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Antioxidant packaging with encapsulated green tea for fresh minced meat.

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Abstract

A novel approach to incorporate green tea extract into polyethylene by extrusion technology has been studied. Green tea entrapped by inorganic capsules was incorporated into melted material and extruded without compromising its quality. The shelf-life of fresh meat from two types of active packaging was significantly extended for 3 days compared to blank samples. Significant difference was observed after 9th day of study in case of results of CIE L*a*b*, MetMb and organoleptic assay. Migration study of antioxidants from the materials was also performed by UHPLC-MS/TQ using food simulants. Amount of migrated catechins was in the range between 6.3 ± 3.3 and 228.4 ± 15.2 μg/Kg (ratio 6:1). Regression coefficients (R) between 0.9925 and 0.9989 were obtained. Minimum LOD (0.001 μg/g) and LOQ (0.004 μg/g) were obtained for epicatechin gallate and catechin gallate while maximum LOD (0.057 μg/g) and LOQ (0.191 μg/g) were obtained for catechin.

Keywords: green tea; active packaging; encapsulation; polyethylene; minced meat; migration

1. Introduction

The development of new films for extending the shelf-life of fresh food products is important for the food industry as packaging plays an essential role in limiting the deterioration of food (Prasad & Kochhar, 2014). Recently a lot of attention is given to active packaging (Soares, Silva, Medeiros, Carelli, & Espitia, 2011). Therefore, different active packaging technologies have been developed and applied to decrease meat decay (Contini, Álvarez, O'Sullivan, Dowling, Gargan, & Monahan, 2014; Lorenzo, Batlle, & Gómez, 2014; Muppalla, Kanatt, Chawla, & Sharma, 2014; Nerin, et al., 2006) and also to limit environmental pollution connected with packaging (McMillin & Belcher, 2012). Although the great interest and the amount of scientific publications dealing with active packaging, there are only a few packaging materials commercially available. This lack of commercial materials can be attributed to either the difficulties in producing the active materials at industrial scale, the low efficiency of most of the developed materials in *in vivo* tests and the degradation of active agents in extrusion processes.

One of the promising multi-component technologies that can be applied to protect the active ingredients during the packaging production is encapsulation. Encapsulation involves the isolation of a compound from its external environment by entrapping it into an additionally material, which has a protective function. This way the active compounds can be incorporated into melted material and extruded without compromising its quality. A lot of work about encapsulation directly applied into food products, processing and production can be already found in the scientific literature (Gibbs, Kermasha, Alli, & Mulligan, 1999; Nedovic, Kalusevic, Manojlovic, Levic, & Bugarski, 2011; uidam edo ic , 2010). However, the use of active capsules in polymers for extending the shelf-life of food has not been extensively used to date (Augustin & Sanguansri, 2010; Haidong, Fang, Zhihong, & Changle, 2011). The most popular materials used for encapsulation are cyclodextrins that let encapsulate compounds quickly and efficiently (Fuenmayor, et al., 2013; Haidong, et al., 2011; Silva, Figueiras, Gallardo, Nerin, & Domingues, 2014; Sun, et al., 2014). However, inorganic capsules consisting of crystalline microporous aluminosilicates, as those used in this study, can be perfect carriers because of the micropores present on the shell, which facilitate the incorporation and protection of the active agents. Moreover, inorganic capsules are more thermo-resistant and the antioxidant performance could be

observed in high humidity environment (Wei, Wang, Xiao, Zhang, Chen, & Ding, 2013; Yu & Xu, 2010).

Natural antioxidants such as alpha-tocopherol (Noronha, Granada, de Carvalho, Lino, Maciel, & Barreto, 2013), eugenol (Woranuch & Yoksan, 2013), thymol and cinnamaldehyde (Ponce Cevallos, Buera, & Elizalde, 2010) have been successfully encapsulated with further possibility of incorporation into plastic films. Catechins are powerful antioxidants present in cocoa, green tea, grapes and green algae (Arts, van De Putte, & Hollman, 2000; Haidong, et al., 2011; Ioannone, Di Mattia, De Gregorio, Sergi, Serafini, Sacchetti, 2015; Rusak, Komes, Likić, Horžić, Ko a č, 2008) among others. They include compounds such as (+)-catechin, (−)-epicatechin, (-)-gallocatechin, (−)-epigallocatechin, (−)-epigallocatechin gallate, (−)-epicatechin gallate, (−)-catechin gallate and (-)-gallocatechin gallate. They are efficient free radical scavengers due to the presence of phenolic hydroxyl groups (Colon & Nerin, 2012). As has been previously demonstrated (D. Carrizo, Gullo, Bosetti, & Nerin, 2014; Daniel Carrizo, Taborda, Nerín, & Bosetti, 2016; Nerin, 2010) when scavenging free radicals the release of antioxidants is not required, as they can be active and efficient antioxidants without direct contact with the food (Daniel Carrizo, et al., 2016; Nerin, 2010). Two mechanisms explain the antioxidant capacity (CAOX) of catechins: hydrogen-atom transfer and single electron transfer (Quideau, Deffieux, Douat-Casassus, & Pouysegu, 2011). In both systems direct contact with the food is not required and thus the use of catechins and other compounds with similar behaviour is a non-migrating performance. Although the chemical activity and antioxidant capacity of individual catechins are not equal, antioxidant synergy of catechins mix was scientifically proved (Sanna, et al., 2015). This way, the use of a natural mixture such as green tea, is better option than applying any single catechin.

The stability of green tea during processing and storage can be significantly reduced as they can easily undergo oxidation and thermal decomposition (Ananingsih, Sharma, & Zhou, 2013; Li, Taylor, & Mauer, 2011). Therefore, protection in a capsule would be highly desirable to fully utilize their potential benefits. Several systems based on encapsulation of green tea have been proposed in the literature (Gadkari & Balaraman, 2015) and the properties of catechins as free radical scavengers have been already applied in active packaging to extend the shelf-life of storage products (D. Carrizo, et al., 2014; Daniel Carrizo, et al., 2016; Colon & Nerin, 2014; Kaewprachu, Osako, Benjakul, & Rawdkuen, 2015; López de Dicastillo, Castro-López, López-

Vilariño, & González-Rodríguez, 2013; Wrona, Bentayeb, & Nerín, 2015).

Without a doubt, the quality of fresh minced meat is crucial for consumer's acceptance and one of the most visible decay is the fade of red colour of the meat. That turns brown by oxidation. Deterioration of minced meat is caused by protein oxidation characterised by the red color loss and lipid oxidation characterised by off-flavours and off-odours formation (Kaewprachu, et al., 2015; Nerin, et al., 2006). Therefore, the application of active packaging based on natural antioxidants working as free radical scavengers can be a good strategy for improving the quality and freshness of minced meat.

The aim of this work was to develop a new active packaging made by extrusion with encapsulated green tea as free radical scavenger, for extending the shelf-life of fresh meat without compromising its quality. Moreover, the proposed packaging was based on natural active agent rich in catechins that was protected by encapsulation, incorporated into melted polyethylene and extruded as a film, which was further used for packaging fresh minced meat. A pilot plant was used to prepare the new active film. Thanks to the encapsulation step the stabilization of green tea extract and preservation of its antioxidant property were ensured. In vivo test with minced fresh meat demonstrated the feasibility of the new material.

2. Materials and methods

2.1. Reagents

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; 98%, CAS 53188-07-1), fluorescein (3,6-dihydroxypiro[isobenzofuran-1[3H],9[9H]-xanthen]-3-one; Standard Fluka, CAS 518-47-8), AAPH (2,2'-azobis(2-methyl-propionamidine)dihydrochloride; 97%, CAS 2997-92-4), DPPH (α,α – difenilpicrilhidrazil radical, CAS 1898-664), caffeine (CAS 58-08-2) (CAF), (+)-catechin (>99.0% (HPLC), CAS 154-23-4) (C), (−)-epicatechin (>95% (HPLC), CAS 490-46-0) (EC), (−)-epicatechin gallate (>98% (HPLC), CAS 1257-08-5) (ECG), (−)-catechin gallate (>98% (HPLC), CAS 130405-40-2) (CG), (−)-epigallocatechin (>95% (HPLC), CAS 970-74-1) (EGC), (−)-gallocatechin (>98% (HPLC), CAS 3371-27-5) (GC), (−)-gallocatechin gallate (>98% (HPLC), CAS 4233-96-9) (GCG) and (−)-epigallocatechin gallate (>95% (HPLC), CAS 989-51-5) (ECG) were from Sigma-Aldrich (Madrid, Spain). Disodium hydrogen phosphate dihydrate (99.5%, CAS 10028-

24-7), and sodium dihydrogen phosphate monohydrate (99%, CAS 7558-80-7) were purchased from Merck (Madrid, Spain). Methanol (LC-MS, CAS 67-56-1), ethanol absolute (GC-MS, CAS 64-27-5) and acetone (HPLC grade, CAS 67-64-1) were from Sharlau (Barcelona, Spain). The ultrapure water was obtained from a Millipore Milli-Q_{PLUS} 185 system (Madrid, Spain).

2.2.Apparatus

Ultra-Turrax T18 from IKA (Staufen, Germany) was used to homogenize the meat samples. Ultrasonic bath Branson 3510 (Branson Ultrasonic Corporation, USA) and centrifuge CENTROMIX model S-549 were also utilized.
Nikon Coolpix 4300 camera (Tokyo, Japan) and Konika Minolta chroma meter C-400 (Tokyo, Japan) were used for colour evaluation.

2.3.Equipment

UV-1700 of Shimadzu Pharmaspec Ibérica (Madrid, Spain) spectrophotometer was used for antioxidant capacity methods.

Non-volatile compounds were analysed by AcquityTM system with use of an Acquity UPLC BEH C18 column of 17-µm particle size (2.1 mm×100 mm), both from Waters (Milford, MA, USA). MS detector consisted of an hexapole, a quadrupole, a colli- tion cell and time of flight analyser (Xevo G2) from Waters (Milford, MA, USA).

Volatile compounds were analysed by CTC Analytics CombiPal autosampler coupled to an Agilent 6890 N gas chromatograph with an MS mass spectrometer detector. All instruments were from Agilent Technologies (Palo Alto, CA, USA). Chromatographic separations were carried out on a BP-20 (30 m × 0.25 mm × 0.25 µm) from SGE analytical science (Madrid, Spain). DVB/CAR/PDMS fiber from Supelco (Bellefonte, PA, USA) for SPME adsorbstion was used.

Antioxidants were analysed by Acquity Ultra Performance LC TQ detector (triple quadrupole; Waters). An ESI probe was used in positi e (ESI+) and in negati e (ESI−) mode as the ionization source. Chromatography was carried out in the Acquity system using an Acquity UPLC BEH C18 column of 1.7 µm particle size (100 mm × 2.1 mm) from Waters. MassLynx (v. 4.1) software (Waters) was used to acquire and process the chromatographic and MS data.

2.4.Samples

Samples of minced pork meat were bought in a local supermarket. Samples were obtained on PP trays closed with a lid of a high barrier plastic. The trays were provided with absorbent pad. All samples were stored in a refrigerator at 4 °C.

2.5.Active packaging

Active polyethylene (PE) films with incorporated capsules loaded with green tea extract were provided by NUREL. Processes of preparation of inorganic capsules and active materials are protected by the patents EP1564242B1 and EP1923423B1. Two different types of capsules developed by the company were incorporated into the polymer structure: polyethylene with type I capsules (PE I) and polyethylene with type II capsules (PE II). PE I consisted of 20% of masterbatch containing active capsules while PE II consisted of 40% of masterbatch containing active capsules. Both materials were prepared by extrusion. These materials with green tea extract at concentrations of 6.4 mg/g (mg of active agent per g of material) in case of PE I and 12.8 mg/g (mg of active agent per g of material) in case of PE II were tested. The same materials but without green tea were used as blank samples. 22 g of minced pork meat were placed in small plastic Petri dish (d = 5 cm). Then meat was covered by PE active film (6 cm x 6 cm) and packed at vacuum bag (7 cm x 9 cm) under normal atmosphere using a thermo-sealer. The proposed packaging is shown in figure 1.

<<Figure 1. >>

In this case the active material was in contact with the meat. Also blank samples were prepared using PE without active capsules. Meat was randomly taken from different trays to avoid meat from the same animal being allocated to the same treatment that would affect the replication. Each sample was prepared in triplicate. Replicates were alternatively prepared, that is to say, one replicate of each type of active film was prepared at a time to avoid experimental bias. The packages were stored in a refrigerator at 4 °C for 13 days. Constant and uniform temperature was ensured to avoid differences in the performance of the series of replicates. Additionally, replicates of the

same type of packaging were interspersed in the refrigerator. Figure 2 shows a scheme of the applied procedure for active packaging development.

<<Figure 2 >>

2.6.Quality of meat

The quality of meat was tested during the experiments by the following measurements: colour measurement, Met myoglobin (MetMb) measurement and organoleptic (visual and aroma) content. Also photographs were taken. All measurements were performed after 0, 6, 9, 12 and 13 days of experiment.

2.6.1.Photographs and colour measurement

Photographs and colour evaluation were carried out 30 min after opening the package to allow blooming. Photographs were taken by the camera set at 15 cm height and at an angle of 45˚. The samples were lightened from abo e. The camera settings were adjusted as follows: mode, manual; flash, cancelled; focus mode, macro close-up; image quality, fine; image size, 1600 pixels; sensitivity, ISO auto; metering, matrix. White balance was manually adjusted using a standard white chromameter.

Colour measurement was performed using a portable chromameter. To quantify colour change CIE L*a*b* colour space was used. Parameter a*, responsible of redness, was recorded. Positive values of a * indicate red colour and negative values indicate green colour.

2.6.2.MetMb content

The method proposed by Krzywicki (Krzywicki, 1979) was applied to determine MetMb content responsible for the brown colour of meat. Breafly, 5 g of meat were homogenised with 25 ml of iced cold phosphate buffer (40 mM , pH 6.8) at 18000 rpm using an Ultra-Turrax. Then all samples were maintained at 4 ° C for 60 minutes. After that time samples were centrifuged and the supernatant was filtered using LAB® PTFE-filter (diameter: 25 mm, pore size: 0.45 m). The absorbance of the filtrate was measured at 525 nm, 545 nm, 565 nm and 572 nm against the blank sample (solution of phosphate buffer). MetMb concentration was determined from an equation given by Krzywicki.

2.6.3. Organoleptic assay

The organoleptic test based on the evaluation of visual appearance and smell of the samples was performed. Five testers were invited to participate in the organoleptic evaluation of meat. All of them underwent training in which the different stages of minced meat deterioration were shown.

Four different evaluations were prepared. Firstly, the colour of blank samples and the color of samples from antioxidant packaging were compared (pair comparison test). Then a ranking test was carried out where a value from colour scale: 1 to 5 (1 was nasty and 5 was very good) was assigned to each type of sample. During the third assessment the aroma was assigned to each type of sample. Then a value from odour scale: 1 to 5 (1 was nasty and 5 was very good) was assigned to each type of sample. Both the ranking test and pair comparison test were based on ISO norms (ISO 8587:2006 ("Sensory analysis. Guidelines for the use of quantitative response scales. (ISO 412:2003),"), ISO 5495:2005 and ISO 5495:2005/Cor 1:2006 (Zotarelli, Porciuncula, & Laurindo, 2012)).

2.7. Quality of active packaging

2.7.1. Antioxidant capacity

Two methods were applied to determine the antioxidant capacity of the materials under study. The first method was DPPH. For that, 1 dm^2 of each active and blank film was accurately weighed and placed in 18 mL vials. Then 6 g of methanol were added to each vial. The extraction was performed in the ultrasonic bath for 15 min. Each sample was prepared in triplicate. Also the antioxidant capacity of the pure extract and the capsules were evaluated by addition of 6 g of methanol to 0.05 g of analyte in 18 mL vial. Again the extraction was performed in the ultrasonic bath for 15 min.

The antioxidant activity was evaluated by preparing 5 different film extracts dilutions in methanol. The concentrations of dissolutions of extracts were expressed in μg/g taking into account extraction yields (EY) for each type of polymer. EY was determined gravimetrically comparing dry extract with amount of active agent incorporated in the material. Also mass of dry extract of blank material (with empty capsules) was prepared and used in calculations. EY was expressed in percentage. The

reaction was conducted by adding 100 µL of each extract dilution to 3.5 mL of 76 µM solution of DPPH in methanol. Also blank solution (DPPH in methanol without extract) was prepared. All samples were left for 15 minutes in darkness. After this time the absorbance of samples at 515 nm was measured using the UV-VIS spectrophotometer. The spectrophotometric measurement was made against the blank (methanol). Additionally, a calibration for checking DPPH concentration was carried out. For this purpose, standard solutions of DPPH at concentrations between 4 and 64 µg/g were prepared in methanol. DPPH solution was prepared daily.

The antioxidant capacity of the samples was expressed as percentage of inhibition of DPPH (I%) and it was calculated according to the following formula $I\% = [(A_0\text{-}A) / A_0] \times 100$ where A_0 and A are the absorbance values of the blank (DPPH in methanol) and the extract sample (DPPH with extract), respectively. The value of percentage of inhibition after 15 min was plotted against the concentration of antioxidant. In this way a linear regression was applied to obtain inhibition concentration (IC_{50}) that is commonly used for comparison of antioxidant capacities of natural extracts measured by DPPH method. It indicates the antioxidant concentration necessary to decrease initial concentration of DPPH by 50%. The strongest antioxidant is, the lower value of IC_{50} is obtained. Therefore, IC_{50} value is inversely proportional to the antioxidant activity.

The second method used was oxygen radical absorbance capacity (ORAC) assay that was performed by the method optimised by Bentayeb et al. (Bentayeb, Vera, Rubio, & Nerin, 2009). A piece of 1 dm^2 of active film was cut into 4 pieces and extracted with 6 g of acetone using ultrasonic bath for 15 min. Also blank samples were analysed. All samples were prepared in triplicate. The antioxidant capacity of both pure extract and capsules was evaluated by addition of 6 g of acetone to 0.05 g of analyte or capsule in 18 mL vial. Again the extraction was performed in the ultrasonic bath for 15 min. The antioxidant capacity of the resulting solution was measured using the protocol described above.

The standard solutions of fluorescein (2.3 µg/g) and AAPH (34 mg/g) were prepared in sodium phosphate buffer (75 mM, pH 7.0). The ORAC assay was performed by mixing 0.2 ml of the antioxidant extract with 1.9 ml of fluorescein in a spectrophotometer cuvette of 10 mm optical path. The reaction was triggered by addition of 1.4 ml of AAPH solution. Then the mixture was briefly stirred and the absorbance at 490 nm was monitored using spectrophotometer. The buffer solution was used as reference. The reaction was carried out at 40 ° C for 60 min using the kinetic

mode. Immediately before testing, the corresponding volumes of reagent solutions were incubated at 40 ° C for 15 min to minimize irreproducibilities related with temperature. All results were expressed as grams of Trolox per square meter of active film. Therefore, external calibration was performed by measuring Trolox solutions in the range of 25 mg/g to 250 mg/g. A 1000 mg/g Trolox solution in buffer was previously prepared to draw the calibration curve.

2.7.2. Migration test

Migration assays have been performed for fresh minced meat in accordance with the parameters contained in the legislation for food contact materials EU/11/2011 ("Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food," 2011). 10% and 95% solutions of ethanol were used as simulants. Migration assay was carried out during 10 days at 20 ºC. Total immersion of active films was performed. 2 cm x 3 cm of each material was accurately weight and placed in a 20 mL vial with 18 g of simulant. The samples of 95% ethanol were five times concentrated under gentle stream of nitrogen at 40° C. Three replicates of each sample and blank sample consisting of pure simulant were prepared. The volatile compounds were analysed using SPME-GC-MS and non-volatile compounds were analysed using UHPLC-QTOF-MS^E (negative and positive mode). Chromatographic conditions for both types of analysis (volatiles and non-volatiles) proposed by Canellas et al. (Canellas, Vera, & Nerin, 2015) were applied.

Moreover, the prepared samples were analysed by UHPLC-MS/TQ to quantify the amount of released active agents. The analysis was carried out according to the chromatographic method developed by Colon and Nerin (Colon, et al., 2014). Quantification of the standards and the samples was carried out by the selected ion recording (SIR) acquisition method. Moreover, calibration of external standards was performed and analytical parameters were determined. Stock solutions of each catechin and caffeine at concentration 50 μg/g in methanol were prepared. The linearity of the calibration curve was determined by preparing seven working standards from the stock solutions in the range of 0.1–50 μg/g. Blank solutions of pure solvent (methanol) were also analysed by UHPLC-MS/TQ.

The total concentration of all detected antioxidants was calculated and was expressed as that corresponding to 6 dm^2 of material per 1 Kg of food simulant (10% or

95% ethanol).

The analytical features of the method such as the linear range, limits of detection (LOD) and quantification (LOQ) were determined for catechins and caffeine. LOD and LOQ were determined using signal-to-noise method.

2.8.Statistic

All the presented results were expressed as mean ± standard deviation. Student t test has been performed to check if there are significant differences between the analysed films with different concentrations of the capsules. The null hypothesis was adopted if the analysed samples were the same. When experimental value of t was greater than t value given in the table, the difference between the samples was significant and the null hypothesis was rejected.

3. Results and discussion

3.1.Quality of meat

3.1.1.Photographs and colour measurement

Analysing the photographs from all days of experiments it can be concluded that until day 6 there wasn´t any difference between the blank samples and the samples from both types of active packaging. Significant difference was observed after 9^{th} day of study. Figure 3 shows the meat samples after 9 days of experiment. All replicates look similar and satisfactory reproducibility was obtained during the experiments. It means that samples, blank material and active materials were homogeneous. Total spoilage resulting in brown color of meat can be observed in the case of samples from blank packaging, whereas the samples from active packaging stayed red and fresh.

<<Figure 3 >>

Redness of meat is described by a* value. The higher a* value is the more red and desirable meat colour. It is obviously what most influence the consumer acceptance of product. Other CIE L*a*b* values such as L* and b* were not taken into account due to the lack of significant information. The obtained a* values are presented in figure 4.

<<Figure 4 >>

It can be clearly seen that there is significant difference ($t_{BK\ vs\ PE\ I}$ = 8.23; $t_{BK\ vs\ PE\ II}$ = 8.96; t_4 = 2.78 (P = 0.05)) between a* value of meat from the blank packaging and a* value of meat from both types of active packaging after 9 days of refrigerated storage. Values of a* after 9 days for blank packaging, PE I and PE II were 13.1 ± 0.8; 17.0 ± 0.3; 17.2 ± 0.2 respectively. Moreover, a* values of meat from 9 days active packaging corresponds to a* values of meat from 6 days blank packaging. Therefore, proposed packaging extends shelf life of meat for 3 days. There was no significant difference between values of a* of PE I and PE II active packaging.

3.1.2. MetMb content

Myoglobin is the protein within the muscle fibre that takes oxygen from hemoglobin. When meat is oxidized (oxidation of iron) the pigment cannot bind oxygen and MetMb is created. Therefore, MetMb is responsible for the brown color of meat and is a good indicator of its deterioration. The results of MetMb content are shown in table 1. The percentage of MetMb in the meat from 9th day in the blank packaging is 1,5 times higher than the percentage of MetMb from 9th day active packaging (PE I and PE II). All samples had the same MetMb value before 9 days of analysis. Moreover, after this time there was no significative difference between blank samples and antioxidant samples. Student test t has shown significant difference between blank packaging and two types of active packaging ($t_{BK\ vs\ PE\ I}$ = 6.08; $t_{BK\ vs\ PE\ II}$ = 8.54; t_4 = 2.78 (P = 0.05)). Thus, redness of meat from active packaging was higher. This indicates that the developed active packaging preserves the red color of meat. These results agree with the results obtained during CIE L*a*b* measurement and confirms that the proposed antioxidant material extends the shelf life of meat for 3 days. There was no significant difference between values of a* of PE I and PE II active packaging. After day 9 meat is deteriorated and no significant differences were found between active and blank samples.

Table 1. Results of MetMb content for different types of packaging during the whole experiment.

Time (days)	BK	RSD%	PE I	RSD%	PE II	RSD%
0	11% ± 1%	9%	11% ± 1%	9%	11% ± 1%	9%
6	14% ± 1%	4%	15% ± 1%	7%	15% ± 1%	7%
9	27% ± 2%	6%	18% ± 2%	12%	18% ± 1%	6%
12	34% ± 6%	16%	34% ± 5%	13%	36% ± 5%	12%
13	38% ± 6%	14%	35% ± 6%	16%	40% ± 1%	2%

3.1.3. Organoleptic assay

The influence of developed active packaging into sensory attributes such as colour and aroma evaluated by testers is summarized in tables 2 and 3. Testers have seen difference between BK samples and PE I and PE II samples only after 9 days of experiment. There wasn´t any difference between samples before day 9 because during this time meat was still fresh. Then, after day 9 all samples were equally deteriorated. Similar results were obtained in case of evaluation of aroma of meat. All samples maintained aroma of fresh meat till day 6. In case of 9th day blank samples were characterised by vinegar aroma while the aroma of samples from active packaging was not changed. Vinegar aroma can be an indicator of meat fermentation and the beginning of spoilage reaction. Vinegar aroma is associated with acetic acid coming from amino acids degradation and also from hydrolysis of triglycerides (Hernandez-Macedo, et al., 2012). Obviously this sour odour disqualifies the meat as an acceptable product for the consumer. Aroma of all samples was described as yeast odour after 12th day and after 13th day of experiment, and an unpleasant spoiled meat odour was perceived.

Table 2. Results of ranking test for color and odour of meat samples during whole experiment.

Evaluation	Sample	Scale				
		Day 0	Day 6	Day 9	Day 12	Day 13
Visual	BK	5.0	5.0	3.0	2.0	2.0
Visual	PE I	5.0	5.0	4.5	2.0	2.0
Visual	PE II	5.0	5.0	4.5	2.0	2.0
Odour	BK	5.0	5.0	3.0	2.0	2.0
Odour	PE I	5.0	5.0	4.5	2.0	2.0
Odour	PE II	5.0	5.0	4.5	2.0	2.0

Table 3. Type of aroma of meat samples according to testers during whole experiment.

Sample	Aroma				
	Day 0	**Day 6**	**Day 9**	**Day 12**	**Day 13**
BK	fresh meat	fresh meat	vinegar	yeast	spoiled meat
PE I	fresh meat	fresh meat	fresh meat	yeast	spoiled meat
PE II	fresh meat	fresh meat	fresh meat	yeast	spoiled meat

3.2.Quality of active packaging

3.2.1.Antioxidant capacity

Extraction yield for both materials was 99% ± 1% and it was taken into account when calculating IC_{50}. Antioxidant capacity using DPPH method obtained for blank PE I was IC_{50} = 286 ± 6 μg/g and for blank PE II it was IC_{50} = 257 ± 7 μg/g. Results pointed out a significant difference between polyethylene samples. Thus, higher antioxidant capacity was obtained for PE II. Blank sample of polyethylene did not change the absorbance of DPPH, so obviously it has not got any antioxidant properties.

ORAC was the second method used for the determination of antioxidant capacity ($CAOX_{ORAC}$) where results were expressed as grams of Trolox per square meter of active film. Here the strongest antioxidant is, the higher value of antioxidant capacity is. Antioxidant capacity obtained for blank PE I was $CAOX_{ORAC}$ = 2.20 ± 0.08 g Trolox/m^2 and for blank PE II it was $CAOX_{ORAC}$ = 2.48 ± 0.09 g Trolox/m^2. The results obtained by ORAC assay where coherent with DPPH results.

Moreover, it was observed that the antioxidant capacity of active agent decreased together with its application into active material. As a result, active capsules had lower CAOX than the pure extract (IC_{50} = 99 ± 11 μg/g; $CAOX_{ORAC}$ = 2.05 ± 0.15 g extract/ g Trolox) and consequently active materials containing the capsules had lower antioxidant capacity than the active capsules (PEI: IC_{50} = 117 ± 4 μg/g; $CAOX_{ORAC}$ = 2.63 ± 0.06 g Trolox/m^2; PEII: IC_{50} = 118 ± 12 μg/g; $CAOX_{ORAC}$ = 2.56 ± 0.10 g Trolox/m^2). Amongst other contributing factors, the efficiency of extraction procedure can be also important.

3.2.2.Migration test

Results of specific migration for all materials from SPME-GC-MS and UHPLC-QTOF-MS^E have shown that there were not detected any migrant neither volatile nor non-volatile coming from the plastic films. Furthermore, analysing the results of

migration for all materials from UHPLC-MS/TQ shows presence of components of green tea. The used materials were food contact plastics. The results of quantification are presented in the table 4.

Table 4. Results of migration study and analytical characteristics of analytical method.

Compound	Ratio 6:1 (µg/Kg)				r	LOD (µg/g)	LOQ (µg/g)
	10%EtOH PE I	10%EtO PE II	95%EtOH PE I	95%EtOH PE II			
EGC	-	32.0 ± 0.7	-	-	0.9937	0.002	0.006
GC	-	101.9 ± 3.5	-	-	0.9968	0.043	0.144
C	-	-	-	43.5 ± 16.4	0.9983	0.057	0.191
EC	-	-	97.6 ± 64.3	6.3 ± 3.3	0.9989	0.011	0.038
EGCG	-	88.1 ± 2.0	94.3 ± 85.3	228.4 ± 15.2	0.9925	0.003	0.011
GCG	17.9 ± 13.2	19.4 ± 7.1	139.7 ± 87.9	55.6 ± 6.5	0.9967	0.008	0.027
ECG	36.2 ± 11.9	8.6 ± 1.5	181.5 ± 34.6	90.8 ± 6.8	0.9975	0.001	0.004
CG	75.9 ± 12.9	53.8 ± 2.1	77.0 ± 29.2	-	0.9965	0.001	0.004
CAF	67.8 ± 0.9	66.0 ± 0.8	10.8 ± 4.2	6.4 ± 1.3	0.9973	0.020	0.060

The performed study let to determine the releasing properties of each catechin and caffeine from the active film samples. It can be seen that a higher diversity of compounds was observed during migration from PE II in both types of simulant. However, at the same time higher amount of specific compounds was released from PE I. The highest migration rate was observed for epigallocatechin gallate, gallocatechin gallate and epicatechin gallate in 95% ethanol representing lipophilic food. While, the lowest migration in 95% ethanol was observed for caffeine. Then gallocatechin, epigallocatechin gallate and catechin gallate were the most migrating compounds to 10% ethanol, representing food with high content of water. While, the lowest migration in 10% ethanol was observed for gallocatechin gallate.

According to the European Legislation ("Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food," 2011) food contact materials must not transfer their components into the foods in unacceptable quantities. However, green tea extract used as an active agent is considered as a health claim ("Regulation (EC) No 1924/2006 of the European Parliament and of the council of 20 December 2006 on nutrition and health claims made on foods.," 2006) and it can be used as a food additive without limits, as it is food. It can be concluded that the developed active materials meet current legislation for food contact materials.

Linear ranges from 0.1 µg/g to 50 µg/g with the regression coefficient (r) between 0.9925 and 0.9989 were obtained. In terms of sensitivity of the method, minimum LOD (0.001 µg/g) was obtained for epicatechin gallate and catechin gallate while maximum

LOD (0.057 µg/g) was obtained for catechin. The same trend was observed for LOQ values.

4. Conclusion

Multi-component technology such as encapsulation was successfully used for the preparation of active materials. It was demonstrated that inorganic capsules protected the extract of green tea versus processing temperature by extrusion. The release of a small portion of active agent at ppb level from the active material was confirmed. Then, effectiveness of the developed packaging was checked with in vivo experiments and the extension of shelf-life of fresh minced pork meat was successfully achieved. Parameters such as MetMb, a* and sensory evaluation were significantly better for meat from both types of active packaging compared to meat from blank packaging after 9 days of experiment. Therefore, novel active packaging was developed with high antioxidant properties. Finally migration tests of volatile and non-volatile compounds were carried out. According to the results obtained the migration of active compounds such as catechins and caffeine was observed at ppb level in the analysed samples. As green tea is food, it can be considered that the developed active materials meet the current European legislation.

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Figure 1. Sample of Petri dish with minced pork meat placed in active packaging containing inorganic capsules loaded with green tea extract and vacuum bag.

Figure 2. Scheme of procedure for development of active packaging.

Figure 3. Sample of Petri dish with minced pork meat placed in blank packaging (without active capsules) and active packaging consisted of PE I and PEII active film

Figure 4. Results of a* values from CIE L*a*b* measurement where the blue continuous line with circle markers is meat from blank packaging; red dotted line with the square markers is meat from PE I active packaging and green dashed line with triangular markers is meat from PE II active packaging.

Figure 1

Figure 2

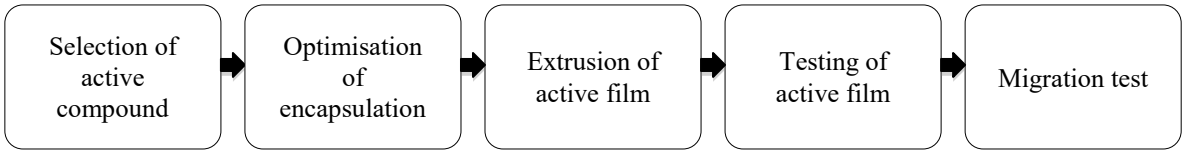
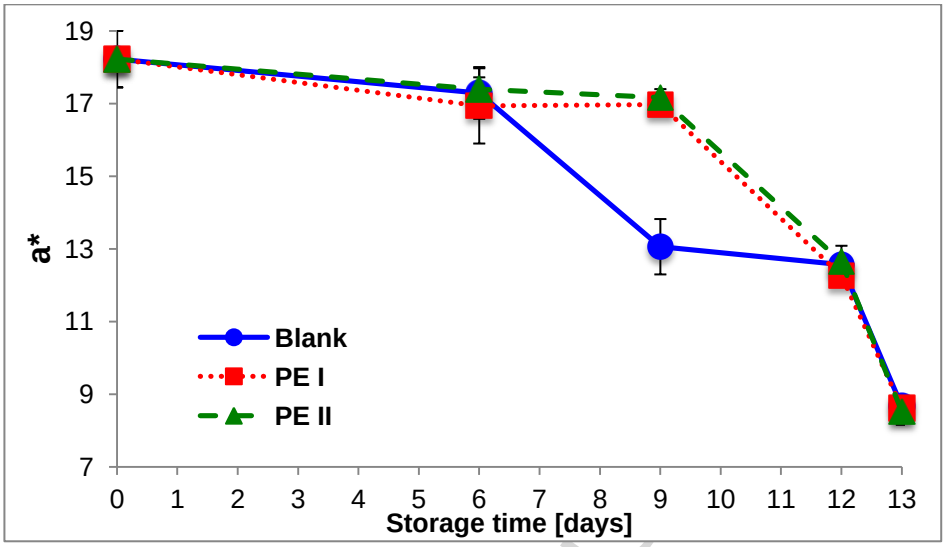


Figure 3

Figure 4



HIGHLIGHTS

- Packaging based on Green Tea Extract (GTE) was developed.
- Green tea entrapped by inorganic capsules was incorporated into melted material.
- Shelf life extension of fresh minced meat was measured and achieved.
- Migration of antioxidants from the materials was studied by UHPLC-MS/TQ.