other previous studies [11, 12, 15-18]. Confirming the resistance and proving the feasibility of extruding spores in polymeric fibers was important as intensive research on potential novel properties from spores that can be incorporated into textiles is being examined. Additionally, spores can possibly be used as carriers of other biological compounds that are not resistant to such extreme conditions, like PMD, chili [19] hence being important in developing novel functional textile materials. This can mean that different biological compounds can be incorporated in fibers despite their intorelence to harsh conditions which can lead to great advancement in the textile industry.

However, extruding spores in PET fibers lead to a decrease in tensile strength, Young's modulus and elongation at break. This trend was attributed to the observed spore agglomerates in the polymer matrix. Spore agglomerates may have induced cracks on the fiber surface, obstructing stress transfer between the spores and the polymer matrix that created weak points in the fibers which resulted in earlier failure [20-23]. Moreover, the optical micrograph of PET fibers extruded with spores was characterized with cracks parallel to the fiber axis. The cracks were attributed to a decrease in macromolecule arrangement of PET along the fiber axis possibly caused by adding the spores in the structure [19, 24]. This suggested poor dispersion of spores in the polymer matrix and poor spore-matrix

interface adhesion bonding [19, 25, 26]. Thermal properties of the resulting spore/PET fibers were higher than those of blank PET.

The optimal process parameters for extruding *B. amyloliquefaciens* spores in PET fibers were 6 wt% spore concentration, a barrel heated at 280°C for the feed stock, 290°C for plasticizing and 295°C for pumping as well as a die heated at 295°C, pressure of 6.0 ±0.2 Mpa and residence time of 5 ±0.5 minutes. Despite the decrease in the mechanical properties of the resulting PET/spore fibers, the reported properties fell within the acceptable range and were of the same mechanical quality with normal PET fibers in the market only that the fibers produced in this work could have a novel functionality from the incorporated bacteria spores. This means that incorporating the spores does not significantly affect the material properties which is good news for production of functional textiles as more biopolymers are being developed using biotechnologically altered bacteria that can secrete important substances useful in textiles for various applications like wound dressing, drug delivery, tissue engineering, pest control, etc. [27-30].

On the other hand, 0 to 16 wt% PMD microcapsules, permethrin and chili oil as well as 0 to 8 wt% catnip oil were successfully electrospun in PVA nanofibers. It was not possible to electrospin higher concentrations of repellents. Electrospinning PMD microcapsules in PVA nanofibers did not lead to any significant effect on the mechanical and thermal properties of the resulting nanofibrous structure ($p>0.05$). This meant that the microcapsules did not offer any reinforcement toward the PVA nanofibers matrix; neither did they introduce early stress failure which could have deteriorated the properties [31]. There seemed to be almost no interaction between the PVA nanofibers and the microcapsules and, as SEM images indicated, the microcapsules were located in the spaces between the fibers in the nanofibrous structure. However, the standard deviations of the mechanical properties increased with increasing microcapsule concentration meaning that the more microcapsules were incorporated in the nanoweb the more they filled up the available spaces between the fibers possibly bending the fibers thus changing the material properties.

For PVA nanofibers incorporated with permethrin, chili and catnip oil, the tensile and thermal properties were not significantly affected though samples with incorporated catnip showed a slight decrease in the material properties. This decrease was attributed to a bad emulsion which may have resulted in formation of beads in the nanofibrous structure that lead to the decrease in the mechanical properties. The presence of PMD microcapsules, permethrin, chili and catnip oil in the PVA nanofibers was confirmed by Raman spectroscopy and repellency tests. The repellency tests showed that all the incorporated repellents significantly reduced the number of mosquito landings compared to the control.

These results present possible slow release capabilities for volatile biological mosquito repellents not present in other textiles. The direct integration of repellents into an emulsion during electrospinning gives a big advantage for the industry as it is a very simple and cheap possible encapsulation process that results in a very effective high quality product with a high protection against mosquitoes. This can lead to an increase in efficiency of the repellant therefore providing an improved protection against

mosquito bites. The produced nanofibrous structure can also be integrated into existing textiles to develop a new generation of mosquito repellent textiles.

Furthermore, results in this dissertation give enlightenment on how incorporated biological compounds can affect the fiber properties. With this knowledge, the industry now knows how and where to improve the melt extrusion and electrospinning process for production of quality functionalized textile materials. Additionally, the study shows the concentration limits that foreign compounds can successfully be incorporated in the fibers and still acquire the desired functionality. This gives a base for understanding how much functionalizing compounds may be needed for production of a novel functionalized textile material. This is important for determining the feasibility of producing a given functionalized textile by determining the cost of production in terms of how much functionalizing compound will be required. With this, the industry has gained new inspiration and knowledge on novel methods of functionalizing textile materials with various compounds for different applications like in personal protective clothing, medical fields, agriculture, etc. This is important because the garment and textile industry is in a continuous search for new techniques to use in developing materials with new functionalities to meet the current increasing demand for textiles with novel functionalities and improved properties.

Therefore, the knowledge generated in this dissertation is an eye opener to new possibilities of integrating a range of mosquito repellents into textiles. Such materials are important owing to the millions of deaths caused by mosquito borne diseases in tropical and sub-tropical regions. Thus, this study shows a great potential in the use of melt extrusion and electrospinning techniques in functionalizing textile materials which will contribute not only to the laboratory research but also in large-scale production of functionalized materials.

To conclude, the results of this research shows a great potential in the use of fiber production techniques in adding novel functionalities in conventional textiles for use in different fields. Both the melt extrusion and electrospinning processes proved to be feasible techniques that can be used for functionalizing textile materials with biological compounds. However, repellent could not be extruded into fibers because they are volatile and can be degraded by the extreme processing condition in the extruder. Therefore, electrospinning technique proved to be more feasible because the repellent could stand the electrospinning process conditions. Additionally, repellents are mostly volatile hence a good and effective mosquito repellent textile material should have a slow release profile to optimize the protection period. Thus, during electrospinning, the nanofibers could have encapsulated the incorporated biological repellents, possibly giving them a slow release mechanism that can prolong their protection time. Moreover, electrospinning is a simple and faster technique thus can produce large quantities of quality mosquito repellent textiles in a short period of time which will enable to meet the high market demand for such materials. Furthermore, nanofibers produced during electrospinning usually have large surface area which can give high functionality hence maximizing protection against mosquito bites. However, electrospinning of bacteria spores was foreseen but we ran out of time. Moreover, electrospinning technique is not industrially available yet unlike melt extrusion and this research work hopes not only to contribute to laboratory research but also for making electrospinning

an industrially available technique. This research work also sets a firm base upon which large-scale production of novel mosquito repellent textiles can be developed and produced in large scale. This will lead to great advances not only in the textile industry but also to the public health sector which is currently threatened by the increasing cases of mosquito borne diseases.

Recommendation for future research

This research work has proved the possibility of incorporating mosquito repellents in textile materials during melt extrusion and electrospinning. However, during care and use of the product, the materials will be exposed to abrasion, detergents, and water which can affect their performance. Therefore, it is important to test the resistance of the produced textile to use (abrasion, UV light and sweat) and washing conditions (detergent, abrasion, heat and water) to ascertain their practical use. Additionally, the study aimed at developing a slow release mechanism that extends the repellent activity to maximize protection against mosquito bite. Thus, it is important to study the period of time the incorporated repellent can stay active in the textile.

Moreover, PVA is a water soluble polymer which means it cannot be washed or used in the rain without modifying to be water insoluble. Currently, Glutaraldehyde (GA) solutions are used in crosslinking PVA to make it water insoluble. However, we do not know if this crosslinking can affect the efficacy of the incorporated repellents. Thus, further studies need to be done to examine effects of crosslinking the PVA/repellent nanofibrous structure on the efficacy of the incorporated repellent.

Finally, this research proved electrospinning as the most feasible technique for incorporating bipropellants in textile fibers. However, this technique has not been industrialized yet it has great potential in making good quality functionalized textiles. Therefore, there is a need to industrialize electrospinning as it is a fast technique that can be used in making mosquito repellent thus helping in the fight against mosquito born disease.

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Annex – List of Figures

List of Figures

Figure 1. A schematic diagram showing the outline of the dissertation

Figure 2. A scheme demonstrating the development of functional textile materials

Figure 3. The schematic diagram showing the melt extrusion equipment

Figure 4.Cross section of extruder barrel. A) Single-screw extruder, B) Twin-screw extruder

Figure 5. A Scheme of electrospinning setup with indication of the process parameters

Figure 6. The anatomy of mosquito

Figure 7. A schematic diagram representing the life-cycle of a mosquito

Figure 8. The structure of a bacteria spore

Figure 9. Device used to test the resistance of spores to high temperature, pressure and residence time: a) Sample holder, b) An opening to connect to pressure vessels

Figure 10. The effect of temperature on the viability of *Bacillus amyloliquefaciens* spores

Figure 11. The effect of pressure on the viability of *Bacillus amyloliquefaciens* spores

Figure 12. The effect of residence time on the viability of *Bacillus amyloliquefaciens* spores; a) under pressure (0.1 MPa) and b) under high temperature (300°C)

Figure 13. A photograph of PET film embedded with spores showing visible spots of spores on the film surface

Figure 14. Viability tests (PET Films inoculated in nutrient medium and incubated for 24 h at 40°C. a) Control PET film with no spores showing no growth, b) PET films extruded with 0.5 wt% spores showing bacterial growth.

Figure 15. SEM micrograph of a PET film embedded with spores. The surface is characterized by spore aggregations

Figure 16. An exponential curve to relate survival rate of spores with concentration of extruded spores.

Figure 17. SEM micrographs showing the distribution of spores in the PET fibers at different spores concentration: a) 6 wt% spores, b) 10 wt% spores.

Figure 18. Additives incorporated in textiles: a) during fiber production, b) as a post-treatment (Padded, Sprayed etc.), c) Chemically bonded on the surface

Annex – List of Figures

Figure 19. Polypropylene fibers with incorporated particulates of average diameter of 2µm: a) SEM micrograph showing voids in the fiber, b) Electronic micrograph showing uneven polypropylene fiber (63x)

Figure 20. Tensile properties of extruded PET fibers with different concentration of spores. a) Tensile strength, b) Elongation at break, c) Young's modulus

Figure 21. Two representative optical micrographs of PET fibers. a) Blank PET fiber (control), b) PET fiber incorporated with 2 wt% spores exhibit cracks parallel to the fiber axis

Figure 22. SEM micrographs for PET fibers. a) Blank PET fiber (control), b) PET fiber incorporated with 2 wt% spores exhibiting spores agglomerates on the fiber surface

Figure 23. TEM micrographs of: a) Blank PET fiber, b) PET fibers incorporated with 6 wt% spores showing spores agglomerates in the polymer matrix

Figure 24. DSC results for thermal properties of fibers embedded with different concentration of spores. a) Melt temperature, b) Crystallization temperature, c) Degree of crystallinity

Figure 25. FT-IR spectrum for blank PET fibers (shown in green color) and PET fibers incorporated with 10 wt% spores

Figure 26. Raman spectrum for PET fibers embedded with different concentrations of spores

Figure 27. Raman spectrums of PET fibers extruded with different concentrations of spores. a) At 1,095 cm^{-1} and 1,120 cm^{-1} band, b) At 1,730 cm^{-1} band

Figure 28. SEM micrographs showing PVA nanofibers characterized with beads electrospun using 2 wt% PVA concentration

Figure 29. Linear regression model showing the influence of electrospinning process parameters on PVA fiber diameter: a) Effect of PVA concentration, b) Effect of Applied voltage, c) Effect of tip-to-collector distance, d) Effect of flow rate diameter of nanofibers

Figure 30. SEM micrographs of PVA nanofibers: a) Blank PVA nanofibers, b) PVA nanofibers incorporated with PMD microcapsules.

Figure 31. Fiber diameters of nanofibers incorporated with different concentrations of PMD microcapsules

Figure 32. Mechanical properties and two-sample Kolmogorov-Smirnov tests showing the statistical significance of PVA nanofibrous structure incorporated with PMD microcapsules: a) Tensile strength, b) Young's modulus, c) Elongation at break

Figure 33. DSC results for thermal properties and two-sample Kolmogorov-Smirnov tests showing the statistical significance of PVA nanofibrous structure incorporated with different concentrations of PMD

List of Tables

Table 2. Mosquito species and the diseases they transmit

Table 2. Test parameters under which the *Bacillus amyloliquefaciens* spores were tested

Table 3. Results for two-sample Kolmogorov-Smirnov tests for temperature, pressure and residence time on number of viable spores

Table 4. Surviving spores in fibers after sterilizing in soaking in a sodium hypochlorite (NaClO) solution, a Dettol solution (5 %) and in an ethanol solution (96%) for 10 minutes each or by pasteurizing at 80° C for 10 minutes (n: number of replica)