Antibacterial Finishing of Cotton Bed Sheets using Olive Leaves Extract

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Abstract

The main goal of this project consisted of the conceptualization and development of a product related to the olive tree chemical features to monetize the wastes of the olive fruit exploitation. The most abundant by-product of the olive fruit harvest is the olive leaves, used as the raw material in this project. By following the steps of Chemical Product Design, it was decided to pursue the idea of creating antibacterial cotton bed sheets impregnated with olive leaves extract, mainly composed of oleuropein. The production process includes a pre-treatment of the olive leaves, oleuropein extraction, and supercritical impregnation. The estimated amount of leaves required is 39 g/m^2 of cotton, considering a cotton fabric with 115 GSM.

Author Keywords. Olive Tree. Oleuropein. Antibacterial Finishing. Cotton Impregnation.

Type: Research Article

1. Introduction

This work aims to develop a product from the olive tree (raw material) based on the chemical product design steps proposed by Cussler and Moggridge (2011). These steps consist of identifying market needs related to properties or functions of the raw material, creating ideas for innovative products to meet said needs, selecting the products that will be developed based on the assessment of the market, and designing the manufacturing process.

The olive tree is an ancient tree known to produce olive oil, one of the most common vegetable oils used in the Mediterranean diet. The chemical composition of this raw material is the fundamental pillar for the development of the product. After scrutinizing the phenolic compounds' profile in the olive tree, oleuropein was chosen as the key component for this work since it was present in larger quantities.

The selected product to valorize the oleuropein consists of a cotton bed sheet with antibacterial properties, which is achieved by the impregnation of oleuropein. Besides acting as an antibacterial agent, oleuropein is also a natural dye.

Natural dyes are a growing interest in the textile industry due to their lower environmental impact and effect on human health, without a significant price increase when compared to synthetic dyes (Shahmoradi Ghaheh et al. 2014).

Besides oleuropein, other natural dyes can cause an increase in antibacterial resistance due to opportunistic bacterial pathogens (de Kraker, Davey, and Grundmann 2011) and so this reveals the importance of providing extra protection against these bacteria. The study made by de Kraker, Davey, and Grundmann (2011) estimated that more than 8,000 deaths and 62 million euros in extra costs were associated with *Staphylococcus aureus* and *Escherichia coli* blood infections in the European region in 2007. Therefore, the presence of these bacteria in hospitals does not only increases expenses associated with the development of more efficient antibiotics and the increase of patients who extend their stay in public hospitals, but it also is responsible for multiple deaths.

2. Raw Material

The olive tree is an evergreen tree from the *Oleaceae* family and its scientific name is *Olea europaea* L. (Rau 2020). The plantation area of olive trees in the European Union is about 4.6 million hectares, and 62 % of this value corresponds to the Iberian Peninsula: 55 % of the plantation area belongs to Spain and 7 % to Portugal (EUROSTAT 2019).

In 2018, Portugal produced above 725 thousand tons of olive fruits, being Alentejo the region with the highest production, followed by Trás-os-Montes e Alto Douro (Instituto Nacional de Estatística 2019). The olive fruit harvest in Portugal usually takes place from November to January (Mondragão-Rodrigues and Lopes 2020).

Leaves are the most abundant by-products of olive oil production, usually discharged or used for animal feeding (Herrero et al. 2011). During olive harvesting, the weight of leaves collected corresponds to 10 % of the weight of the olives picked (Rahmanian, Jafari, and Wani 2015). This means that around 72.5 thousand tons of leaves are collected during harvesting in Portugal. Seeking the valorization of these residues, the olive leaves were chosen as raw material to extract oleuropein which will be the basis of the product to be developed.

The olive leaves have in their composition phenolic compounds that have interesting properties for different applications. However, Laguerre et al. (2009) refer that the amount of the constituents is variable and depends on the geographical location and conditions of the tree production.

Ghomari et al. (2019) studied the flavonoid and phenolic profile of olive leaves extract using several extraction methods as maceration, sonication, and maceration in two steps. They concluded that oleuropein was the major compound for all extraction methods, and the maximum concentration in dry leaves was 50.86 + 0.20 mg/g. Other phenolic compounds can be identified in lower concentrations, such as hydroxytyrosol, coumaric acid, gallic acid, and flavonoids. Also, they showed that the richness in bioactive compounds such as oleuropein could result in a strong antibacterial activity against a broad spectrum of pathogenic bacteria. In the scope of this work, only oleuropein and hydroxytyrosol, the main compounds of the extract, will be considered.

Finally, the evaluation of the antibacterial activity of different olive leaf extracts against a broad spectrum of pathogenic bacteria was performed in order to explore the relationship between this activity and the content of phenolic compounds and flavonoids of each extract.

The characteristics of oleuropein and hydroxytyrosol are presented in Table 1.

Components	Molecular form	Molecular mass (g/mol)	Properties
Oleuropein	C25H32O13	540.514	Vasodilator and antiatherogenic (Shamshoum, Vlavcheski, and Tsiani 2017; National Center for Biotechnology Information 2005b); protection of neurodegenerative processes (Martorell et al. 2016); antibacterial, antimicrobial, antiviral, antioxidant (Shamshoum, Vlavcheski, and Tsiani 2017) antihypertensive effect (Xu et al. 2018); antihyperglycemic effect (Omar 2010).
Hydroxytyrosol	C8H10O3	154.16	Anti-sclerotic (Catalán et al. 2015); anti-inflammatory, antibacterial, antimicrobial, antifungal, antiviral, antioxidant (Bertelli et al. 2020; Zheng et al. 2015a); protective properties against cardiovascular system diseases, neurodegenerative processes, and retinal problems (Zheng et al. 2015b)

Table 1: Oleuropein and hydroxytyrosol main characteristics

Hydroxytyrosol can be formed from oleuropein by hydrolysis. There are two possible mechanisms through which hydrolysis occurs: (1) enzymatic hydrolysis with two enzymes: β-glycosidase and esterase; this is related to the natural process happening in the olive tree; (2) the synthetic process that implies acidic hydrolysis (De Leonardis et al. 2008).

3. Chemical Product Design (CPD)

Some market needs were identified based on olive tree components and their properties. There were some ideas to fill those needs. A pre-selection was made in a first evaluation, and some weaker or impossible ideas were eliminated. The criterium for that elimination was the feasibility of those ideas. Table 2 presents the market needs and the final ideas associated with those.

Needs	Ideas
Antioxidant products for skincare protection	An anti-ageing concealer with antioxidant properties A sunscreen with oleuropein
Anti-inflammatory and antiviral products	Honey enriched with oleuropein for tonsillitis
Products for the mitigation of the muscle pain	Post-workout snacks to relieve muscular pain
Products used in degenerative and chronic diseases	Chocolate for pregnant women to fight neurogenesis and cognitive functions of babies submitted to prenatal stress
A functional product that can be used to prevent and fight bacterial infections	Bed sheets impregnated with oleuropein for hospital beds and baby cribs

Table 2: Market needs and ideas for the application of natural compounds extracted from olive trees

For comparison between the ideas in Table 2, five selection criteria were established (Table 3). The ideas were evaluated by classifying them on a scale of 1 to 5, regarding five criteria with different levels of importance, according to the authors' knowledge as health safety (30%), scientific maturity (25%), minimum risk (20%), low environmental impact (15%) and low cost (10%), resulting in a score that determined the main idea.

Health safety was the criterion with the highest weighting factor since all the ideas are products directly related to human health.

Scientific maturity was the second most rated criterion because it was considered important to have reliable knowledge about manufacturing methods and processes.

Minimum risk comes in third, followed by the environmental impact. It was considered that the risk of investment was more important than making a greener and more eco-friendly product because the idea needed to be economically viable.

More points were attributed to the product that would be advantageous accordingly to the criteria in question.

In Table 3 is presented the selection matrix with the punctuation for all selection criteria and ideas, as well as the weighting factor.

Selection Criteria	Weighting factor	Concealer	Sunscreen	Bed sheets	Snack	Honey
Health Safety	0.30	2	1.75	4.5	3.5	3.5
Scientific Maturity	0.25	3	4	3.5	4	2.5
Minimum Risk	0.20	1.75	3	3.5	3	2.25
Low environmental impact	0.15	1.5	4	4	3	2
Low cost	0.10	2.5	3	2.5	4	3
Score		2.18	3.03	3.78	3.50	2.73

Table 3: Selection matrix for new applications of the active principles of the olive tree

The most rated idea was the bedsheets impregnated with oleuropein, which has an antibacterial effect. To put this idea into practice, some specific needs must be assured, which can be divided into three categories: essential needs, such as guaranteeing the antibacterial properties, not causing cutaneous and respiratory allergies, and possessing a long-lasting effect; desirable needs, like having a low environmental impact, easy stain removal and made of a natural dye; and useful needs, for example, being comfortable, made of breathable material and having a competitive price.

4. Manufacture

The manufacturing process is outlined in Figure 1. Once the leaves have been purchased and delivered, they will be dried and crushed to reduce them to powder. This will allow better conservation of the leaves during storage and an easier extraction process. Each step of the process (i) extraction with a mixture of ethanol: water, (ii) impregnation of fabric are described below.

The quantities presented in the following sections were extrapolated from research articles without in-house laboratory experiments to sustain them.

4.1. Extraction method

Oleuropein can be extracted by several processes, such as Soxhlet, cold solvent, supercritical fluid, pressurized fluid, and ultrasound-assisted extraction (Otero et al. 2020).

The Soxhlet conventional method was the chosen process since it can extract oleuropein with high efficiency and it is easily implemented in the industry (Otero et al. 2020). To optimize oleuropein extraction from the raw material and assure a high yield, a solvent with 80 % ethanol and 20 % of water (% v/v) is needed (Yateem, Afaneh, and Al-Rimawi 2014).

Yateem, Afaneh, and Al-Rimawi (2014) concluded that the temperature should be set at 60 °C so that it is possible to extract 19 mg of oleuropein per gram of dry leaves. After the extraction process, a concentration of oleuropein of 0.95 g/L is obtained. This study also shows that the extraction time should be 4 hours and that the ratio of the solvent's volume to the mass of dry leaves should be 20 mL/g. The Soxhlet process requires high consumption of water and ethanol, and to counter this, the final extract will be used as is in the impregnation process.

The oleuropein will not be separated from the rest of the extract since the other compounds extracted from the olive tree leaves could enhance the antibacterial activity of oleuropein. Besides that, the separation would require more investment, lowering profits.
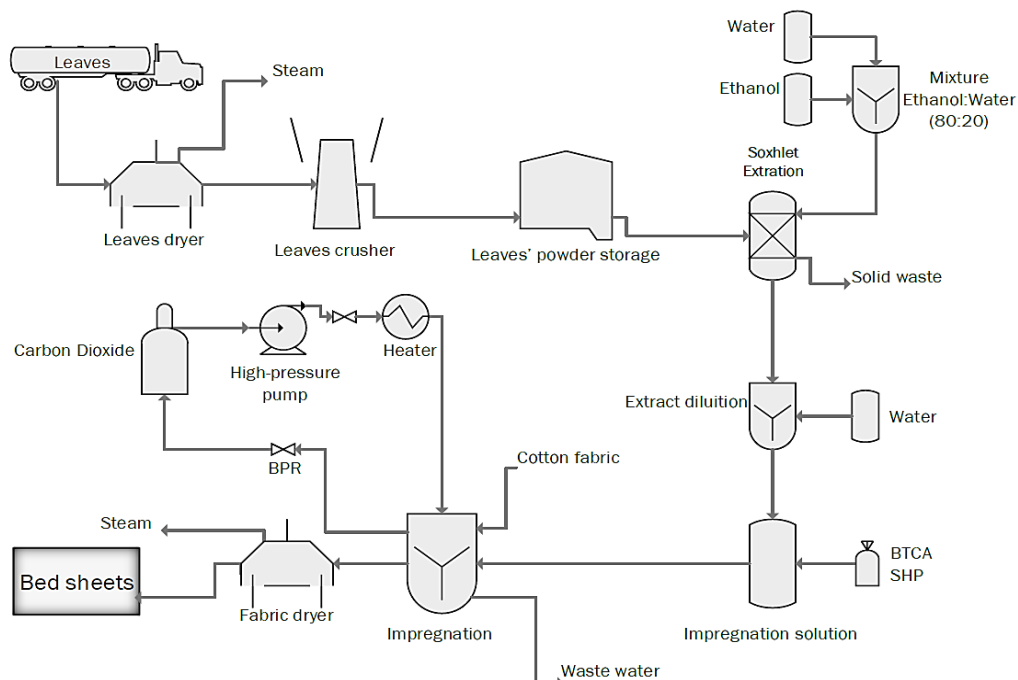

Figure 1: Flow diagram for production of antibacterial finishing of cotton bed sheets using oleuropein extracted from olive leaves

4.2. Impregnation process

The antibacterial effect of oleuropein in cotton fabrics was studied by Yılmaz and Bahtiyari (2020). In this study, the extraction of oleuropein was performed at a pH of 7. The temperature was not mentioned, so it was assumed to be ambient conditions (~25 °C). The test was made with 1 L of water and 40 g of dry leaves. Under these conditions, it is possible to extract 0.16 mg of oleuropein per gram of dry leaf (Yateem, Afaneh, and Al-Rimawi 2014).

Yılmaz and Bahtiyari (2020) used a liquor ratio of 1:60 to dye the tissue, which means that 60 mL of liquor (water) is required to impregnate 1 g of cotton fabric. If one assumes that all the oleuropein extracted will be used in the impregnation, 736 mg of oleuropein per square meter of cotton will be impregnated, considering that cotton fabric has 115 GSM. According to Yateem, Afaneh, and Al-Rimawi (2014), the concentration of oleuropein present in the extract is 6.56 g/m^2 of bed sheet, which is equivalent to 0.95 g/L, as mentioned before. So, the extract needs to be diluted by a factor of 8.9.

The study made by Fernández-Ponce et al. (2018) demonstrates that impregnation is more efficient when using 6 % of ethanol. However, the percentage obtained in the diluted extract is 9 %, but since it is a volatile compound and the variation is not significant, this percentage will be used in the impregnation process of the bed sheets.

A supercritical impregnation procedure will be used. The equipment consists of high-pressure pumps for CO$_2$, an isothermal stainless reactor, and a back-pressure regulator (BPR) (Fernández-Ponce et al. 2018). The impregnation vessel will be loaded with the diluted solution that contains oleuropein, BTCA (1,2,3,4-Butanetetracarboxylic acid), and SHP (sodium hypophosphite), as will be mentioned below. The cotton fabric will be placed at the bottom of this equipment in a spiral stainless steel which will serve as support.

After the heater is set to 45 °C, CO_2 will be injected and the vessel pressure will be raised at 5 bar/min until it reaches 300 bar, a process which will take 1 h. The impregnation process will last 24 h at constant pressure. Then, the system will be depressurized at 5 bar/min for 1 h with the heater switched off. At this point, CO_2 will be completely desorbed, and the fabric will be impregnated with oleuropein (Fernández-Ponce et al. 2018). The presence of CO_2 improves the mass transfer phenomena, favoring the kinetic desorption of analytes and providing an inert environment that prevents the degradation of sensitive compounds (Fernández-Ponce et al. 2018). It is important to mention that CO_2 used in the pressurization stage will be recovered to minimize costs.

4.3. Crosslinkers selection and washing cycles

To understand the durability of the antibacterial activity of cotton fabric impregnated with oleuropein, Bilgiç and Uğur (2015) studied the effect of 10 washing cycles at 40 °C for 30 minutes. Impregnations with additional crosslinking agents to increase the adhesion between oleuropein and cotton were also tested. The combinations were (i) 10 g/L of oleuropein, (ii) 5 g/L of oleuropein + 5 g/L of BTCA + 10 g/L of SHP and (iii) 5 g/L of oleuropein + 5 g/L of N- methylolacrylamide.

Bilgiç and Uğur (2015) concluded that due to the addition of crosslinkers, even with smaller amounts of oleuropein, the antibacterial activity against Staphylococcus Aureus and Escherichia coli remains the same or has a small decrease in its efficiency after the washing cycles. In the study of Yılmaz and Bahtiyari (2020), it was determined that the reduction of activity of Escherichia coli was 99.99 %, and the reduction of activity of Staphylococcus Aureus was 95 % at 80 °C, using only olive leaf extract without any crosslinker.

Bilgiç and Uğur (2015) observed alterations on cotton's surface by Scanning Electron Microscopy, which shows the oleuropein's linking to cotton fabric and the formation of a thick film. The addition of crosslinkers promotes a more effective formation of this film.

The best crosslinker was N-methylolacrylamide. However, due to lack of information about the use of this compound in the antibacterial finishing of fabrics and its hazards to human health, according to the National Center for Biotechnology Information (2005a), it was decided that the cotton fabric would be impregnated with BTCA and SHP.

These two agents were also tested by Montazer and Afjeh (2007) with chitosan and N-(2-hydroxy)-propyl-3-trimethyl ammonium chitosan chloride (HTCC) in a similar process. In this study, one washing cycle equals five home washing cycles at 40 – 50 °C. It was shown that after 15 launderings in testing conditions (75 home laundering cycles), the antibacterial activity would still have an efficiency of at least 99 % for different BTCA contents.

It was extrapolated that using the same mass correlation of BTCA and SHP with oleuropein in cotton fabric impregnation would keep at least 99 % of antibacterial efficiency after 50 washing cycles. The bed sheets should be washed at 40 °C to 50 °C for 35 minutes. If more washing cycles would be performed the antibacterial efficiency will decrease.

With the information collected so far, it was determined that the proportion of oleuropein, the BTCA and the SHP in the impregnation solution would be 1:1:2.

Table 4 summarizes the relevant quantities used in the entire process.

	Quantity
Oleuropein content per m² of bed sheet	7.4 g
Mass of dry leaves used per m² of bed sheet	387 g
Volume of ethanol used in the exaction of oleuropein per m² of bed sheet	6.2 L
Volume of water used in the exaction of oleuropein per m² of bed sheet	5.7 L
BTCA required for impregnating per m² of bed sheet	7.4 g
SHP required for impregnating per m² of bed sheet	14.7 g

Table 4: Summary of quantities used to impregnate 10 sqm of fabric

It is to highlight that, in this work, there were no carry out experimental tests to determine the other compounds present in the extract do not cause allergic reactions. However, Erbay and Icier (2010) concluded that olive leaves could be used in a wide range of applications without inconvenience. In this work, the authors considered there are not any allergic reactions.

5. Economic Analyses

5.1. Production volume

An exploitation scenario for the bed sheets is analyzed to assess the potential of the raw material valorization. It was defined that in the first years of production, the bed sheets would be exclusively sold in Portugal. The product will have as final consumers hospitals, nursing homes and all the population, and it can be for double size beds and baby's cribs. The expected sales for each product are shown in Table 5.

	Public Hospitals*	Private Hospitals	Cribs	Private Nursing Homes	Double-size beds for general population	Babies' cribs for general population
Institutions	230[1]	114	-	729[1]	-	-
Beds	35 429[1]	11 281[2]	3 443[3]	22 000[1]	10 251 984[1]	86 579[1]
Institutions to be equipped, (%)	30	50	-	50	-	-
Beds to be equipped, (%)	75	75	20	30	0.5	15
Packs to be sold	7 972	4 230	689	330	51 260	12 987

*It was not possible to find the number of public nursing homes in Portugal and either not the number of total beds in this type of institution, so it will not be considered a target market. Data: [1] 2020, [2] 2018, [3] 2019

Table 5: Expected sales

5.2. Profit margin

After determining the amount of raw materials needed for production, it is possible to estimate their cost. The prices of each raw material, as well as the amount needed and their cost for each square meter, are resumed in Table 6. Note that the prices for olive leaves, ethanol, BTCA and SPH were based on average prices from industrial suppliers. The cotton fabric price was provided by *Confeções Valverde de Maria Manuela Pinheiro* (2020), Portugal. For the water supply, it was considered Oporto's water supply tax.

Raw material	Price	Quantity (per m² of fabric)	Cost (€/m²)
Olive leaves	1 €/kg	0.039 kg	0.039
Cotton fabric	3.298 €/kg	0.115 kg	0.379
Ethanol	0.70 €/L	0.62 L	0.434
Water	0.002 €/L	0.57 L	0.001
BTCA	25 €/kg	0.0007 kg	0.018
SPH	5 €/kg	0.0015 kg	0.008
		TOTAL	0.878

Table 6: Raw materials quantities and respective cost per m² of fabric

With the perspective of conceiving a product for sale, two different sizes of bed sheets were designed: the double-size (2.40 m x 2.90 m) and the crib size (1.80 m x 1.20 m).

Considering a lower price for the health institutions, the value defined for a pack of bed sheets was 20 € and 12 € for the double-size bed sheets and cribs' bed sheets sold directly to hospitals and nursing homes, respectively. The bedsheets sold to the general population would cost 24 € for the double-sized bed and 20 € for babies' cribs. These values were based on some basic market research.

Thus, the profit margin would be 40 % on double-size bedsheets sold to health institutions, 70 % on crib bed sheets for nurseries, 50 % on double-size bed sheets and 80 % on crib bed sheets for the general population.

6. Conclusions

This project was born with the goal of assessing new chains of value from natural residues, such as olive leaves, which may help industrialization and increase economic activity in rural areas. After identifying the market's needs, creating ideas, and selecting the more viable product, it was concluded that the best option consisted of cotton bed sheets impregnated with oleuropein to reduce antibacterial activity. The product would have as final consumers hospitals, nursing homes and the general population.

The reduction of antibacterial activity is an important factor since bacterial textile infestations may also affect those using it. The bed sheets with antibacterial finishing would be a useful product to contain and prevent bacterial proliferation and create safer and cleaner environments.

It is ensured a wide quantity of raw material since around 72.5 thousand tons of leaves are collected during harvesting in Portugal, which are usually discharged.

The oleuropein can be extracted by a Soxhlet conventional method using water and ethanol as solvents. The concentration of oleuropein obtained in the extract is 0.95 g/L.

A supercritical impregnation procedure is used, where 736 mg of oleuropein, 736 mg of BTCA and 1472 mg of SHP are impregnated in a square meter of cotton.

The bed sheets keep at least 99 % of antibacterial efficiency after 50 washing cycles and should be washed at 40 °C to 50 °C for 35 minutes.

To obtain better results and improve the bedsheets, it will be necessary to study their antibacterial effect on bacteria other than Staphylococcus Aureus and Escherichia coli. Also, the product's manufacture can be optimized and readjusted, which will imply further research and development work on this subject.

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