Design of new natural antioxidant active packaging: Screening flowsheet from pure essential oils and vegetable oils to *ex vivo* testing in meat samples

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ABSTRACT

Two essential oils and seven vegetable oils were tested in an *in situ* generator of hydroxyl radicals for determining their antioxidant capacity. Then twenty-two different active LDPE films were prepared by incorporating both the essential oils and vegetable oils. The antioxidant capacity, heat-sealing properties and aroma of developed films were determined. The films with the best antioxidant properties, containing flaxseed oil, ginger essential oil, grape seed essential oil and rose oil, were selected and applied as active packaging for fresh meat and tested at industrial scale. The optimum packaging corresponded to 50 μm LDPE film with flaxseed oil which was able to extend by 22% the shelf-life of fresh meat. It was proven that the concentration of flaxseed oil was crucial for antioxidant capabilities of film. Finally, derivatisation was applied to evaluate stability of flaxseed oil packaging. Stability assays suggest that active films should be kept in closed aluminium bags to prevent flaxseed oil oxidation by air and light.

1. Introduction

Oxidation is the main non-microbial cause of quality deterioration in meat and meat products (Ribeiro et al., 2019). This is a process that begins with slaughtering of the animal

and continues progressively until the final product being consumed. Besides reducing the nutritional value of meat due to the loss of essential fatty acids and vitamins (Amaral, Silva, & Lannes, 2018), oxidation also causes a gradual loss of the sensory qualities such as changes in colour, texture, appearance of rancid off-odours and flavours, leading to an overall reduction in consumer acceptance. Therefore, it is of paramount relevance that all meat processing steps, from handling through storage, are controlled to prevent oxidation reactions from occurring (Villalobos-Delgado et al., 2019).

In terms of packaging, the first technologies that arose were vacuum and modified atmospheres (MAP) that aimed to control the inner gas environment of the packaged food and thus delay oxygen-dependent oxidation processes (Kerry, O'Grady, & Hogan, 2006; Arvanitoyannis & Stratakos, 2012). Despite the success attained by such approaches, the food industry still demands for newer and better performing technologies to decrease food losses and extend even further the shelf-life of the packaged products. This resulted in the introduction of active packaging (Fang, Zhao, Warner, & Johnson, 2017). As antioxidant active packaging the two main approaches are: the incorporation of independent devices (sachets, labels or pads) to conventional packages (Brody, Bugusu, Han, Sand, & McHugh, 2008), and antioxidant packaging materials, such in the present study, where the active agent is incorporated within the container or onto the surface of the packaging films.

According to European regulation (EC) No 450/2009, active packaging systems "deliberately incorporate components that would release or absorb substances into or from the packaged food or the environment surrounding the food" (European Commission, 2009). In terms of antioxidant packaging technologies, the two main types available are oxygen scavengers/absorbers and free radical scavengers (Yildirim et al., 2018). Synthetic free radical scavengers like selenium nanoparticles (Vera, Canellas, & Nerin, 2018), butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tert-butylhydroquinone (TBHQ), or natural ones such as plant extracts and essential oils react with free radicals, so that they are unable to engage in further oxidative chain reactions (Borzi, Torrieri, Wrona, & Nerin, 2019; Yildirim et al., 2018).

Over the past decades, antioxidant compounds from natural origin (Brewer, 2011) such as α-tocopherol and resveratrol, essential oils such as the ones from ginger, rosemary, clove, cumin, cinnamon (Pateiro et al., 2018) and natural extracts such as kombucha tea, green tea, olive oil and mango peel have been successfully applied to prevent oxidation in foods such as fatty fish, poultry meat, read meat, oil, mushrooms (Silva, Becerril, & Nerin, 2019). However, many of these natural compounds present challenges to the incorporation in packaging materials due to their high volatility, light oxidation, low thermal stability and incompatibility with common packaging materials (Nerin, Silva, Manso, & Becerril, 2016).

In this paper we describe the incorporation of both essential oils (rose seeds and ginger root, mainly composed of volatile compounds, polyphenols and others) and vegetable oils

extracted from seeds/fruits (with fatty acids as major components) with high temperature thermal stability and non-polar character such as the ones from avocado, flaxseed, grape seed, milk thistle, pomegranate, starflower and walnut in low-density polyethylene (LDPE) packaging materials. The resulting packaging materials were evaluated in terms of antioxidant capacity and *ex vivo* effectiveness in meat samples for retail.

2. Experimental part

2.1. Chemicals

Acetic acid (CAS 64-19-7), hydrogen peroxide (30%, CAS 7722-84-1), margaric acid (CAS 506-12-7), potassium hydroxide (CAS 1310-583), sodium salicylate (CAS 54-21-7) and sodium acetate (CAS 127-09-3) were purchased from Sigma-Aldrich (Madrid, Spain). Ethanol absolute (CAS 64-27-5), hexane (CAS 110-54-3), heptane (CAS 142-82-5), hydrochloric acid (37%, CAS 7647-01-0) and *ortho*-phosphoric acid (85%, CAS 7664-38-2) were from Scharlab (Barcelona, Spain). HPLC grade methanol (CAS 67-56-1) was from Honeywell (Madrid, Spain). Ultrapure water was produced in a Wasserlab Ultramatic GR system (Barbatáin, Spain).

2.2. Active agents

Avocado pulp virgin oil, flaxseed organic extra virgin oil, grape seed, organic extra virgin oil, milk thistle seed organic extra virgin oil, pomegranate seed organic extra virgin oil, starflower seed organic extra virgin oil, walnut organic extra virgin vegetable oil and rose (*Rosa eglanteria*) seed essential oil were purchased from Terpenic Labs (Barcelona, Spain). Ginger (*Zingiber officinalis*) root essential oil was from Aromium (Madrid, Spain).

2.3. Active film preparation

NUREL company (Zaragoza, Spain), part of SAMCA group, made the incorporation of the active agents to the LDPE Alcudia® PE-003 from Repsol (Spain) following a blending and extrusion process. Briefly, for the masterbatch, LDPE pellets and active agents were fed to an extruder, mixed and melted together at temperatures of 120–150 ºC. This temperature was changed depending on the active agent to ensure optimal mixing and fusion of the active film. The extruded material was cooled in a water bath and cut into granules. For the manufacturing of the final active films, the appropriate masterbatch granule concentration and blank LDPE pellets were fed to a blown film extrusion machine, operated at similar temperatures as the ones used for the manufacturing of the masterbatch, for the obtaining of films. Film thickness was adjusted by controlling the velocity of the stretcher rollers. The prepared samples are described in Table 1.

Table 1

Characteristics of prepared active samples of LDPE.

Sample	Active agent	Active masterbatch concentration (%)	Film thickness (μm)
A1	avocado oil	25	51 ± 3
A2	avocado oil	50	52 ± 2
A3	avocado oil	50	31 ± 2
F1	flaxseed oil	25	49 ± 3
F2	flaxseed oil	50	32 ± 3
F3	flaxseed oil	50	51 ± 2
F4	flaxseed oil	100	50 ± 3
G1	ginger essential oil	25	48 ± 2
G2	ginger essential oil	50	21 ± 2
G3	ginger essential oil	50	27 ± 2
GS1	grape seed oil –composition A	25	50 ± 2
GS2	grape seed oil –composition A	50	49 ± 3
GS3	grape seed oil –composition B	50	48 ± 3
M1	milk thistle oil	25	50 ± 2
M2	milk thistle oil	50	49 ± 2
P1	pomegranate oil	50	53 ± 2
R1	rose essential oil	25	49 ± 2
R2	rose essential oil	50	51 ± 3
S1	starflower oil	25	50 ± 3
S2	starflower oil	50	49 ± 4
W2	walnut oil	50	53 ± 2

2.4. Thickness measurements of active films

The measurement of film thickness was performed according to ISO 13385-1:2019 norm (International Organization for Standardization, 2019) with a calliper from Holex-Hoffmann Iberia (Madrid, Spain) at 20 ºC and 50% humidity. Active films were measured in three different points.

2.5. Antioxidant capacity of pure active agents and active films

In situ generator of hydroxyl free radicals (OH·) designed and optimised by Pezo et al. (Pezo, Salafranca, & Nerin, 2006, 2008) was applied to evaluate the antioxidant capacity (CAOX) of both pure active agents and active films. To perform evaluation of active agents, 0.05 g of sample were placed in specially designed Pasteur pipettes containing 0.3 g of glass wool. The inlets of the pipettes were then connected to the OH· generator. Measurement was performed against blanks (Pasteur pipettes containing 0.3 g of glass wool).

To perform evaluation of active films, 1 dm^2 of each sample was placed in a 40 μm (12 × 12 cm) LDPE bag with micropipette tip inlet and outlet. Afterwards, the inlets of the bags were connected to the OH· generator. Measurement was performed against blanks (1 dm^2 of neat LDPE).

The reaction chamber used to generate radicals from aqueous solution of hydrogen peroxide (0.29 M) consisted of a quartz tube with UV radiation. Amber bottles with 50 g of an aqueous solution of sodium salicylate (2 μg mL^{-1}, pH 4.5) were used to capture the stream of OH· radicals for 24 h. During this time the fluorescent compound 2,5-dihydroxybenzoic acid was generated as major product due to the reaction of sodium salicylate with free radicals. The compounds formed in these solutions were further analysed by HPLC with fluorescence detection. The samples were directly injected into a Waters HPLC Alliance 2795 Separation module (Milford, MA, USA) coupled to a Waters 474 Scanning Fluorescence Detector. Excitation and emission wavelengths were, respectively, 324 and 448 nm. Chromatographic separation was attained by passing a mobile phase consisting of 10% of methanol with 90% of acetic/acetate buffer (35 mmol L^{-1}, pH 5.9) at a flow rate of 1 mL min^{-1} in isocratic mode through a Waters Atlantis™ dC_{18} column kept at 25 ºC. The samples were also stored at a temperature of 25 ºC prior and post-analysis.

2.6. Heat-sealing properties of active films

The heat-sealing properties of active films were tested using an impulse sealer PFS-200 Zhejiang Dongfeng Packing Machine Co. (Wenzhou, China). The films were sealed in peel-test geometry at 105 ◦C.

2.7. Odour tests of active films

Odour tests were performed according to quantitative descriptive analysis (Gao et al., 2010) where testers are 20 or more consumers analysing products to determine differences between them by sensory means. However, for practical purposes, a reduced set of four well-trained testers, 4 women with age 33–65 years old recruited from the Institute of Research and Engineering of Aragon (Spain) carried out the odour evaluation. They smelled several pieces of each active film and evaluated the acceptance of film odour using two-point scale (acceptable and not acceptable) and type of aroma. Sniffing sessions were performed in the sensory room at 25 ◦C. The evaluation was performed against blank LDPE samples.

151 *2.8. Meat samples*

152 *Ex vivo* tests with meat samples were performed at industrial scale. Fresh beef packaged
153 in MAP (70% O_2 + 30% CO_2) commercial trays was applied. Selected active films were
154 placed on the top of the tray. The packaged meat was stored at 4 ◦C during the test. In all
155 cases, blank samples were simultaneously tested.

156 *2.9. Quality of fresh meat samples*

157 The quality tests of fresh meat packaged with the new active films were performed by a
158 local meat company during its shelf-life by their visual and sensory panel. Different tasters,
159 workers of the quality control department (7 women and 5 men, with ages between 26 and 59
160 years) were asked to evaluate the appearance and smell of fresh meat samples. It was assumed
161 that standard time of shelf-life for fresh meat from blank packaging was 6–7 days.

162 *2.10. Stability of flaxseed active films*

163 To check the content and stability of flaxseed oil in films, a quantitative analysis of
164 linolenic acid methyl ester was performed using the well-known derivatisation procedure
165 (Nerin, Tovar, & Salafranca, 2008). Film F1 recently prepared and after 12 months was
166 tested. For that purpose, 4 g of the active film containing flaxseed oil was extracted with 300
167 g of hexane for 24 h using a Soxhlet extractor. The solvent was completely evaporated using a
168 rotary evaporator and the residue was derivatised. For this purpose, 1 mL of methanol and 100
169 µL of 1 M KOH in methanol were added to the sample residue. 0.02 g of solid margaric acid
170 was added as internal standard in each determination, in order to reproduce the entire process
171 of esterification. Then the mixture was boiled until the obtaining of a transparent sample (10
172 min) and left to cool to room temperature. Then, 5 mL of heptane and 10 mL of ultrapure
173 water were added to the cooled solution. These samples were shaken and centrifuged for 10
174 min at 5000 rpm for the phase separation and the organic phase was collected. The aqueous
175 phase was re-extracted with 5 mL of heptane. As before, the samples were shaken and
176 centrifuged for 10 min at 5000 rpm. The organic extracts resultant from the two extractions
177 were combined and concentrated under a gentle stream of nitrogen.

178 The samples were analysed by an Agilent 6890 N gas chromatograph with 5975 Mass
179 Spectrometer (Palo Alto, CA, USA) using an Agilent HP5 column (30 m long, 0.25 mm i.d.,
180 0.25 µm film thickness) under the following chromatographic conditions: injection of 1 µL in
181 splitless mode, temperature of injector was set to 250 ºC, the carrier gas was helium with a
182 flow rate of 1.0 ml min-1. The initial temperature of the oven was set to 40 ºC, maintained
183 during 5 min, and then increased up to 300 ºC at 10 ◦C·min-1 and maintained for 5 min. The
184 MS detection was performed in SCAN mode (50–400 m/z) with a solvent delay of 7 min. MS
185 source and quadrupole temperatures were 230 and 250 ºC, respectively.

186 Different amounts of flaxseed oil (0.01–0.5 g) were used for the calibration curve and the
187 derivatisation procedure was undertaken according to the one previously described. Linolenic

188 acid methyl ester has been used as a marker for the presence of the oil.

189 *2.11. Statistics*

190 All samples were analysed in triplicate. The presented data were calculated as the average
191 values of obtained results. Error was calculated as standard deviations.

192 Student t-test was applied to determine significant differences ($p\ 0.05$) between analysed ≤
193 samples. At the beginning hypothesis that samples are the same was taken into account.
194 Therefore, experimental and theoretical values of t were compared. If two values were the
195 same or experimental value was lower than theoretical one, the hypothesis was accepted and
196 if experimental value was higher than theoretical one, the hypothesis was rejected, and it was
197 concluded that samples are significantly different.

198 **3. Results**

199 *3.1. Selection of active agents*

200 For this study, two essential oils and seven vegetable oils not yet studied for their CAOX
201 were selected. Previous researches have explored the possible benefits of combining different
202 natural antioxidants in order to exploit their synergistic effects, such as the mixture of
203 rosemary extract and alpha-tocopherol (Wada & Fang, 1992) or rosemary and sage extracts
204 (Korczak, Janitz, & Nogala, 1996). Nevertheless, another study concluded that, in most cases,
205 near additive properties occurred when comparing essential oils of cinnamon, oregano,
206 carvacrol and thymol with their main chemical components (Bentayeb, Vera, Rubio, & Nerin,
207 2014). Finally, in some cases an antagonistic effect was noticed (Yanishlieva, 2006).
208 Considering that these effects are frequently concentration-dependent, and the absence of
209 previous data for the selected set of active agents, in this study only individual oils were
210 considered for evaluation.

211 The analysis of free OH· radical scavenging capacity in an *in situ* generator of hydroxyl
212 free radicals was carried out. The obtained results are shown in Table 2 as the percentage of
213 hydroxylation (the lower hydroxylation percentages, the higher CAOX). This assay was used
214 as an initial screening to select the most promising antioxidant active agents for its subsequent
215 incorporation into the LDPE polymer. The highest CAOX were obtained for avocado oil,
216 grape seed oil and milk thistle oil with no significant difference among the three samples
217 (significance level $p < 0.05$). However, it was rejected due to its strong orange colour with
218 staining properties and, therefore, not ideal for a fresh meat packaging application. The
219 remaining oils were subsequently incorporated into LDPE films (Table 1) and their CAOX
220 was tested.

Table 2

Results of hydroxylation percentage of pure active agents. Higher hydroxylation percentages correspond to lower antioxidant capacity.

Active agent	Hydroxylation (%)	RSD%, n = 3
avocado oil	56 ± 2 [a]	3%
flaxseed oil	74 ± 2 [a]	3%
ginger essential oil	89 ± 2 [c]	3%
grape seed oil	58 ± 1 [b]	2%
milk thistle oil	56 ± 3 [b]	1%
pomegranate oil	69 ± 1 [a]	4%
rose essential oil	87 ± 11 [c]	13%
starflower oil	77 ± 1 [a]	1%
walnut oil	68 ± 7 [a]	10%

[a, b, c] different letters indicate statistically significant differences between samples ($p \leq 0.05$).

3.2. Antioxidant capacity of active films

CAOX is widely used as a parameter to characterise different substances with the ability of scavenging or neutralising free radicals. Commonly applied methods such as oxygen radical absorbance capacity (ORAC) or 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH) can be adapted to the evaluation of CAOX of polymers but they are indirect methods that require sample extraction (Bentayeb, Vera, Rubio, & Nerin, 2009; Pyrzynska & Pę;;kal, 2013) and thus they do not provide a true analysis of the actual film, but the performance of the extraction yield of the extract. To overcome this, direct analysis of potentially active polymers was applied in this study using a method involving the *in situ*, vapour-phase generation of OH· radicals and their quantification in the presence and absence of potential antioxidant extracts. The obtained results are presented in Fig. 1.

Similarly, to the analysis of pure essential oils and vegetable oils, the CAOX was expressed as percentage of hydroxylation, as above explained.

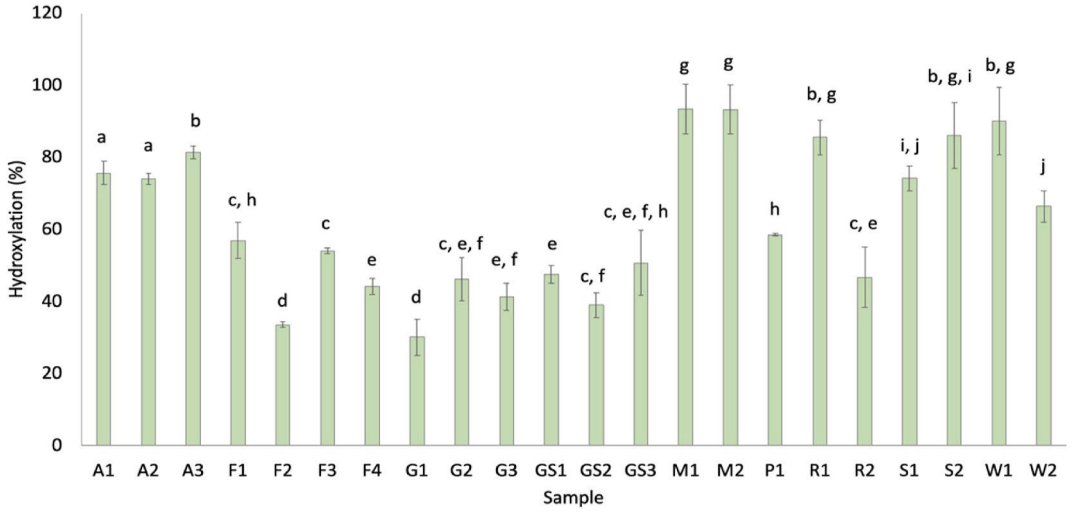


Fig. 1. Results of CAOX of active films evaluated by *in situ* generator of OH· radicals; where a, b, c, d, e, f, g, h, i, j different letters indicate statistically significant differences ($p \leq 0.05$). Error bars correspond to standard deviation.

Several samples presented high RSD values as a consequence of lack of homogeneity among replicates. The best CAOX was obtained for LDPE films with incorporated flaxseed oil, ginger essential oil, grape seed oil and rose oil. When comparing different concentrations and thicknesses of film with flaxseed oil, the 50 μm films with 25% (F1) and 50% (F3) of masterbatch had the same CAOX, and the one with the highest concentration of active agent (F4, 100% masterbatch) was only slightly better than films described before. Finally, the best CAOX among flaxseed films was obtained for the 30 μm film with 50% of masterbatch (F2). This is due to the fact that thinner film facilitates the penetration of OH· free radicals and their scavenging by the film. The results are consistent with those obtained by Sangatash et al. (Sangatash, Niaz-mand, Jamab, & Modaressi, 2016) when assessing the oxygen transmission rate in antioxidant active LDPE films containing sodium ascorbate. The outcomes obtained in this step were used to choose the best active films for subsequent *ex vivo* tests with fresh meat.

3.3. Heat-sealing properties of active films

The heat-sealing properties of developed active films were determined in comparison to a commercially available and easily sealable polymer such as LDPE. The film thicknesses ranged from 20 to 50 μm. The results of heat-sealing properties are shown in Table 3. It can be observed that all developed polymers were thermosealable with one exception: sample with pomegranate oil (P1). It could be explained by the macroscopic physical properties of the surface of this material, which was observed to be very oily. Therefore, sample P1 was rejected for further investigation.

3.4. Odour tests of active films

The results of odour tests consisting of determining the type of aroma of the produced films and its classification in a two-point scale are presented in Table 3. The aim of these tests was to detect any odour that was stronger than normal or either unusual or unpleasant. Samples with rancid smell (avocado oil, grape seed essential oil, milk thistle oil, pomegranate oil, starflower oil and nut oil) were classified as not acceptable. Besides being undesirable, rancid smell is highly rejected by the consumers. This rancid smell (Morales, Luna, & Aparicio, 2005) is caused by the formation of both saturated and unsaturated aldehydes due to the rapid oxidation of the active agent in the polymer.

Table 3

Results of heat-sealing properties and odour tests of active films.

Sample	Heat-sealing properties	Aroma	Two point scaling
A1	Yes	Rancid	Not acceptable
A2	Yes	Neutral	Acceptable
A3	Yes	Neutral	Acceptable
F1	Yes	Fresh	Acceptable
F2	Yes	Fresh	Acceptable
F3	Yes	Fresh	Acceptable
F4	Yes	Fresh	Acceptable
G1	Yes	Ginger	Acceptable but smell is specific
G2	Yes	Ginger	Acceptable but smell is specific
G3	Yes	Ginger	Acceptable but smell is specific
GS1	Yes	Rancid	Not acceptable
GS2	Yes	Rancid	Not acceptable
GS3	Yes	Citric	Acceptable
M1	Yes	Rancid	Not acceptable
M2	Yes	Rancid	Not acceptable
P1	No	Rancid	Not acceptable
R1	Yes	Rose	Acceptable but smell is specific
R2	Yes	Rose	Acceptable but smell is specific
S1	Yes	Rancid	Not acceptable
S2	Yes	Rancid	Not acceptable
W1	Yes	Plastic	Not acceptable
W2	Yes	Rancid	Not acceptable

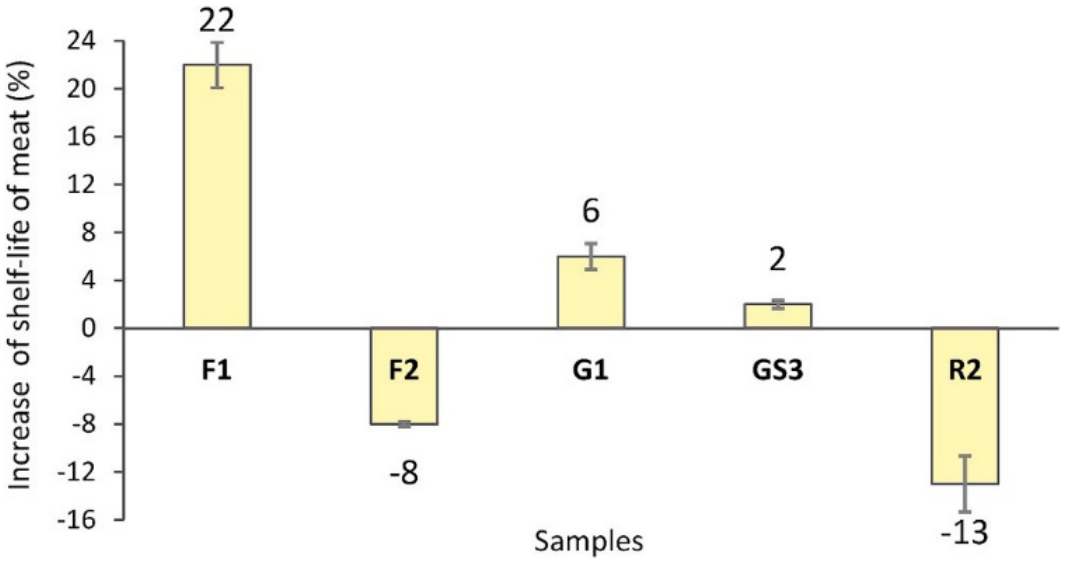
Moreover, two tendencies related to the concentration of active agent in polymer can be observed. On the one hand, high concentration of active agent (nut oil) gave rancid smell to polymer while the same film with low concentration of nut oil had acceptable smell. Consequently, when using high concentrations of nut oil, its incorporation into the structure of polymer is not complete, and the excess remaining on the surface of the polymer can be easily oxidized, mainly due to the high temperature (Mahesar, Sherazi, Khaskheli, Kandhro, & Uddin, 2014) reached during the extrusion procedure. On the other hand, when considering avocado oil, it can be seen that low concentrations gave rancid smell to polymer while films with high concentration had acceptable smell. This phenomenon indicates quick oxidation of a portion of avocado oil, but at high concentration the rancid smell is masked and cannot be well perceived. This fact was considered as a drawback and thus, these samples were also rejected for further investigation.

Polymers with both ginger essential oil and rose oil had a very strong and characteristic smell, meaning that their application in food packaging is limited to specific types of food compatible with those types of aroma.

3.5. Quality of fresh meat samples

After analysing all the results from previous experiments, the following samples were chosen for the application as active packaging for fresh meat at industrial scale: F1 (flaxseed oil, 25% masterbatch, 50 μm), F2 (flaxseed oil, 50%, 30 μm), G1 (ginger essential oil, 25%, 50 μm), GS3 (grapeseed oil, composition B, 50%, 50 μm) and R2 (rose essential oil, 50%, 50 μm). Films with flaxseed oil, ginger essential oil, grape seed essential oil and rose oil were chosen because of their good OH· scavenging properties and to omit aroma. Two different films with flaxseed oil were chosen to compare the influence of active agent concentration and film thickness on the shelf-life of fresh meat. The obtained results are shown in Fig. 2. Standard shelf-life time of fresh beef meat packed in blank packaging was 7 days. Increase or decrease in the shelf-life of meat stored in active packaging was calculated in relation to this value and expressed as percentage.

From the analysis of the results from Fig. 2, it can be seen that the best antioxidant packaging, that extended by 22% the shelf-life of freshmeat, was 50 μm LDPE film with 25% of flaxseed oil masterbatch. Opposite effect was obtained in the case of 30 μm LDPE film with 50% of flaxseed oil masterbatch, thus suggesting that there is an optimal concentration of flaxseed oil able to yield a positive influence of the film in the shelf-life of meat. Similar behaviour has been found in previous studies (Estevez & Cava, 2006; Zhou, Jongberg, Zhao, Sun, & Skibsted, 2019) where the concentration of rosemary, green tea and mate extracts greatly influenced the antioxidant or prooxidant activities on meat products.



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312 **Fig. 2.** Increase of shelf-life of meat stored in selected active packaging. F1 (flaxseed oil, 25%
313 masterbatch, 50 μm), F2 (flaxseed oil, 50% masterbatch, 30 μm), G1 (ginger essential oil,
314 25% masterbatch, 50 μm), GS3 (grapeseed oil, composition B, 50% masterbatch, 50 μm) and
315 R2 (rose essential oil, 50% masterbatch, 50 μm). Error bars correspond to standard deviation.

316 Additionally, packaging with ginger essential oil and grape seed essential oil LDPE films
317 also showed a positive effect in the freshness of meat, extending its shelf-life by 6% and 2%,
318 respectively. In contrast, active film with rose oil had a negative influence in meat shelf-life
319 causing its accelerated oxidation.

320 The decrease in CAOX properties from pure agents to polymers (Pezo, Salafranca, &
321 Nerín, 2008) with these agents and from *in vitro* to *ex vivo* studies with meat samples was
322 observed. The most likely reason is that active agents incorporated into the polymer are
323 dispersed and partially polymer coated, therefore their availability is limited. Then, ratio food
324 surface/volume is different in both cases (*in vitro* and *ex vivo*). Besides, food handling is
325 different on an industrial scale. Therefore, the efficiencies of active film vs. pure antioxidant
326 and the *in vitro* antioxidant capacity of active film vs. its efficiency with meat are expected to
327 decrease.

328 *3.6. Stability of flaxseed active films*

329 As the best antioxidant effects in meat samples were observed for the films F1 with
330 flaxseed oil, the stability of these LDPE films was further evaluated. Flaxseed oil is rich in
331 linolenic acid, a non-volatile compound that can be analysed quantitatively using GC-MS
332 after its derivatisation. Fig. 3 shows the obtained chromatograms of flaxseed active film and
333 flaxseed oil standard, both after derivatisation.

334 Real concentration of flaxseed oil in film F1 (50 μm LDPE film with 25% of
335 masterbatch) was 1.25% (1.25 g of active agent/100 g of film). When comparing the results of

films analysed after 0 and 12 months, it was noticed that the concentration of flaxseed oil dropped to 37 ± 3 µg of active agent/100 g of film. It clearly indicates oxidation of developed packaging after 1 year of storage in free air. Therefore, it would be highly recommended to keep the active film with flaxseed oil in airtight aluminium bags to avoid direct contact with air during storage. Furthermore, the oil could also be oxidized from the active LDPE package, thus decreasing its concentration, as flaxseed oil is known to be prone to oxidation due to their high polyunsaturated fatty acids (PUFA) content (Lu et al., 2020).

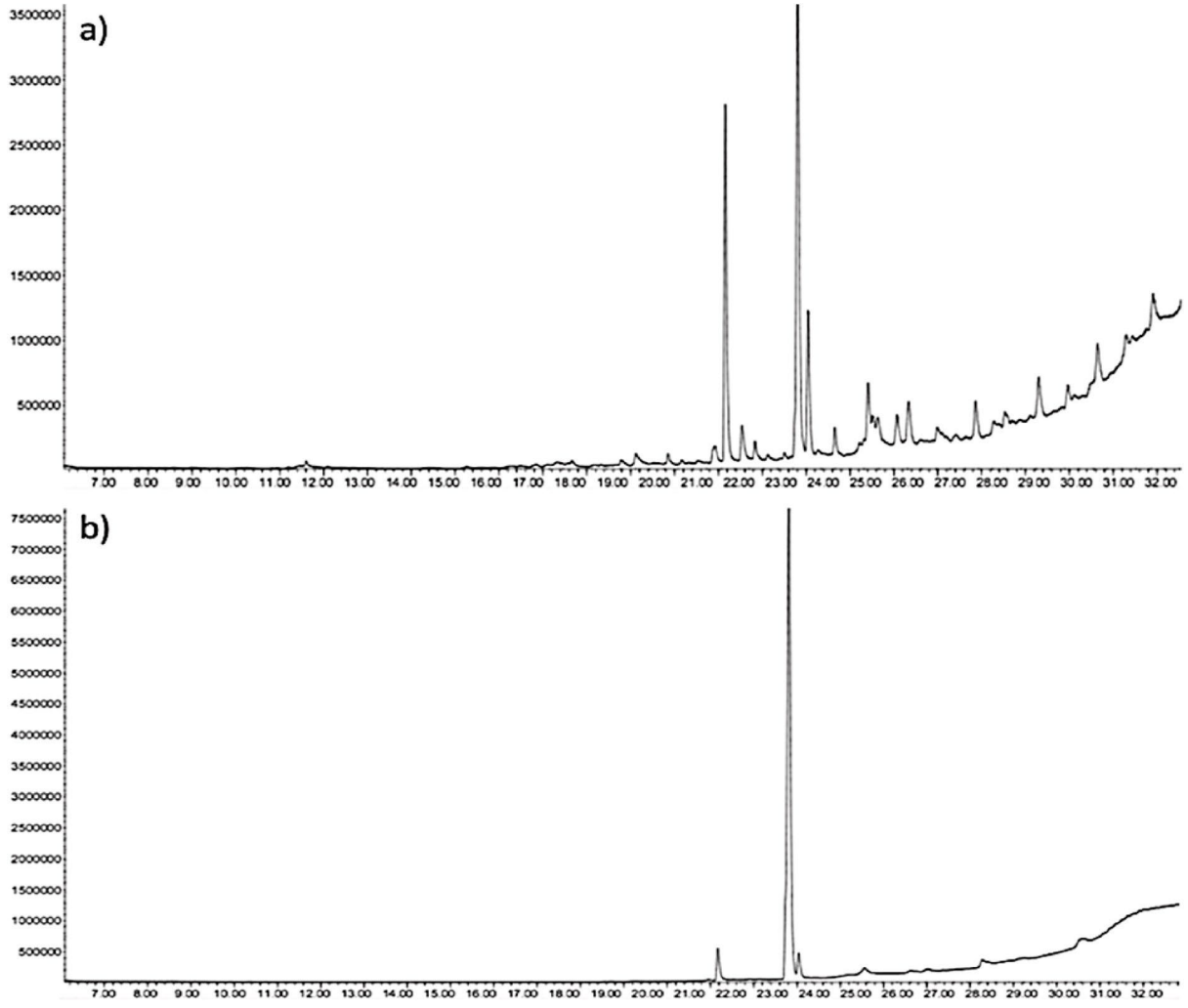

Fig. 3. Chromatograms of a) sample of flaxseed active film after derivatisation; b) standard of the flaxseed oil after derivatisation. The large peak at 23.80 min corresponds to linolenic acid methyl ester.

4. Conclusions

Theoretically, the most promising antioxidant agents for further incorporation into LDPE were avocado oil, grape seed oil and milk thistle oil, well promoted as good antioxidants. Nevertheless, the results of CAOX of active agents incorporated into polymer showed different tendencies. In this case, the best CAOX was obtained for LDPE films with incorporated flaxseed oil, ginger essential oil, grape seed oil and rose oil. However, the specific smells of both ginger and rose oil active packaging definitely limits their application to specific types of food compatible with them.

After testing the selected active packaging films with fresh meat at industrial scale, the best antioxidant packaging which extended by 22% the shelf-life of fresh meat was 50 μm LDPE film with 25% of masterbatch with flaxseed oil. Opposite effect was obtained in the case of 30 μm LDPE film with 50% of masterbatch with flaxseed oil, meaning that the active agent concentration should always be subjected to optimisation. Finally, it was recommended to store active film with flaxseed oil in airtight aluminium bag due to its proven oxidation after 1 year of storage in an open space.

Overall, the results show that the incorporation of these agents is possible in LDPE films by extrusion. In most of the cases, CAOX of the polymer is greatly increased without compromising its physical properties. Furthermore, storage of foods prone to oxidation, as is the case of fresh red meat, could be improved by using films with flaxseed oil.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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