STSM report – Aleksandra Miletić

I spent 34 days (from 15.10-17.11.2018.) at IRTA Institute, Monnels (Girona), Spain under the supervision of Dr Begonya Marcos. During this period of time the performance of electrospun PLA fibers loaded with different essential oils was tested as antioxidant interleavers to extend the shelf life of sliced dry-sausages vacuum packed in PE/PA bags.

My work was divided into 3 parts. First part was preparation of PLA-based electrospun fibers and characterization of obtained fibers. As antioxidant compounds 4 different essential oils (fennel, juniper, thyme and black cumin) were chosen. Preliminary tests of antioxidant activity were done, where PLA fibers with 5, 10 and 15 wt% of essential oil were prepared and tested *in vitro* against DPPH radicals. The activity against DPPH radicals was expressed as mM Trolox/g of active fibers, and the values for fennel oil-loaded PLA fiber were shown in the following Table. The same trend was observed with all fibers so the concentration of 10 wt% was considered as optimal and taken for preparation of all active films for shelf-life study.

Sample	**DPPH [mM Trolox/g]**	**std**
PLA + 5 wt%	0,002055	0,000346
PLA + 10 wt%	0,002228	0,000821
PLA + 15 wt%	0,002193	0,000271

Five series of fibers were prepared for further examination as listed in the following Table.

No.	Sample
1.	PLA
2.	PLA + 10 wt% fennel oil
3.	PLA + 10 wt% thyme oil
4.	PLA + 10 wt% juniper oil
5.	PLA + 10 wt% black cumin oil

First, morphology of the fibers was observed using SEM analysis. Smooth and regular fibers were observed, without drops and beads. SEM images are shown at Figure 1.

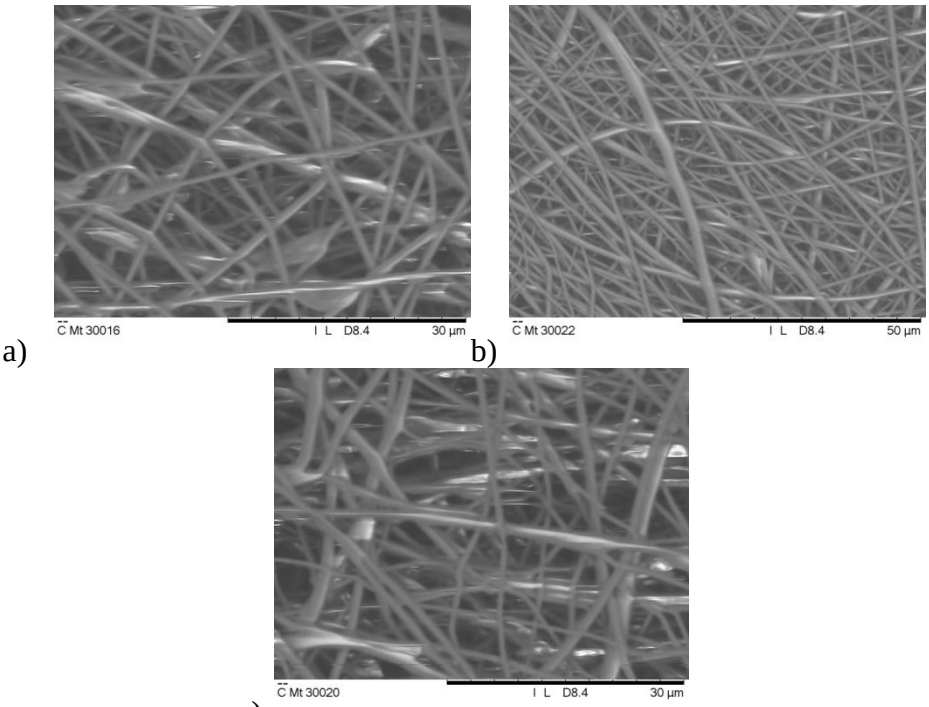


Figure 1. SEM images of a) PLA + Thyme, b) PLA + juniper and c) PLA + Black cumin oil

According to the FTIR analysis, it can be concluded that there is no chemical reaction between polymer and essential oil, because only characteristic peaks for PLA are present at the spectra. Also, it can be considered that the whole amount of essential oil is entrapped into the fibers, and that the amount present at the surface of the fibers is not significant. The comparison between spectra from different fibers is shown at Figure 2.

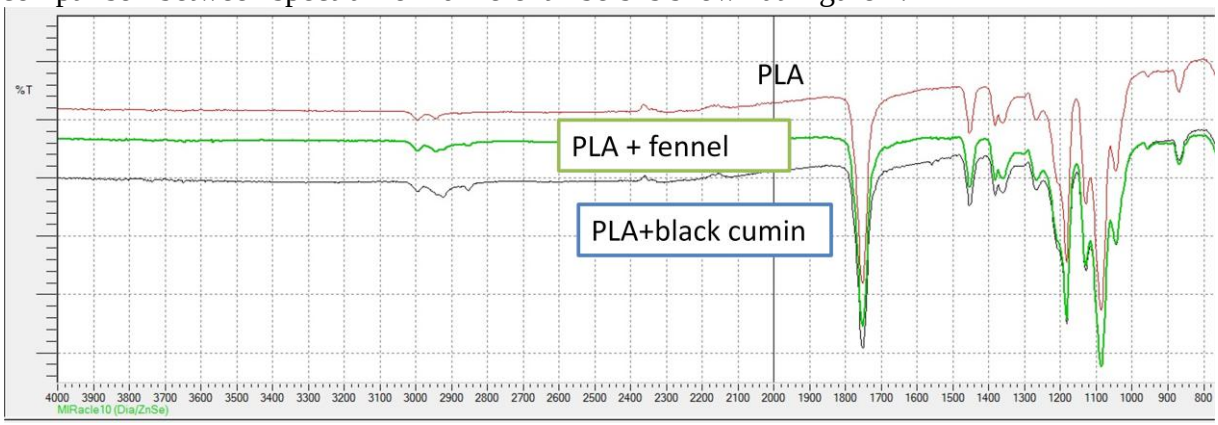


Figure 2. FTIR spectra of prepared PLA-based fibers

DSC thermograms are shown at Figure 3. The analysis of thermal properties of obtained fiber-based materials showed that the addition of thyme, fennel and black cumin oil had significant influence on Tg values, compared to pure PLA fiber, decreasing it in the range of 5 to 8 °C. The addition of thyme oil decreased also cold crystallization temperature Tc for 5.5 °C, while the addition of fennel and black cumin oil did not have the influence on the crystallization. The addition of juniper oil did not have the influence on Tg and melting temperature, but it increased the Tc temperature, compared with Tc of pure PLA fibers. 10 wt% of thyme oil decreased melting temperature for 5 °C, while the presence of other essential oils didn't have the influence on melting temperature.

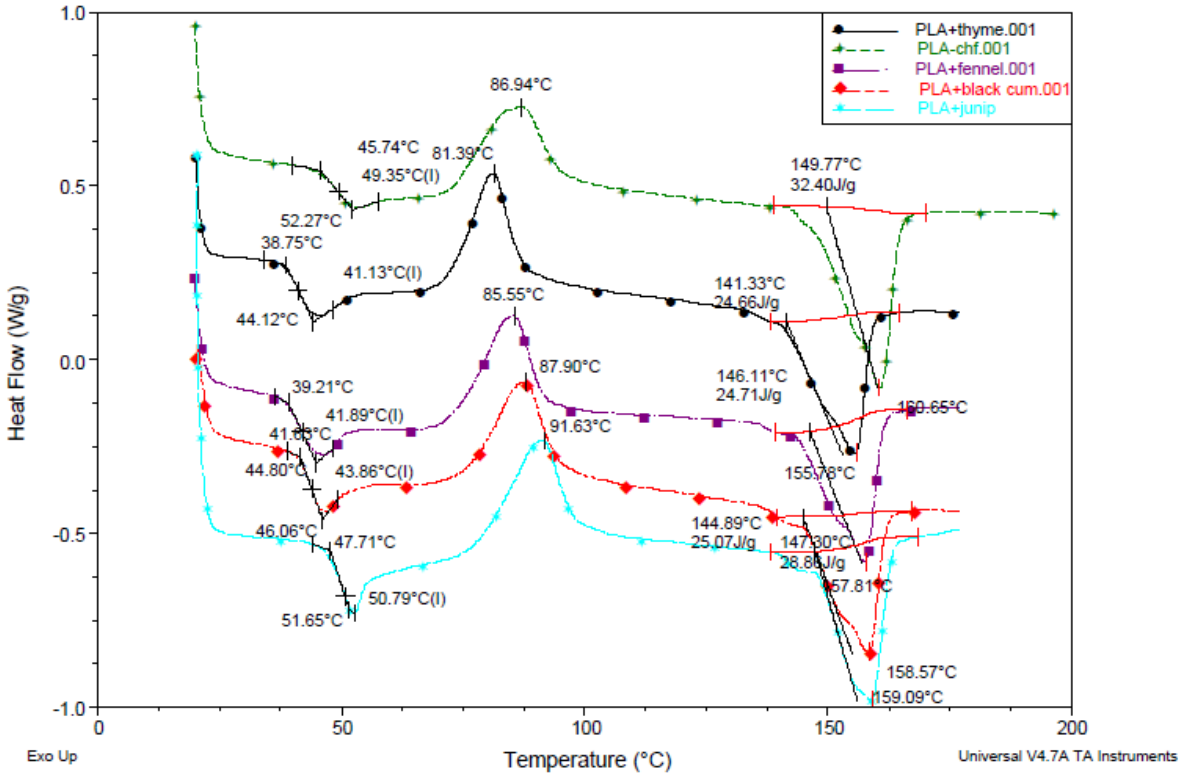


Figure 3. DSC thermograms of obtained fibers

After characterization of films, slices of dry-cured sausage were packed into commercially available PE/PA bags using vacuum packaging technique (Figure 4). Three samples from each batch for each sampling time were packed and put into a display cabinet where retail conditions were simulated. Samples were kept 12 hour at light and 12 hour in dark at around 8 °C. Sampling days were day 0, 7, 14, 21 and 28, and at each sampling day samples were taken from the cabinet and analyzed. Color measurement using Conica Minolta colorimeter, imaging and hyperspectral imaging were done on samples. Also, sensors for oxygen rate were put into the bags for the sampling day 28 and oxygen rate was measured at each sampling time. Water activity was measured for samples without interleaver and ones packed with pure PLA films.

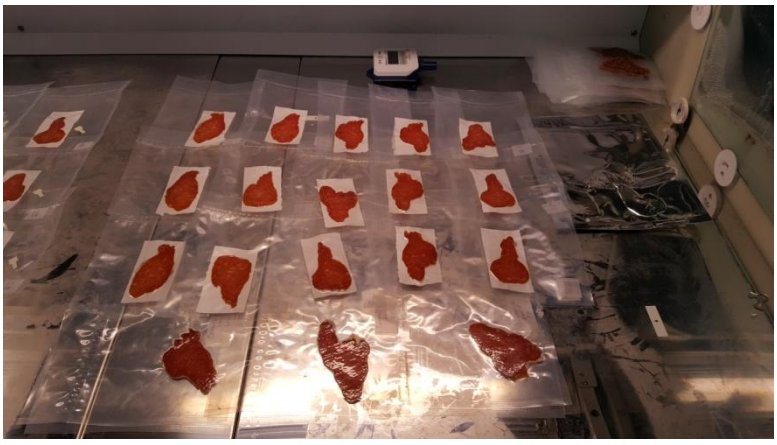
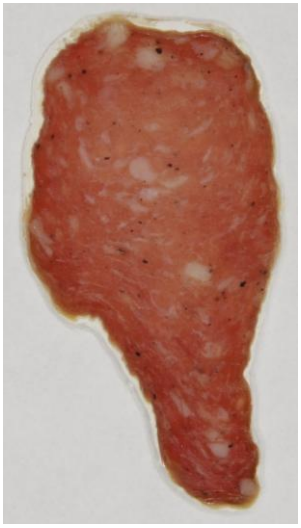
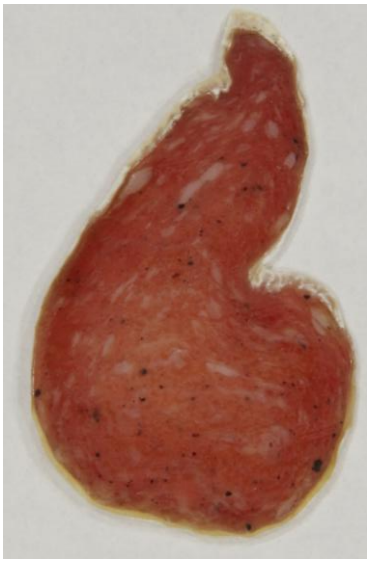
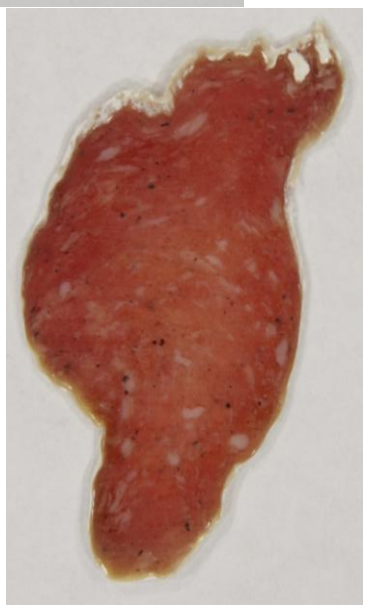


Figure 4. Vacuum-packed samples into the display cabinet

At each sampling time visual aspect and the smell of the samples were examined, and it is observed that at day 28 color changes can be detected by inspection. At the same time, no

changes in smell of the samples couldn’t be detected which indicates that the use of this kind of active packaging doesn’t change sensorial characteristics of the samples.
Changes in color (photos are taken with imaging system with calibrated lights) of the samples are shown at the Figure 5, following particular PLA + juniper oil during the time. It can be seen that color changed from nice red color to dark red-brown, and that the change occurred from the borders of the samples. It is considered that the color change is due to water loss from slices.

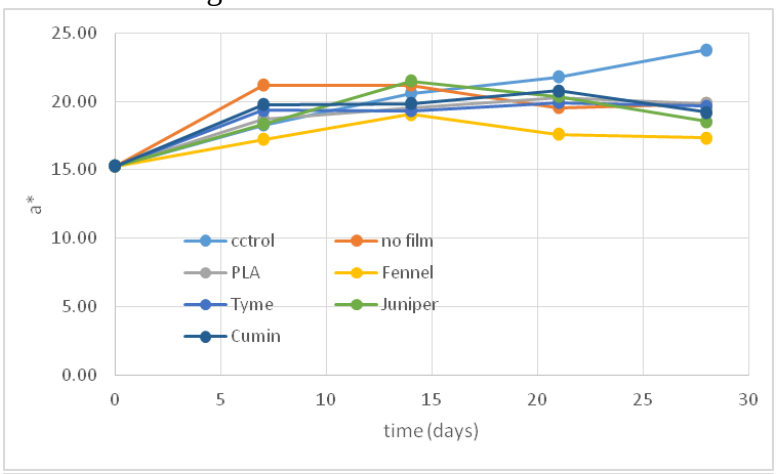
a)  b) 

c)  d) 

e) 

Figure 5. Color changes of PLA + juniper series samples a) t0, b) t7, c) t14, d) t21 and e) t28

Within colorimetric measurement, L*, a*, b* and R570/R650 values (according to the standard lower value points to less fade sample) were measured and examined. Following graphs are showing the trend of changes within different batches.

a) 

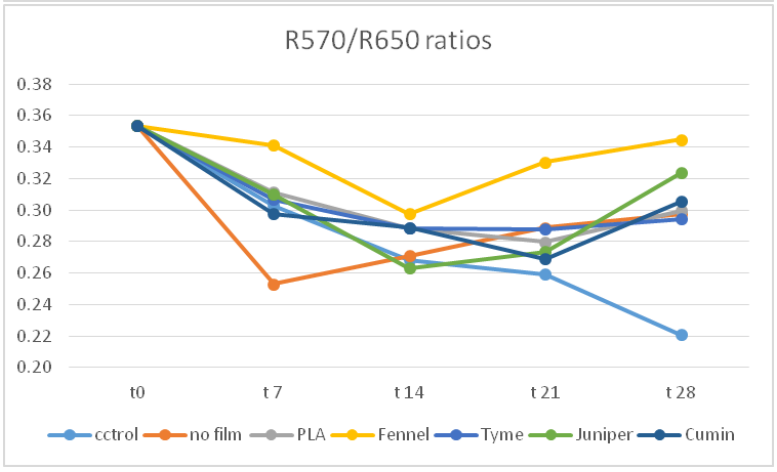
b) 

Figure 6. Comparison between a) a* values and b) R570/R650 values during time

Statistical analysis of results obtained from hyperspectral imaging, colorimetric measurement and oxygen rate was done to examine if there is statistically significant difference between the activity of PLA-active interleavers between different batches and during the time. It is shown that a* values and R570/650 for slices packed into bags with PLA+fennel oil film are

significantly different compared to others. Juniper oil has the highest influence on b* values of the samples at time t21, and at time 28 there is significant difference in R570/R650 values within group of samples packed with fennel-, juniper- and black cumin oil- loaded PLA fibers compared to the rest of batches. Those changes might occur due to interaction between meat and components of essential oils, which consist of variety of chemically different active compounds.

Changes in the oxygen rate into the packaging during the time are shown at Figure 7. Into the packaging where interleavers were present, decrease of the oxygen rate is observed, which is considered to be the result of microbial growth activity, and the slightly increase after sampling day 21 is considered to be from the permeability of the PE/PA bag which is not 100% oxygen barrier. There might be also some oxygen scavenging activity of PLA-based interleavers, because when compared to oxygen rate into control packaging, big differences can be seen. Higher oxygen rate within control packaging without film can be due to disbalance between used and diffused oxygen.

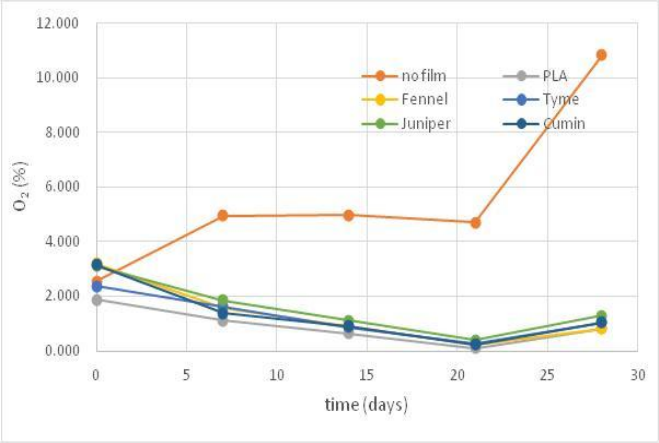


Figure 7. Changes in oxygen rate during the time

The antioxidant effect of the films on the dry-cured sausages was evaluated by means of the TBARS (thiobarbituric acid reactive substances) analysis TBARS extracts were obtained and kept at -80 °C. Due to a technical problem with the Alliance Analyser at IRTA, extracts could not be analyzed. Analysis of the extracts will be performed by Dr. Marcos once the technical problems are solved.

Results of water activity of the samples have following values: slices packed without any film: 0.852 and slices packed with PLA-film: 0.815, which indicates that porous interleavers absorb certain amount of water and dehydrate sausage. This is in accordance with the color change of slices during the time.

All used active pads were collected for the post analysis of main properties (Figure 8). SEM imaging was done on used samples and it was observed that pores are filled with fat (which is also confirmed using FTIR analysis).

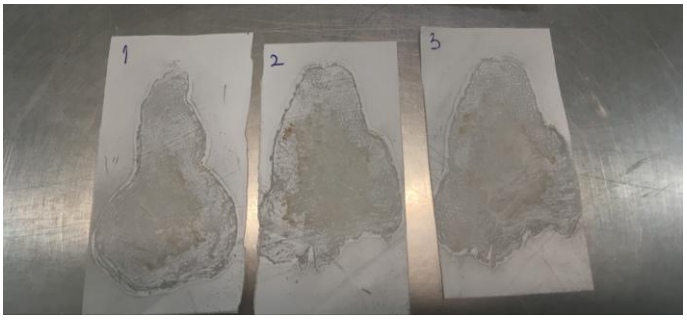


Figure 8. Used PLA-based interleavers

There is no difference between morphology of active pad used 7 and 28 days, which indicates that the biggest changes occurred in the first week of the shelf life study. SEM Images of different fibers are shown at Figure 9. It can be seen that drops of the fat are entrapped between the fibers, but fibers remained smooth and the diameter of fibers did not change, so fibers themselves did not absorb fat, it just diffused through pores.

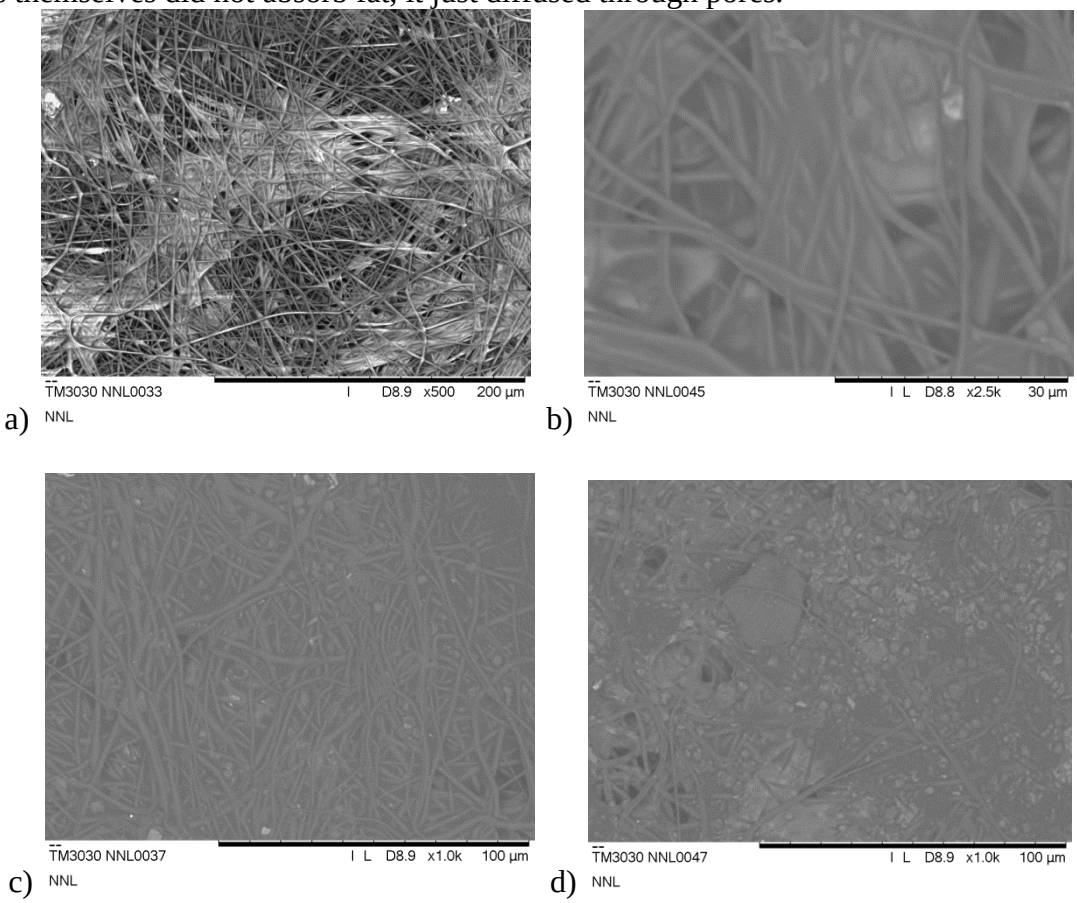
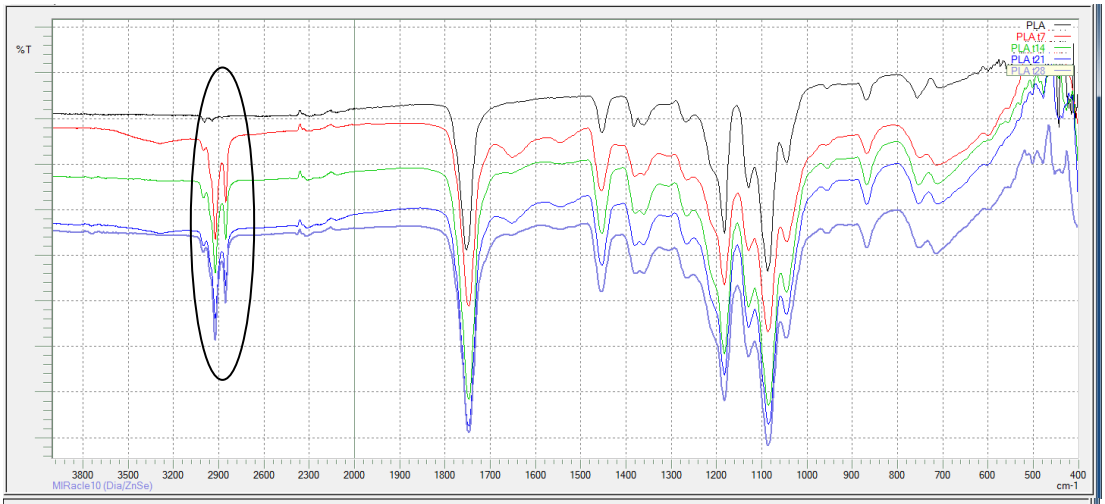


Figure 9. SEM images of used active pads a) PLA + fennel t28, b) PLA + juniper t28, c) PLA t28 and d) PLA + thyme t28

At FTIR spectra of used active pads, there are some changes compared to spectra of unused materials. The presence of two very sharp peaks around 2900 nm is observed and the comparison between spectra from unused and used films was shown at Figure 10.

a)



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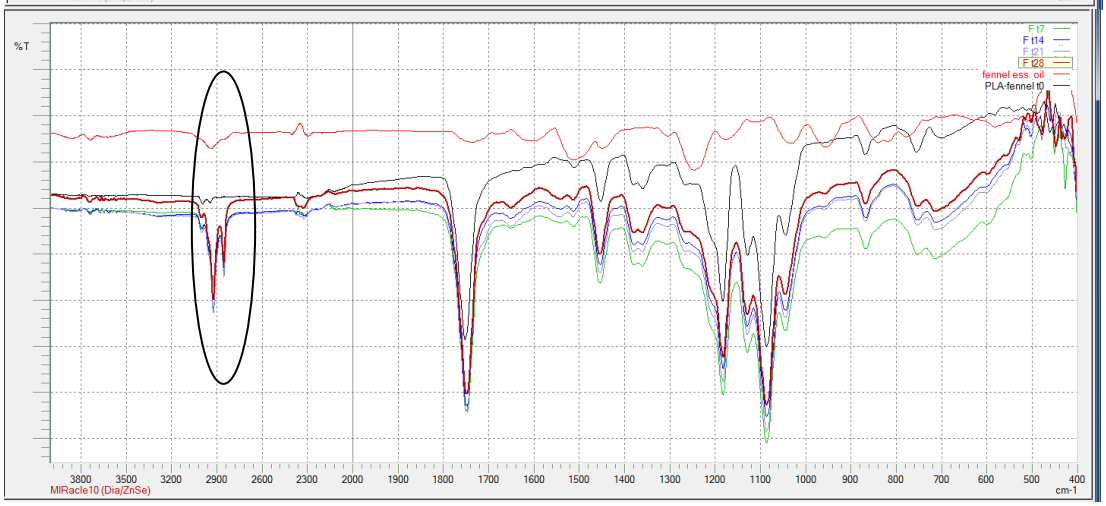
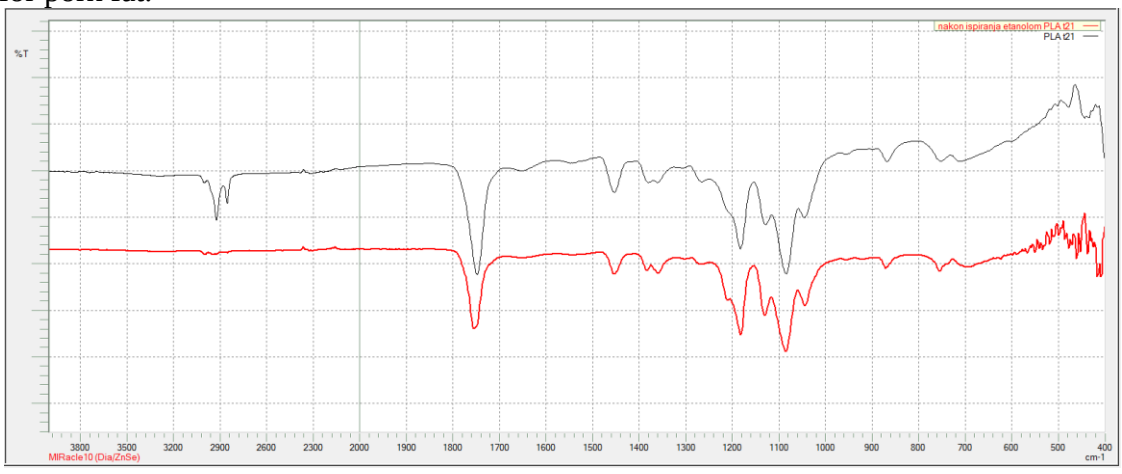


Figure 10. FTIR spectra of a) PLA series and b) PLA + fennel oil series

For proving the nature of those peaks, materials used to pack sliced dry-sausages were washed with ethanol, and after washing FTIR analysis was repeated on fibers and also on the residue obtained after evaporation of ethanol. Those spectra are shown at Figure 11. It can be seen that peaks around 3000 nm disappeared after washing, which indicates that this was just physical entrapment of fat. Peaks at 2860 and 2940 nm represent vibrations of C-H and are typical for pork fat.

a)



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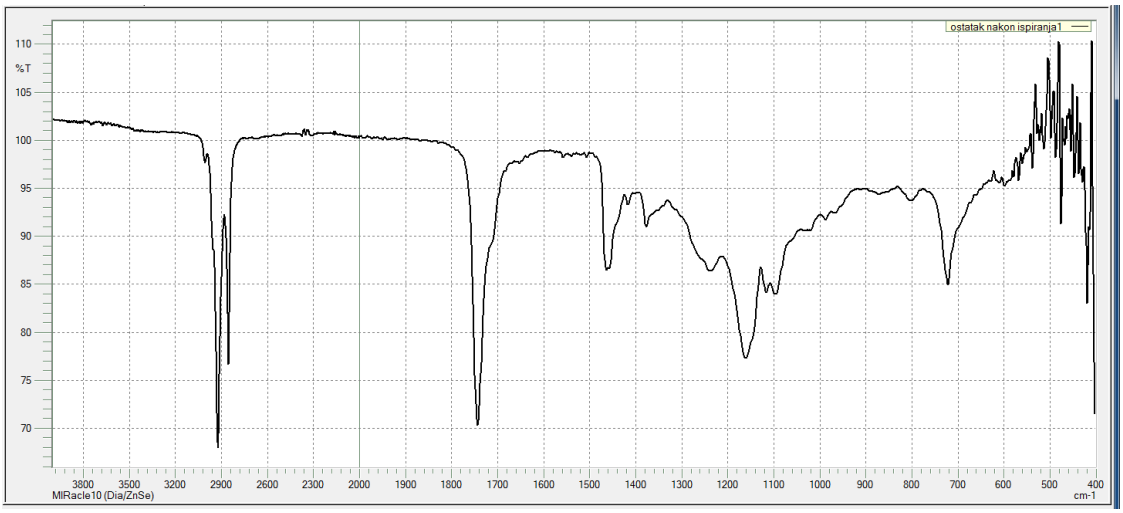
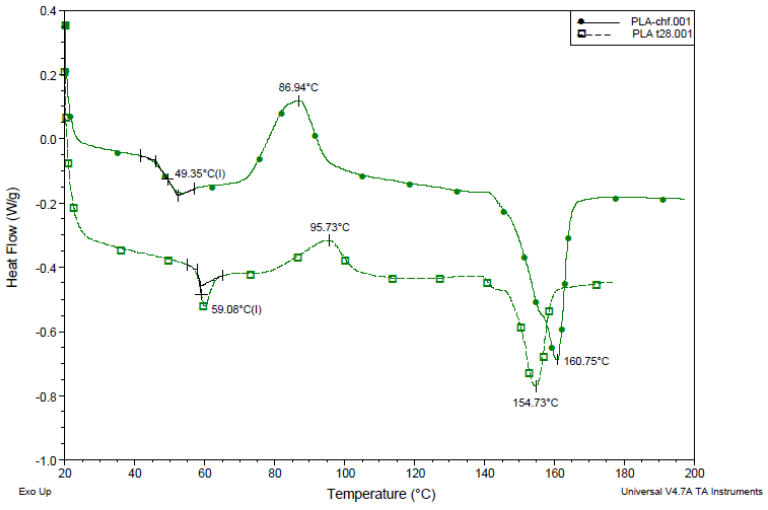


Figure 11. FTIR spectra of a) PLA fibers t21 (black) and PLA fibers t21 after washing and b) residue after evaporation of ethanol

Changes of thermal properties are observed using DSC analysis. Graphs of comparison of pure PLA, unused and used active pads are shown at Figure 12. Significant increase of Tg values and decrease of enthalpy of crystallization were observed. Increase of Tc values is also present, and it is followed with decrease of melting temperatures. This might happen due to presence of fat which has low melting temperature and also due to immiscibility of PLA and fat.

a)



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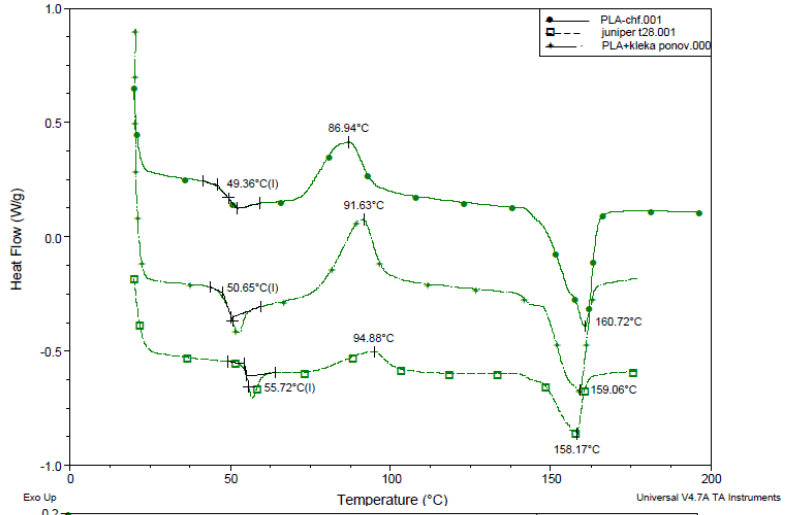

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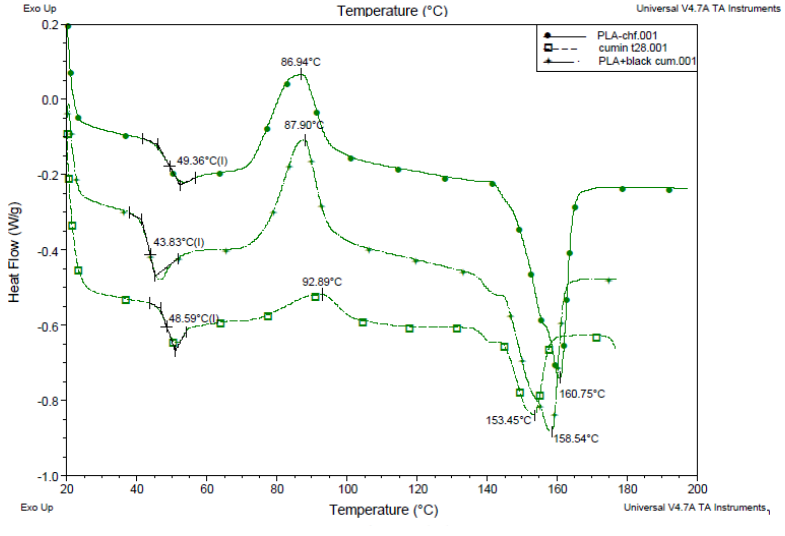

Figure 12. DSC thermograms of a) PLA series, b) PLA + juniper series and c) PLA + black cumin series

According to the results presented above, it can be concluded that this kind of porous interleavers can induce unwanted effect of dehydration of the products, so they can be used for packaging of the products where water is not desired, ex. fresh meat or dried fruits. This characteristic can have a bad influence like color change which is not due to the spoilage of the product but due to the water loss. At the same time, fat from the product also diffused between the pores of the material, which has influence on thermal properties of material, decreasing the melting point. There is no chemical change of the PLA-based material during the time, which indicates that there is no degradation of the material and that it can be considered for the long term use.

At the end, I would like to thank COST Action ActInPak FP1405 for supporting my STSM and enabling me to perform such a huge work on examination of *in vivo* activity of materials I prepared. This is a great example of applied science and scientific results and multidisciplinary approach. I would also like to thank to my great host dr Begonya Marcos, who shared a lot of her knowledge and experience with me and I really learned a lot during my STSM. Results obtained during this study will be processed and discussed for publication in high impacted journal, which will open collaboration between two research groups.