Preliminary data on the antimicrobial effect of *Cannabis sativa* L. variety Futura 75 against food-borne pathogens *in vitro* as well as against naturally occurring microbial populations on minced meat during storage

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Abstract

In the present study, the antimicrobial effect of *Cannabis sativa* Futura 75 was evaluated both *in vitro* against foodborne bacterial pathogens, and on food against naturally occurring microbial groups of minced meat stored for 8 days at 4°C. Ethanol extraction was performed on the grind of the inflorescence. After extraction, ethanol was completely evaporated and substituted by water. Serial dilutions of the extract, the grind and cannabidiol 99% were added to Nutrient Agar and spotted with *Listeria monocytogenes*, *Salmonella* Typhimurium, *Escherichia coli* and *Staphylococcus* spp. Regarding the evaluation on food, 50 mL of extract, characterised by CBD at concentration of 322,70 µg/mL, were added to 2.5 kg of minced beef meat. Meat was divided into aliquots and stored for 8 days at 4°C. At 0, 1, 2, 3, 4, 7, and 8 days, aerobic bacteria, enterobacteria, coliforms and *E. coli* were enumerated. All tested products were efficient against Gram +. In particular, extract corresponding to CBD concentration of 0.017 and 0.3 mg/mL were effective against *L. monocytogenes* and *Staphylococcus* spp. respectively. After 8 days of storage at 4°C, treated minced meat showed a bright red colour in comparison to a brownish control meat. Moreover, *Enterobacteriaceae* and coliforms were significantly reduced of 2.3 log CFU/g and 1.6 log CFU/g respectively in treated meat in comparison to the control. Although preliminary, the present study suggests the antimicrobial properties of the extract of *Cannabis sativa* both *in vitro* and in minced meat.

Introduction

In Europe, *Cannabis sativa L.* varieties can be legally cultivated if it is registered in the EU Plant variety database of agricultural plant species and its tetrahydrocannabinol (THC) content does not exceed 0.2 % (w/w) hereafter addressed as industrial hemp (EC, 2019a). These plants find different applications in pharmacotherapy (Bonini *et al.*, 2018), agronomy (Das *et al*., 2017), food industry (Radočaj *et al.*, 2014; Zając *et al*., 2018), cosmetic (Nuutinen, 2018), sustainable building (Arizzi *et al.*, 2019), animal production (Khan *et al.*, 2010; Neijat *et al.*, 2016; Vispute *et al.*, 2019), broiler meat and chicken egg production (Goldberg *et al.*, 2012; Jing *et al.*, 2017). In Italy the cultivation of industrial hemp is specifically regulated by law N° 242 of 2016, which specifically promotes the use of this plant in research, cosmetics, food industry, biomaterials, sustainable building.

As food or food ingredient, industrial hemp is regulated at European level as novel food and needs pre-market authorisation before commercialisation (EC, 2019b; Regulation EU 2015/2283). However, some products such as hemp seed, seed flour and oil have a long history of consumption and cannot be considered as novel. For them, the use is regulated at national level. With the exception of Romania, where hemp seed and oil can be used without any limit, in other countries such as The Netherlands, Germany, Danmark and Italy, the maximum limits of THC in hemp seed, oil, flour and other foods and drinks is specifically regulated. In particular for Italy, the 30th of October 2018, the Ministry of Health submitted to the European Commission a draft regulation, not yet approved, including the following maximum limits of THC: hemp oil – 5 ppm; hemp seeds and seed flour – 2 ppm; dietary supplement including hemp derivatives: 2 ppm (Italian Ministry of Health, 2018). According to this draft regulation, for all the other hemp derivatives, for example hemp inflorescence, food business operators shall provide evidences on the THC concentration in foodstuffs to competent authorities of official control taking into account specific concentration or dilution factors linked to the food processing (Commission Regulation (EC) No 1881/2006).

Industrial hemp inflorescence, especially resin secreted from the trichomes of female plants, are rich in phytocannabinoids. In *C. sativa*, cannabinoids are biosynthesized and accumulated as cannabinoid acids, and subsequently decarboxylated into their neutral forms (Bonini *et al*., 2018). At present around 100 molecules have been characterised with Cannabidiol (CBD) as the one of the most represented in industrial hemp. Properties such as anti-inflammatory, immunomodulation, anti-arthritic have been attributed to CBD (Bonini *et al.*, 2018). Along with these properties, CBD has been described as an antimicrobial agent since the 70ies when CBD was shown to be effective against Gram + bacteria, although not against Gram - (Van Klingeren and Ham, 1976). More recently, MICs of 0.5-1 µg/mL were registered for CBD against different strains of multiresistant and methicillin resistant *Staphylococcus aureus* (MRSA) (Appendino *et al.*, 2008). Antimicrobial activity of CBD against *S. aureus* was observed also by other authors (Burstein *et al.*, 2015). Along phytocannabinoids, also terpenoids are found in *C. sativa*. More than 200 terpenoids have been characterised with limonene, β-myrcene, α- and β-pinene as the most common. Terpenoids act synergistically with phytocannabinoids in the defense strategy against predators (Bonini *et al.*, 2018; Russo *et al.*, 2011). Antimicrobial properties were specifically observed for α- and β-pinene against both Gram + and Gram – bacteria as well as fungi namely, *Pseudomonas aeruginosa*, *Escherichia coli,* MRSA and *Candida albicans*, (da Silva *et al*., 2012; Dai *et al*., 2013; Leite *et al*., 2007).

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Due to the presence of different antimicrobial compounds acting synergistically, the antimicrobial activity of *Cannabis sativa L.* might be potentially higher than those of the single molecules (Russo *et al.*, 2011; Blasco-Benito *et al.*, 2018; Andre *et al.,* 2016). Different types of extracts of seeds or female inflorescence, and resin of industrial hemp have been tested against bacteria, fungi and moulds. For a comprehensive and detailed review please refer to Tandon *et al.* (2017). With particular reference to bacteria, although with broad diverse results related to plant varieties, extraction methods and parts of the plant tested, industrial hemp extracts showed to be effective against microbial pathogens such as *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphyloccocus aureus*. Among all varieties tested, Futura 75 might be addressed as a promising antimicrobial. Futura 75 variety showed superior antimicrobial properties in comparison to other varieties. In particular, higher MIC values were registered against *Enterococcus faecium* and *Clostridium botulinum* (Futura MIC values of 1.55 and 1.76 %v/v respectively) (Nissen *et al.*, 2010). More recently, the water flower extract of *Cannabis sativa L.* variety Futura 75 was described as effective also against *E. coli*, *P. aeruginosa* and *S. aureus* (Ferrante *et al.*, 2019). Unfortunately, all information on antimicrobial activity of *Cannabis sativa* were collected from experiments performed *in vitro* against food-unrelated microbial pathogens. Since the great application of industrial hemp as food ingredient for nutraceutical purposes, knowledges are missing on food safety and hygiene and in particular on the antimicrobial properties of this plant against foodborne-pathogens *in vitro* as well as against bacteria related to the hygiene of food processing (Commission Regulation (EC) No 2073/2005).

The research question of the present study was to investigate the antimicrobial activity of an extract of the female fluorescence of *Cannabis sativa L.* variety Futura 75 *in vitro* against *E. coli*, *Salmonella* Typhimurium, *Listeria monocytogenes* and *Staphylococcus* spp. and against microbial populations occurring in minced beef stored for 8 days at 4°C.

Materials and Methods

Cannabis sativa L. variety Futura 75

Cannabis sativa L. variety Futura 75 was cultivated without the use of fertilizers, biocidal chemicals or irrigation (ESSENZESATIVESALENTINE, Sogliano Cavour, Italy). Plants were harvested on the first hours of the day light when the amount of resin is maximised. After natural dry out, inflorescences were manually trimmed and grinded.

Preparation of Ethanol extract

The ethanol extract was prepared as previously described with few modifications (Romano e Hazencamp, 2013). Briefly, in each of 9 flasks, 100 mL of ethanol (96 % v/v) (Carlo Erba Reagents, Cornaredo, Italy) were mixed with 5 g of the grind. All flasks were placed on a shaking platform at 120 rpm for 20 min. The content of each of the 9 flasks was filtered by filter paper (Whatman™ Grade 113 qualitative filter paper, diameter 90 mm, pore size 30 µm, SigmaAldrich, Milan, Italy) and combined. In order to avoid any antimicrobial misleading effect of ethanol, the ethanol content of the filtrate was eliminated by evaporation (Rotavapor R-300, Buchi, Cornaredo, Italy), and the residue suspended in 90 mL of physiological solution (0.9% NaCl).

Bacterial cultures and growth conditions

The antimicrobial effect of the plant was tested *in vitro* against: two strains of *Salmonella* Typhimurium (ST208 and ST63); two strains of *Escherichia coli* (EC ATCC 25922 and EC135), two strains of *Listeria monocytogenes* (LM1 and LM2) and one strain of *Staphylococcus* spp. (strain S661). For both *E. coli* and *S.* Typhimurium, one completely susceptible (ST208 and EC ATCC 25922) and one multiresistant strain (ST63, EC135) were included. Except *E. coli* ATCC 25922, all other strains were from food origin. All strains were kept at -80°C in Brain Heart Infusion (BHI, Thermo Scientific, Milan, Italy), with the addition of 20% glycerol. Upon use, strains were inoculated in BHI and incubated for 24 hours at 37°C.

In vitro evaluation of *Cannabis sativa L.* susceptibility against foodborne pathogens

In order to evaluate whether the potential antimicrobial effect of industrial hemp was solely due to its CBD content or by additional components, the antimicrobial susceptibilities of different concentrations of the grind, the extract and pure CBD (crystals, purity 99%, ECO HEMP TRADING LTD, Villanova Del Ghebbo, Italy), all with comparable CBD content, were evaluated following a previously reported protocol (Duarte et al., 2016). In particular, the concentrations to be tested were selected based on the following assumptions: 1) 50 mg of pure CBD in 150 mL of Nutrient Agar (NA) was previously suggested as an efficient concentration against *Staphylococcus aureus* (Duarte et al., 2016) and 2) concentration of CBD in the autoclaved extract was 1316,63 µg/mL (Table 1). Based on these assumptions 4.6 mL of the extract or 60 mg of CBD was

Table 1. Phytocannabinoids profile of the grind, autoclaved and not-autoclaved extracts of *Cannabis sativa L* variety Futura 75.

Sample	CBDA (%)	CBGA (%)	CBG (%)	CBD (%)	THCV (%)	CBN (%)	Δ9-THC (%)	Δ8-THC (%)	CBC (%)	THCA (%)
Grind (*Cannabis Sativa L.*)	5.40	0.10	0.03	1.59	nd	0.01	0.11	nd	0.07	0.14
Dev.std.	0.15	0.02	0.01	0.05	-	0.00	0.00	-	0.00	0.01
CV%	2.86	15.01	17.72	3.39	-	7.88	2.63	-	5.76	9.49
Sample	**CBDA (µg/mL)**	**CBGA (µg/mL)**	**CBG (µg/mL)**	**CBD (µg/mL)**	**THCV (µg/mL)**	**CBN (µg/mL)**	**Δ 9-THC (µg/mL)**	**Δ8-THC (µg/mL)**	**CBC (µg/mL)**	**THCA (µg/mL)**
Autoclaved extract	89.96	nd	29.19	1316.63	nd	7.34	23.76	nd	38.99	nd
Dev.std.	1.62	-	0.95	13.64	-	0.58	1.04	-	0.45	-
CV%	1.80	-	3.26	1.04	-	7.89	4.39	-	1.15	-
Not autoclaved extract	882.25	15.24	9.36	322.70	nd	2.28	15.04	nd	10.45	8.36
Dev.std.	20.48	1.30	1.10	14.80	-	0.09	0.62	-	0.05	0.21
CV%	2.32	8.55	11.71	4.59	-	3.86	4.14	-	0.43	2.54

added to 100 mL of NA (Thermo Scientific, Milan, Italy) corresponding to a final concentration of CBD in the autoclaved NA of 0.6 mg/mL. Along this concentration, additional 1:2 dilutions were tested in the range 0.017–0.6 mg/mL. The supplements were added to the medium before autoclaving since the sterilisation temperature of 121°C enhances the decarboxylation of the inactive acid form to the active neutral form of cannabinoids. Bacteria suspensions of each food-borne pathogen were prepared in physiological solution and adjusted to a concentration of 1.5 x 10^8 CFU/mL (corresponding to an OD of 0.08 – 0.1 at 625 nm). On supplemented NA plates, 5 µl of each suspension were spotted, and NA plates incubated for 24-48 hours at 37°C. Inoculated and not supplemented NA plates were included as positive controls.

Antimicrobial effect of *Cannabis sativa L.* against microbial populations of minced beef

In order to test the antimicrobial effect of industrial hemp on microbial indicators of food processing hygiene, 5 kg of minced meat beef was purchased at retail after one day from its production. The meat sample was divided in two aliquots of 2.5 kg each. One aliquot act as control, the second was additioned with 50 mL of the extract. Since the concentration of CBD in the not autoclaved extract was 322,7 µg/mL (Table 1) the final concentration of CBD in minced meat was 6.45 µg/g. In order to mimic domestic storage conditions, aliquots of 80 g each were packed and stored at 4°C along with hemp-free aliquots used as controls. At 0, 1, 2, 3, 4, 7 and 8 days of storage three hemp additioned aliquots and three hemp–free aliquots were tested for, *Enterobacteriaceae*, coliforms, aerobic colony count and *E. coli* following standard procedures (International Organisation for Standardisation (ISO), 2001; ISO, 2003; ISO, 2004; ISO, 2006).

Phytocannabinoids detection and quantification

Phytocannabinoids were detected and quantified in the grind as well as in two autoclaved and not autoclaved aliquots of the extract following a previously reported protocol with some minor modifications

Figure 1. Minced beef with extract (ETA) and without (CONTR) after 8 days of storage at 4°C.

Table 2. Susceptibility to CBD and *Cannabis sativa* of S. Typhimurium (strains ST208, ST63), *E. coli* (strains EC ATCC 25922, EC135), *L. monocytogenes* (LM1, LM2) and *Staphylococcus* spp. (strain S661).

CBD Concentration (mg/mL)	ST208	ST63	EC ATCC	EC135	LM1	LM2	S661
Extract							
0.6	+	+	+	+	-	-	-
0.3	+	+	+	+	-	-	-
0.15	+	+	+	+	-	-	+
0.07	+	+	+	+	-	-	+
0.035	+	+	+	+	-	-	+
0.017	+	+	+	+	-	-	+
CBD							
0.6	+	+	+	+	-	-	-
0.3	+	+	+	+	-	-	-
0.15	+	+	+	+	-	-	+
0.07	+	+	+	+	-	-	+
0.035	+	+	+	+	-	-	+
0.017	+	+	+	+	-	-	+
Negative control							
0	+	+	+	+	+	+	+

(Mandrioli *et al.,* 2019). The autoclaved and not-autoclaved aliquots were tested in order to evaluate the exact cannabinoid composition in the in vitro susceptibility and the food experiments respectively. An aliquot of the sample (grind), about 50 mg, was added to 10 mL of methanol-chloroform 9:1 (v/v) extraction solvent. After 10 min on agitation set at 350 oscillations per minute, the sample was ultrasonicated for 10 minutes. After centrifugation for 5 minutes at 1620 xg, the supernatant was collected. The extraction was repeated twice and the two fractions were collected in a 25 mL volumetric flask and brought to volume with methanol/ chloroform (9:1, v/v). The solution was filtered with a 45 µm nylon filter. One mL of this solution was dried under weak nitrogen flow. Dried material was resuspended in 1 mL of acetonitrile, 5.0 µl of which was injected into an RP-HPLC-DAD liquid chromatography system. For the extract (autoclaved and not autoclaved),1 mL was transferred and directly dissolved into a 10 mL flask and brought to volume with acetonitrile. For the HPLC analysis, UV detection was used at 230 nm, gradient elution was used at flow rate of 1.5 mL/min according to the following procedure: eluent mixture A water + 0.1% phosphoric acid, B acetonitrile + 0.1% phosphoric acid; gradient elution: 75% of B up to 0.7 min. 85% of B to 2 min. 100% of B to 3.0-3.5 min and 100% of B to 3.6-5.0 min. Data were acquired using Chemstation software for LC3D (Rev.A.08.03 Agilent Technologies, USA). The quantification of analytes was carried out using the external standard method, through the construction of calibration curves prepared with standard reference compounds of chromatographic purity. Standard references of CBDA, CBGA, CBG, CBD, THCV, CBN, Δ9-THC, Δ8-THC, CBC, and THCA were diluted wit acetonitrile in a concentration range between 0.1-100 µg/mL. Eight dilutions of each cannabinoid was used to build the calibration curve. The standard solutions were stored away from light at a temperature of −20 ◦C. The equations of the calibration curves were as follows:

CBD, y=6.5473x-1.6742 (r2=0.9999); CBG, y=5.9001x-0.4766 (r2=0.9964); Δ9-THC, y=6.3627x-0.2229 (r2=0.9962); CBN, y=14.792x-0.6310 (r2=0.9990); CBC, y=15.619x-0.788 (r2=0.9989); CBDA, y=10.018x+1.1843 (r2=0.9981); CBGA, y=9.5259x-1.1675 (r2=0.9959); THCA, y=9.154x-0.9992 (r2=0.9954); Δ8-THC, y=4.8513x+1.0499(r2=0.9979); THCV, y=4.9225x+1.883 (r2=0.9987).

Results

Semiquantitative evaluation of CBD and THC

In Table 1, the concentrations of CBDA, CBD, Δ9-THC and THCA in the grind as well as in the autoclaved and not autoclaved extract are reported.

The grind of *Cannabis sativa L.,* included in the present study, was characterised by 5,40% of CBDA and 0.11 % of Δ9-THC confirming previously reported data (Palmieri et al., 2019) (Table 1). Other cannabinoids, identified at low percentages, were CBGA (0.10%), CBN (0.01 %) and CBC (0.07%). THCV and Δ8-THC was not detected. Not surprisingly, in the extract a significant shift of cannabinoids from their acid form to their neutral form was observed comparing the autoclaved *vs* the not autoclaved extract. In particular, in the latter CBDA and CBD were 882.25 µg/mL and 322.70 µg/mL respectively. In the autoclaved extract CBDA was significantly reduced to 89.96 µg/mL , whereas CBD increased up to 1316.63 µg/mL. Moreover, in the autoclaved form, the overall concentration of CBGA+CBG, CBN and CBC increased in comparison to the not autoclaved extract.

In vitro evaluation of *Cannabis sativa* susceptibility against foodborne pathogens

The antimicrobial properties of *Cannabis sativa L.* variety Futura 75 were investigated *in vitro* in comparison to pure CBD against *S.* Typhimurium, *E. coli*, *L. monocytogenes* and *Staphylococcus* spp. (Table 2). At the tested concentrations, hemp

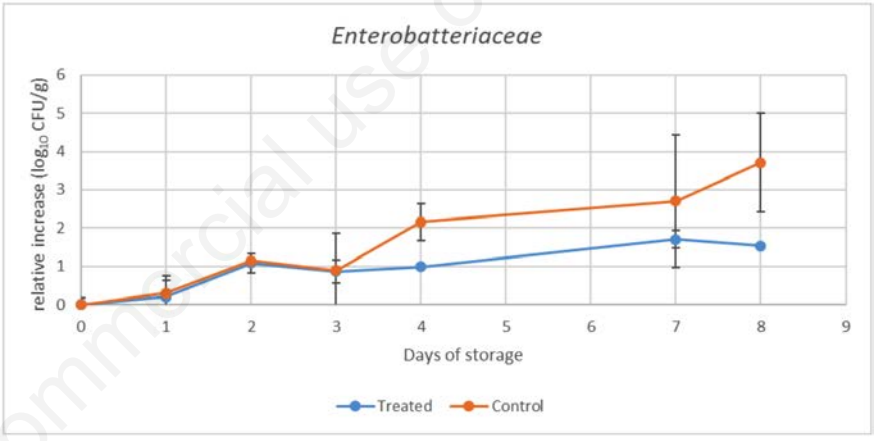

Figure 2. Enumeration of enterobacteriaceae in minced meat with extract (treated) and without (control) during storage at 4°C for 8 days.

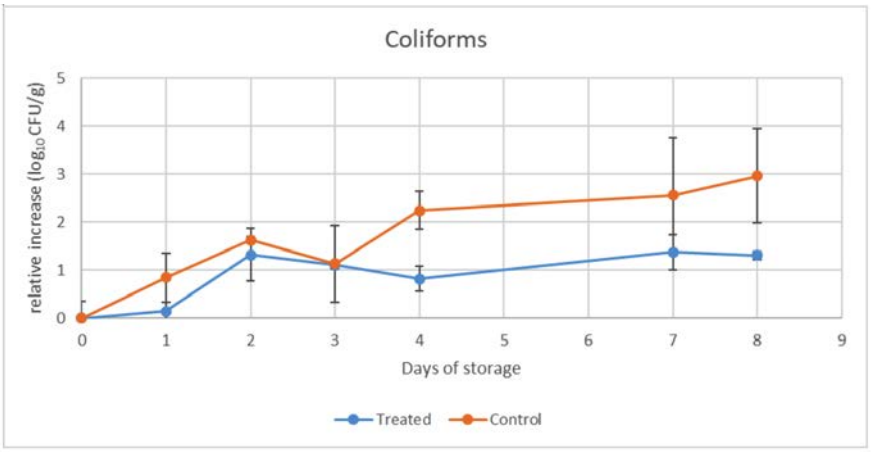

Figure 3. Enumeration of coliforms in minced meat with extract (treated) and without (control) during storage at 4°C for 8 days.

as well as pure CBD were effective in inhibiting Gram positive bacteria but not Gram negative ones (ST208, ST63, EC ATCC and EC135). In particular, all tested concentrations were effective against *L. monocytogenes* (LM1 and LM2) and 0.3 mg/mL was effective against *Staphylococcus* spp (S661). This result confirmed previous published data (Duarte *et al.*, 2016).

Antimicrobial effect of *Cannabis sativa* on microbial populations of minced beef

The antimicrobial effect of hemp was evaluated on microbial populations naturally occurring on minced beef and representing indicators of food hygiene. For this purpose, hemp extract was added to minced beef which was stored at 4°C for 8 days. The final concentration of CBD in the meat was 6.45 µg/g two order of magnitude lower than the one tested in the *in vitro* experiment. Surprisingly, after the storage period, visual assessment revealed that hemp extract impact on the colour of minced meat which appeared light red in comparison to dark brownish red colour of the control (minced meat without the extract) (Figure 1). Although red colour after storage is not an indicator of hygiene per se, this observation might suggest an effect of the extract in inhibiting spoilage bacteria such as those belonging to the strict aerobic Pseudomonas genus and/or an antioxidant effect of cannabinoids. Specific reductions were registered on *Enterobacteriaceae* and coliform enumerations. Both microbial groups showed a significant reduction in minced beef with hemp extract in comparison to control already after 4 days and reaching 2.3 and 1.6 $\log_{10}$ CFU/g reduction respectively after 8 days of storage at 4°C (Figures 2 and 3).

Aerobic colony count showed a slowing down of the growth rate in treated minced beef samples in comparison to control ones at day 4. Subsequently, similar increases up to 2.4-3.0 $\log_{10}$ CFU/g were reached at day 8 in treated and control samples respectively (Figure 4).

E. coli was under the detection limit until day 3. From day 4 to day 8 *E.coli* increased of 2.4 $\log_{10}$ and 2.9 $\log_{10}$ CFU/g in treated and control samples respectively without significant differences (Figure 5).

Discussion

Cannabis sativa variety 75 was investigated as antimicrobial against foodborne pathogens as well as against microbial populations of minced beef. Results *in vitro* confirmed previously reported data on inhibitory properties of this plant against *Staphylococcus aureus* (Appendino *et al.*, 2008; Duarte *et al.*, 2016). Both *L. monocytogenes* tested strains (LM1 and LM2) were susceptible to the whole range of CBD and plant concentrations tested. In literature, data are available on oil extract efficacy against this microorganism. Nevertheless controversial results are reported with moderate (Minimum Inhibitory Concentration (MIC) > 2048 µg/mL) to high efficacy (MIC 2-32 µg/mL) (Iseppi *et al.*, 2019; Marini *et al.*, 2018). In contrast, *S. Typhimurium* and *E. coli* strains were resistant to both CBD and hemp extract. Efficacy against Gram negative bacteria and in particular *E. coli* is controversial since both susceptibility and resistance to hemp were described (Ferrante *et al.*, 2019; Tardon *et al.*, 2017). In particular, oil, aqueous and ethanol extracts were described as inactive or slightly active against *E. coli*, whereas high activity was attributed to methanol and petroleum hemp extracts (Tardon *et al.*, 2017). In the present study, results confirmed the lack of efficacy of ethanol extract against *E. coli* and they extended the same observation to *S. Typhimurium*, which was never tested before to the best of author's knowledge. Moreover, the lack of activity was observed independently from the antibiotic susceptibility profile of selected strains. *E. coli* and *S. Typhimurium*, multiresistant as well as antibiotic susceptible strains were equally resistant to *Cannabis sativa* and CBD at the tested concentrations.

Industrial hemp seeds and seed oil have

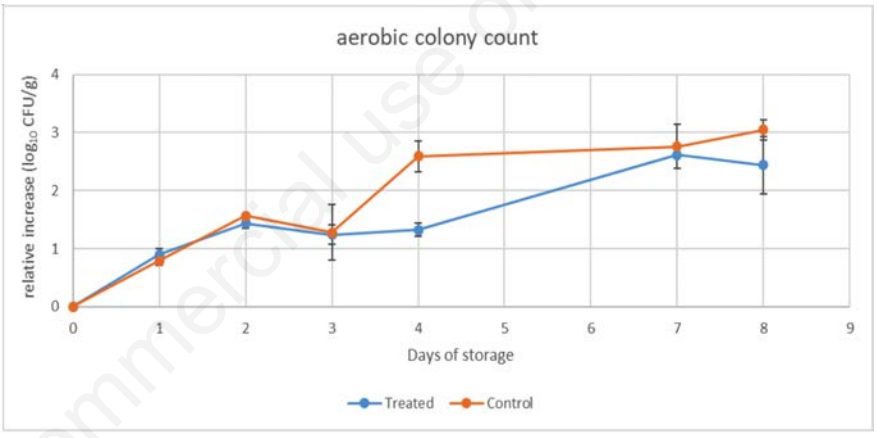

Figure 4. Aerobic colony count in minced meat with extract (treated) and without (control) during storage at 4°C for 8 days.

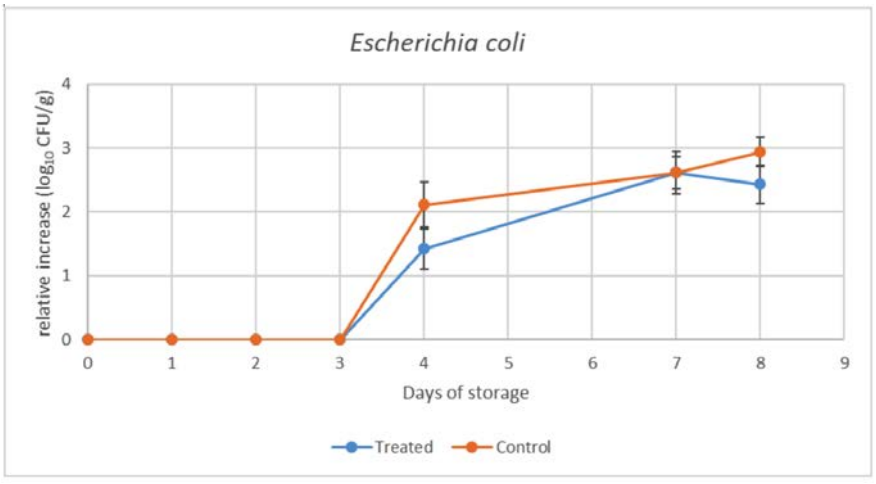

Figure 5. *Escherichia coli* enumeration in minced meat with extract (treated) and without (control) during storage at 4°C for 8 days.

been recently applied as functional food or functional food ingredient thanks to their significant contribution in polyunsaturated fatty acids, optimum Omega-6 and Omega-3 ratio, fibres and amino acids (Apostol *et al.* 2015; Frassinetti *et al.,* 2017; Radočaj *et al.*, 2014). However, to the best of author's knowledges, the effect of hemp extract as natural food preservative was never investigated before. In particular, in the present study the antimicrobial effect of hemp extract was investigated against microbial indicators of food hygiene of minced meat. After one week of storage at 4°C, a significant reduction was achieved for *Enterobacteriaceae* and coliforms respectively, along with a delay on the growth of aerobic colony count. Moreover, a striking lighter red colour of the minced meat was observed in comparison to the control, suggesting: i) hemp antioxidant properties as previously described (Bonini *et al*., 2018); ii) reduction of strict aerobic spoilage bacteria of the Pseudomonas genus.

Interestingly, the hemp extract showed antimicrobial properties both after heat treatment due to autoclaving in the *in vitro* experiment and without heat treatment in the food experiment. In particular, in the food experiment a final CBD concentration of 6.45 µg/g was enough to significantly impact on the growth of tested bacterial groups. As demonstrated by chemical results heat treatment allows the decarboxylation of cannabinoids compounds which shifted from an inactive acid form to an active decarboxylated form. In particular 90% of CBDA and 100 % of THCA and CBGA shifted to their decarboxylated forms CBD, THC and CBG respectively. Additionally, heat treatment might be responsible of the loss of other compounds such as volatile terpenes. Further analyses should be performed in order to detect and quantify the exact profile of terpenoid compounds characterizing the hemp extract as well as the antioxidant activity of cannabinoids in minced meat and their inhibitory effect on spoilage bacteria such as Pseudomonas.

Conclusions

The ethanol extract of industrial hemp (*Cannabis sativa* L. variety Futura 75) showed *in vitro* antimicrobial properties against foodborne pathogens such as *L. monocytogenes* and *Staphylococcus* spp. but not against *S. Typhimurium* and *E. coli*. These results along with the antimicrobial properties observed for hemp extract when mixed to minced beef, suggest that hemp extract might have promising applications as natural food preservative which deserves further investigations especially on the specific molecules as well as their mechanisms of action linked to antimicrobial activity.

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