COST ACTION FP1405 ActInPak

SHORT-TERM SCIENTIFIC MISSION (STSM):

DEVELOPMENT OF ELECTROSPUN NANOFIBROUS MATS WITH ENTRAPPED SAGE EXTRACT (*SALVIA OFFICINALIS* L.) AS AN ACTIVE FOOD PACKAGING

COST is supported by the
EU Framework Programme Horizon 2020

COST action:	Active and intelligent fibre-based packaging - innovation and market introduction (ActInPak) FP1405
STSM title:	Development of electrospun nanofibrous mats with entrapped sage extract (*Salvia officinalis* L.) as an active food packaging
STSM period:	17.01.-16.04. 2018.
Grantee name:	Ana Salević ana.salevic.as@gmail.com
Home institution:	Department of Food Technology and Biochemistry Faculty of Agriculture University of Belgrade Belgrade, Serbia
Home supervisor:	Prof. Dr Viktor Nedović
Host institution:	Novel Materials and Nanotechnology research group Institute of Agrochemistry and Food Technology, Spanish National Research Council, Valencia, Spain
Host supervisor:	Prof. Dr José María Lagarón Cabello

BACKGROUND

Petrochemical-based plastics have been intensively used as food packaging materials due to their availability, cost and desirable functional properties. Currently, there are high demands for the restriction of their use, since these materials are non-biodegradable and represent a serious environmental issue. Therefore, the food packaging industry has expressed a high interest towards substitution of traditionally used plastics by alternative, eco-friendly materials (Siracusa et al., 2008; Souza & Fernando, 2016).

Another environmental issue, related to the food industry, is a food waste. Namely, one-third of the produced food is lost or wasted, globally. One of the reasons is spoilage and deterioration caused by limitations in the packaging systems (FAO, 2011). Consequently, one of the trends in the food industry is focused on the development of novel preservation techniques that would allow production of healthier and safer products (Gómez-Estaca et al., 2014).

In recent times, active food packaging concept, based on various active compounds incorporated into biodegradable materials, has been studied as a response to abovementioned demands and trends. Namely, this concept offers the possibility to increase the quality and shelf life of products, avoid the use of synthetic additives, as well as to reduce the environmental impact associated with plastic packaging waste (Atarés & Chiralt, 2016). In this regard, herbal extracts are focus of the food and packaging industry as a source of natural active compounds. For example, sage (*Salvia officinalis* L.) extracts are widely used for many applications due to their significant antioxidant and antimicrobial properties (Glišić et al, 2010). Among various techniques, electrospinning attracts a great interest for encapsulation of active compounds within polymeric fibres. Continuous fabricating capability, facile operating process and intrinsic properties of the fibres indicate a high potential of the electrospun materials for the active packaging development (Echegoyen et al., 2017). Poly(ε-caprolactone) (PCL) is a promising packaging material due to its desirable characteristics, such as biodegradability and short degradation time, good chemical resistance and easy processing (Siracusa et al., 2008).

PURPOSE OF THE STSM

The purpose of the STSM was to provide an opportunity for the PhD student Ana Salević to broaden her ongoing studies on encapsulation of plant extracts performed at the Faculty of Agriculture (FA), University of Belgrade, with the techniques and approaches developed and used at the Institute of Agrochemistry and Food Technology (IATA) of the Spanish Council for Scientific Research (CSIC) in the research group Novel Materials and Nanotechnology leaded by Prof. Dr José María Lagarón Cabello. The goal of the performed STSM was to develop a novel active packaging intended to preserve quality and prolong shelf life of food products by preventing bacterial growth and delaying oxidation processes. In that respect, the main objective of this STSM was to investigate a potential of the electrohydrodynamic process for design of ultrathin structures based on biodegradable material, with the focus on polycaprolactone (PCL), with entrapped sage extract, as well as to scrutinize the effect of the extract addition on the functional and physical characteristics of the films. In this way, specific objectives were defined as follows:

- to become familiar with the electrospinning technique and its possibility for encapsulation of natural plant extracts;
- to get essential knowledge and experience regarding effects of the processing parameters on properties of the fibrous mats;

- to evaluate potential of PCL to be used as a replacement for conventional plastic packaging and a matrix for the natural herbal extract;
- to learn principles and techniques developed and available at IATA and to apply them to determine the characteristics of the prepared samples, with the focus on the effect of the sage extract addition on the functional and physical characteristics of the films and
- to evaluate the potential for continuation of the study performed during STSM and set a basis for establishment of future collaboration between the two groups.

DESCRIPTION OF THE WORK CARRIED OUT DURING THE STSM

The scientific methodology included in the STSM can be divided into the following working steps:

- Preparation and characterization of the sage extract (SE);
- Preparation of PCL based films;
- Characterization of the PCL based film.

Preparation of the sage extract as a natural food preservative and its characterization.

Preparation of SE. Sage (*Salvia officinalis* L.) was purchased from the Institute for Medicinal Plants Research "Dr. Josif Pančić" (Belgrade, Serbia) and used to prepare the extract. Maceration on the orbital shaker (Stuart SSL1, Staffordshire, UK) was applied as extraction technique. An aqueous solution of ethanol was used as solvent. The extract was stored under refrigeration conditions prior to use.

Characterization of SE. The possibility of SE to be used as a natural food preservative was scrutinized and compared to a commercially available sage essential oil in terms of antimicrobial activity against foodborne bacterial pathogens, as well as antioxidant activity observed as ability to scavenge free radicals.

Staphylococcus aureus (ATCC 6538P) as a gram positive and *Escherichia coli* (ATCC 25922) as a gram negative strain were obtained from the Spanish Type Culture Collection (CECT, Valencia, Spain) and used to evaluate the antimicrobial activity. The effectiveness of SE against these bacterial strains was tested following the plate micro-dilution protocol, as described in the Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard - Tenth Edition (M07-A10) by the Clinical and Laboratory Standards Institute (CLSI). The antimicrobial potential was expressed as minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). MIC was designated as the lowest extract concentration that inhibited bacterial growth according to a color change of a metabolic indicator. MBC was determined as the lowest extract concentration for which no bacterial growth was observed after sub-cultivation of the samples designated as MIC.

Antioxidant activity was evaluated according to a slightly modified $DPPH^{\cdot}$ free radical scavenging assay (Brand-Williams et al., 1995). Trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as a standard. The antioxidant capacity was determined by measuring the absorbance at 517 nm and calculated according to the following Equation:

$$I(\%) = \frac{Ac - As}{Ac} \times 100$$

where:

I is percentage of $DPPH^{\cdot}$ inhibition;

Ac is the absorbance of the control sample and

As is the absorbance of the test sample.

Preparation of PCL based films.

Preparation of fibre forming solutions. PCL-based solutions containing different concentrations of SE (5, 10 and 20%) were prepared and designated as PCL + 5%SE, PCL + 10%SE and PCL + 20%SE, respectively. Also, plain PCL solution without SE, designated as PCL, was prepared as a control (Figure 1).

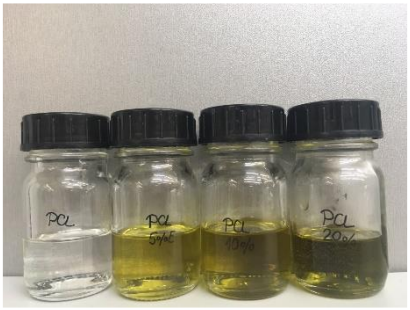

Figure 1. PCL-based solutions

Characterization of the solutions. The prepared systems were characterized in terms of:

- viscosity: a rotational viscometer Visco Basic Plus L (Fungilab S.A., Sane Feliu de Llobregat, Spain);
- surface tension: the Wilhemy plate method in an Easy Dyne K20 tensiometer (Krüss GmbH, Hamburg, Germany) and
- conductivity: conductivity meter (HI98192 portable meter HANNA Instruments, Gothenburg, Sweden), respectively.

All measurements were performed at room temperature.

Film preparation. Fibrous mats were prepared using a Fluidnatek LE-500 pilot line electrospinning equipment (Bioinicia S.L., Valencia, Spain, Figure. 2a). The electrospinning process parameters (voltage, flow-rate, needle tip to collector distance and needle size) were optimized in order to continuously produce the fibrous mats of the predefined systems. Furthermore, the mats were exposed to an annealing step using a hydraulic press (4122 model, Carver Inc., Wabash, Indiana, USA, Figure 2b). The parameters of the annealing process (time, temperature and pressure) were optimized and ultrathin films were obtained.

Figure 2. Electrospinning equipment (a) and hydraulic press (b)

Characterization of the films.

The prepared samples were scrutinized in order to evaluate the effect of the sage extract addition on the physical and functional properties of the films.

Thickness. It was measured at different points of each film employing a digital micrometer (S00014, Mitutoyo Corporation, Kawasaki, Japan).

Morphology. Surface and cryofractured cross-section of each sample, as well as diameters and uniformity of the fibers were analyzed using scanning electron microscopy (Hitachi S-4800 microscope, Tokyo, Japan) and ImageJ program (National Institutes of Health, Bethesda, Maryland, USA).

Optical properties. The light transmission spectrum and transparency of the film samples were determined spectrophotometrically (UV/Vis 4000, Dinko instruments, Barcelona, Spain).

Water contact angle. The films' surface wettability was measured applying optical tensiometer (Theta Lite, Staffordshire, UK).

Mechanical properties. Elastic modulus, tensile strength and elongation at break were determined employing tensile tests machine (AGS-X 500 N model, Shimadzu, Kyoto, Japan).

Thermogravimetric analysis (TGA). TG-STDA thermobalance (TGA/STDA851e/LF/1600 model, Mettler-Toledo, LLC, Columbus, OH, USA) was used to ascertain thermal stability of the films.

Water vapor and aroma permeability. Payne permeability cups (Elcometer Sprl, Hermelle-sous-Argenteau, Belgium) filled with distilled water and D-limonene were used for gravimetrical determination of water vapor and aroma permeability, respectively.

Antimicrobial activity. The Japanese Industrial Standard (JIS) Z 2801:2010 was performed to evaluate antibacterial efficiency against *S. aureus* and *E. coli* on the films' surface. Surface reduction (R) was calculated according to the following Equation:

$$R = \log(B \times A) - \log(C \times A)$$

where:

A is the mean of the viable bacteria counts on the control sample immediately after inoculation;

B is the mean of the viable bacteria counts on the control sample after 24 h and

C is the mean of the viable bacteria counts on the test sample after 24 h.

The antibacterial activity was defined according to the R value as: non significant ($R < 0.5$); slight ($0.5 \leq R < 1$); significant ($1 \leq R < 3$) and strong ($R \geq 3$).

Antioxidant activity. Ability of the films surface to scavenge DPPH$^{\cdot}$ free radical was estimated by measuring the absorbance at 517 nm after incubation of the film surface and free radical solution for 3h. The results were calculated and expressed as it was previously described in the section: Preparation of the sage extract as a natural food preservative and its characterization - Characterization of SE.

DESCRIPTION OF THE OBTAINED RESULTS

The results of the sage extract characterization confirm the extract potential to be used for food preservation and development of active food packaging. Namely, minimal bactericidal and minimal inhibitory concentrations of the sage extract were evaluated performing the broth microdilution method (Figure 3). The obtained results revealed a high antimicrobial activity of the extract, in terms of its efficiency against foodborne pathogens: *S. aureus* and *E. coli*.

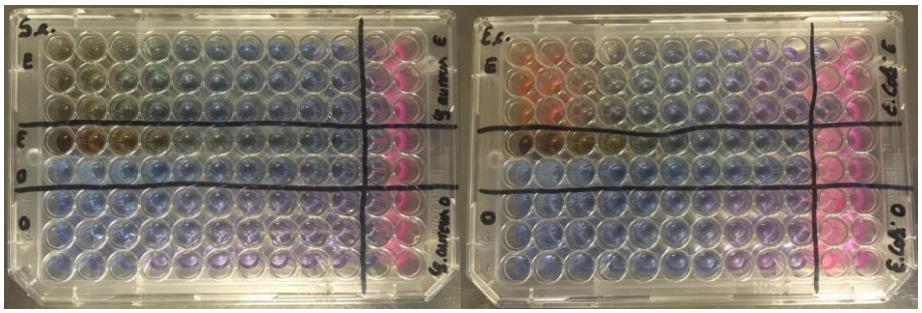

Figure 3. Determination of MIC values

Furthermore, antioxidant activity of the sage extract was evaluated employing DPPH$^{\cdot}$ free radical scavenging assay (Figure 4). A strong ability of the extract to inhibit DPPH$^{\cdot}$ free radical was observed.

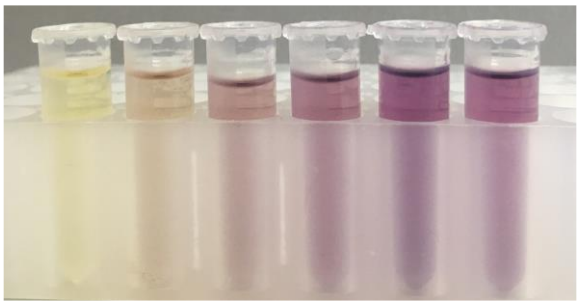

Figure 4. Solutions prepared for the calibration curve

PCL-based fibrous mats with incorporated SE (5, 10 and 20% SE) were prepared by the electrospinning technique (Figure 5). Plain PCL mat was prepared, as well. Afterwards, the mats were converted to the films by the annealing process on the hydraulic press.

Figure 5. Preparation of the fibrous mats

Morphology of the fibrous mats and the films obtained thereof is shown in Figure 6. An effect of the extract addition on diameters of the fibers and thickness of the films was noticed.

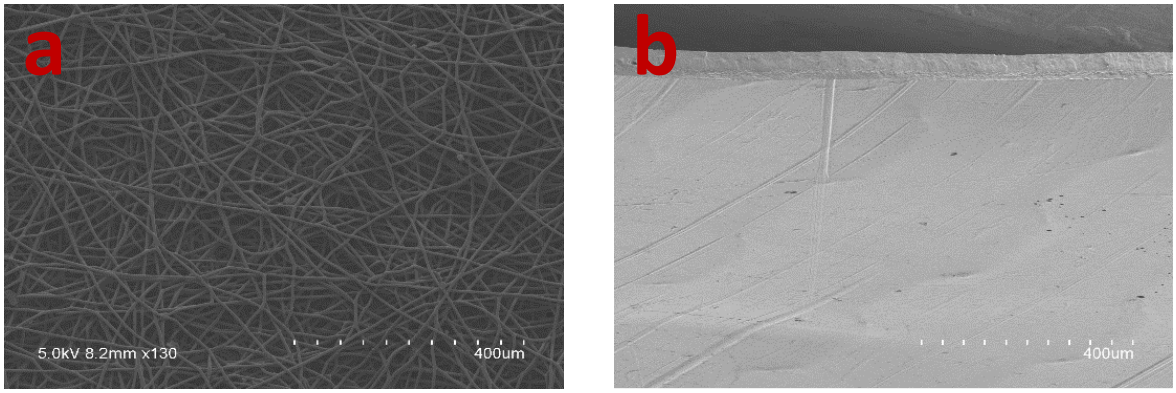

Figure 6. PCL-based fibrous mats (a) and films (b)

The prepared film samples were transparent. However, a change in color (yellowish color) of the samples containing SE was observed (Figure 7).

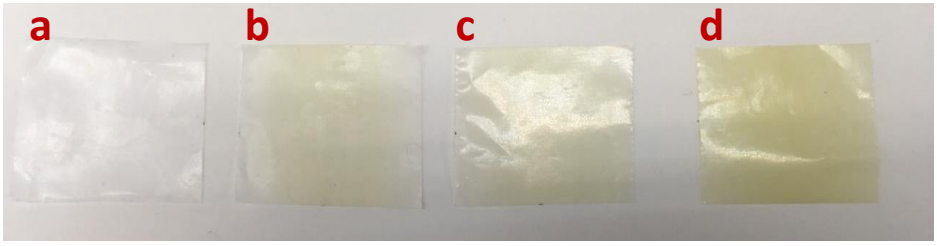

Figure 7. Plain PCL (a) and PCL-based films containing 5 (b), 10 (c) and 20% SE (d)

Characterization of the films in terms of their antimicrobial and antioxidant activity indicated a high potential of the developed ultrathin structures to be used as an active food packaging. Namely, the extract addition contributed to antimicrobial and antioxidant activity of the PCL based films (Table 1, Table 2).

Strong efficiency of the films' surface against *S. aureus* was observed for each formulation containing the extract. Higher efficiency against *E. coli* was achieved by increasing the extract content.

Table 1. Antimicrobial activity of the PCL-based films

SAMPLE	SURFACE REDUCTION *S. aureus*	SURFACE REDUCTION *E. coli*
PCL	R < 0.5	R < 0.5
PCL + 5%SE	R > 3	0.5 ≤ R < 1
PCL + 10%SE	R > 3	1 ≤ R < 3
PCL + 20%SE	R > 3	R >3

The films' surface exhibited high ability to scavenge DPPH$^{\cdot}$ free radical. Stronger ability of the inhibition was determined for the samples containing higher content of the extract.

Table 2. Antioxidant activity of the PCL-based films

SAMPLE	INHIBITION OF DPPH$^{\cdot}$ FREE RADICAL (%)
PCL	5.80 ± 1.13^{a*}
PCL + 5%SE	27.66 ± 1.38^{b}
PCL + 10%SE	41.40 ± 3. 13^{c}
PCL + 20%SE	79.97 ± 5.30^{d}

[*] Results are shown as mean ± standard deviation. Different letters within the same row indicate significant differences among samples. ANOVA, t- test ($p<0.05$).

CONCLUSION AND FUTURE COLLABORATIONS

During her stay at IATA-CSIC, Ana Salević has accomplished the stated aims of the STSM. She has acquired new scientific and operational experience necessary in production and characterization of the fibrous mats with encapsulated herbal extracts.

The STSM realization was effective and resulted in the development of the PCL-based ultrathin structures with encapsulated sage extract. The results reflected a high potential of the prepared films to be used as an active food packaging with the aim to prevent microbiological contamination and oxidative deterioration. Some of the results are under revision and therefore, not shown in the report. However, a scientific publication of the STSM outcome will be publically available very soon.

The performed STSM opens possibilities for further collaboration between the two research groups. There is a high interest in a continuation of the experiments carried out through STSM.

REFERENCES:

Atarés, L., & Chiralt, A. (2016). Essential oils as additives in biodegradable films and coatings for active food packaging. *Trends in Food Science & Technology*, *48*, 51-62.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food science and Technology*, *28*(1), 25-30.

Echegoyen, Y., Fabra, M. J., Castro-Mayorga, J. L., Cherpinski, A., & Lagaron, J. M. (2017). High throughput electro-hydrodynamic processing in food encapsulation and food packaging applications. *Trends in Food Science & Technology*, *60*, 71-79.

FAO. 2011. Global food losses and food waste – Extent, causes and prevention.

Glišić, S., Ivanović, J., Ristić, M., & Skala, D. (2010). Extraction of sage (*Salvia officinalis* L.) by supercritical CO2: Kinetic data, chemical composition and selectivity of diterpenes. *The Journal of Supercritical Fluids*, *52*(1), 62-70.

Gómez-Estaca, J., López-de-Dicastillo, C., Hernández-Muñoz, P., Catalá, R., & Gavara, R. (2014). Advances in antioxidant active food packaging. *Trends in Food Science & Technology*, *35*(1), 42-51.

Siracusa, V., Rocculi, P., Romani, S., & Dalla Rosa, M. (2008). Biodegradable polymers for food packaging: a review. *Trends in Food Science & Technology*, *19*(12), 634-643.

Souza, V. G. L., & Fernando, A. L. (2016). Nanoparticles in food packaging: Biodegradability and potential migration to food - A review. *Food Packaging and Shelf Life*, *8*, 63-70.