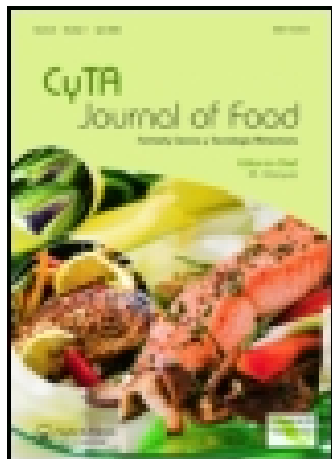


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Antioxidant properties and effect on lactic acid bacterial growth of spice extracts

Mariola Kozłowska^a, Iwona Ścibisz^b, Dorota Zaręba^c & Małgorzata Ziarno^c

^a Department of Chemistry, Faculty of Food Sciences, Warsaw University of Life Sciences (WULS-SGGW), Nowoursynowska 159C St., Warsaw 02-776, Poland

^b Division of Fruit and Vegetable Technology, Faculty of Food Sciences, Warsaw University of Life Sciences (WULS-SGGW), Nowoursynowska 159C St., Warsaw 02-776, Poland

^c Department of Biotechnology, Microbiology and Food Evaluation, Faculty of Food Sciences, Warsaw University of Life Sciences (WULS-SGGW), Nowoursynowska 159C St., Warsaw 02-776, Poland

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Antioxidant properties and effect on lactic acid bacterial growth of spice extracts

Propiedades antioxidantes y efectos sobre el crecimiento de bacterias ácido-lácticas de extractos de especias

Mariola Kozłowska^{a*}, Iwona Ścibisz^b, Dorota Zaręba^c and Małgorzata Ziarno^c

^aDepartment of Chemistry, Faculty of Food Sciences, Warsaw University of Life Sciences (WULS-SGGW), Nowoursynowska 159C St., Warsaw 02-776, Poland; ^bDivision of Fruit and Vegetable Technology, Faculty of Food Sciences, Warsaw University of Life Sciences (WULS-SGGW), Nowoursynowska 159C St., Warsaw 02-776, Poland; ^cDepartment of Biotechnology, Microbiology and Food Evaluation, Faculty of Food Sciences, Warsaw University of Life Sciences (WULS-SGGW), Nowoursynowska 159C St., Warsaw 02-776, Poland

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The total phenolic content (TPC) and antioxidant capacity as determined by 2,2-diphenyl-1-picrylhydrazyl, 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid and ferric reducing antioxidant power assays of spice extracts such as rosemary, lemon balm, hyssop, nettle and cumin seeds were investigated. The effect of spice extracts on the growth of selected lactic acid bacteria (LAB) was also analysed. Lemon balm extract exhibited the highest TPC and antioxidant capacity while the nettle and cumin seeds extracts were characterized by the lowest values. Spice extracts had no impact on the growth of the most tested LAB except for rosemary extract, which showed an inhibitory effect towards all strains of *Lactobacillus acidophilus* and *L. delbrueckii*. These results suggest that spice extracts may be considered as additives in dairy and meat products in which LAB are present.

Keywords: rosemary extract; lemon balm extract; hyssop extract; cumin extract; nettle extract; total phenolics content; DPPH; ABTS; FRAP; *Lactobacillus* spp.

Se investigaron el contenido fenólico total (CFT) y la capacidad antioxidante determinada mediante los métodos DPPH, ABTS y FRAP de los extractos de especias tales como romero, melisa, hisopo, ortiga y semillas de comino. También se analizó el efecto de extractos de especias en el crecimiento de determinadas bacterias ácido-lácticas. El extracto de melisa presentó el mayor contenido total de compuestos fenólicos y la mayor capacidad antioxidante, mientras que los extractos de ortiga y semillas de comino mostraron el menor CFT y la menor capacidad antioxidante. Los extractos de especias no tuvieron ningún impacto en el crecimiento de las bacterias ácido-lácticas examinadas, a excepción del extracto de romero, el cual presentó un efecto inhibitorio con respecto a todas las cepas de *Lactobacillus acidophilus* y *Lactobacillus delbrueckii*. Estos resultados sugieren que los extractos de especias pueden ser considerados como aditivos en alimentos que contienen bacterias ácido-lácticas.

Palabras clave: extracto de romero; extracto de melisa; extracto de hisopo; extracto de comino; extracto de ortiga; compuestos fenólicos; DPPH; ABTS; FRAP; *Lactobacillus* spp.

Introduction

Most consumers are looking for the minimally processed food with no addition of chemical preservatives. They accept the use of spices and herbs or other medicinal plants rather than synthetic antioxidants. Spices and herbs are well known for their antioxidant and antimicrobial activities and are useful for preventing lipid oxidation in living organisms as well as in foods (Ertürk, 2006; Kozłowska, Żbikowska, Gruczyńska, Żontala, & Póltorak, 2014). They are also a source of phenolic compounds that play an important role in human health and disease prevention. Phenolic compounds are a large class of plant secondary metabolites showing a diversity of structure from simple molecules to highly polymerized compounds. Among these, flavonoids and phenolic acids are well known for their antioxidant properties, possessing ideal structural chemistry for radical scavenging activity and being more effective than tocopherol and ascorbate. Some authors have demonstrated a linear correlation between the content of total phenolic compounds and their antioxidant activity (Djeridane, Yousfi, Nadjemi, Boutassouna, Stocker, & Vidal, 2006; Wong, Leong, & Koh, 2006). Extracts

from spices and herbs are also effective in prolongation of the shelf life of food products through the reduction in growth of pathogenic and spoilage microorganisms (Elgayyar, Draughon, Golden, & Mount, 2001). Some spices such as garlic and cardamom are also used for the production of fermented foodstuffs, especially dairy and meat products (Verluyten, Leroy, & Vuyst, 2004). The addition of these to meat may accelerate lactic acid production by lactic acid bacterial starter culture. Lactic acid bacteria (LAB) are commonly used industrially in the fermentation of food and beverage products. LAB strains produce antimicrobial substances with activity against the homologous strain and microbicidal effects against gastric and intestinal pathogens and other microbes (Jacobsen et al., 1999). Various spices and herbal essential oils can influence the growth and activity of LAB in fermentative dairy and meat products. Elgayyar et al. (2001) reported that essential oils obtained from oregano and cardamom inhibited the growth of *Lactobacillus plantarum*, while angelica and basil oils had no inhibitory effect. However, cumin and its essential oil stimulated the growth of *L. plantarum* and acid production (Souza, Stamford, Lima, Trajano & Filho,

*Corresponding author. Email: mariola_kozlowska@sggw.pl

2005). In the present study, aqueous ethanolic extracts from spices and herbs belonging to the Lamiaceae, Apiaceae and Urticaceae families were obtained and their antioxidant activity and total phenolics content (TPC) values evaluated. The aim of this study was to investigate their effect on the growth of selected LAB.

Materials and methods

Materials

Plant materials used were dried leaves of rosemary (*Rosmarinus officinalis* L.), lemon balm (*Melissa officinalis* L.), hyssop (*Hyssopus officinalis* L.) and nettle (*Urtica dioica* L.) and cumin seeds (*Cuminum cyminum* L.) purchased from a local market in Warsaw (Poland). Folin–Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) and 2,4,6-tripyridyl-s-triazine (TPTZ) were obtained from Sigma-Aldrich Chemicals (Poznań, Poland). Potassium persulphate, sodium carbonate and chemical solvents were obtained from POCh (Gliwice, Poland). All solvents and reagents were of the highest purity and were used without further purification.

Microorganisms

The LAB strains used in this study were as follows: *Lactobacillus acidophilus* ATCC 4356, *L. acidophilus* La14, *L. acidophilus* La-5, *L. acidophilus* NCFM, *L. casei* 431, *L. casei* ATCC 393, *L. casei* 01, *L. paracasei* AD 200, *L. rhamnosus* GG, *L. rhamnosus* ATCC 7469, *L. rhamnosus* 573, *L. plantarum* NCAIM B.01834, *L. plantarum* NCAIM B.01149, *L. plantarum* 299 v, *L. delbrueckii* subsp. *lactis* ATCC 4797, *L. delbrueckii* subsp. *bulgaricus* ATCC 11,842 and *L. delbrueckii* subsp. *bulgaricus* LB58. All strains were supplied by the Museum of Clean Cultures of the Division of Milk Biotechnology of Warsaw University of Life Sciences.

Sample preparation

Aqueous ethanolic extracts from plant materials were prepared according to Kozłowska et al. (2010). In the case of cumin extract, before extraction with ethanol (70%), 20 g of seeds were ground to a fine powder in a household grinder and mixed with 200 mL hexane at ambient temperature for 6 h. Then, the solvent was filtrated and the residue dried at room temperature, and extracted with 70% ethanol. The yields of spice extracts were calculated following solvent evaporation to dryness under reduced pressure at 40°C by a rotary evaporator, and were as follows: rosemary 18.02%, lemon balm 23.67%, hyssop 26.06%, nettle 13.32% and cumin 10.98%.

To determine antioxidant activity and TPC, the spice extracts were re-dissolved in 70% ethanol to yield solutions containing 0.5–2.0 mg of extract per mL.

Total phenolics content

TPC was estimated using the Folin–Ciocalteu method described previously (Singleton & Rossi, 1965) with slight modification. Briefly, the appropriately diluted spice extract was mixed with deionized water (20 mL) and Folin–Ciocalteu reagent (0.5 mL). After 30 s, 5 mL of a Na₂CO₃ (20%, v/v) was added. Then, the

solution was incubated at 25°C for 1 h and the absorbance at 765 nm was measured. TPC was expressed as mg gallic acid equivalents per gram of extract using a standard gallic acid calibration curve in the range 1.2–29.4 µmol/L.

DPPH radical scavenging activity

Free radical scavenging activity of the spice extracts was determined by the method of Yen and Chen (1995) with modification. One millilitre of 0.3 mmol/L freshly prepared DPPH-methanol solution was mixed with 0.2 mL of the extract and 3.8 mL of methanol. After 10 min of incubation in the dark at room temperature, the absorbance at 517 nm was measured. Antioxidant capacity based on the DPPH free radical scavenging ability of the extract was expressed as mmol Trolox equivalents per gram of dry matter of extract. A standard curve was obtained using the Trolox standard in the range 8–40 µmol/L.

ABTS assay

Total antioxidant capacity assay was performed according to the method developed by Re et al. (1999). ABTS (14 mmol/L) was mixed with 4.9 mmol/L of potassium persulphate and kept for 12–16 h in the dark at room temperature. For the analysis, the solution was diluted in water to an absorbance of 0.7 ± 0.02 at 734 nm. Then, 40 µL of spice extract and 4 mL of ABTS⁺ working solution were mixed. After 6 min, the absorbance of samples at 734 nm was recorded and compared to that from the calibrated Trolox standard. Results were expressed as mmol Trolox equivalents per gram of dry matter of extract.

Ferric reducing antioxidant power assay

The ferric reducing antioxidant power (FRAP) assay was conducted according to Benzie and Strain (1996) with some modification. The FRAP reagent consists of 20 mmol/L FeCl₃ solution, 300 mmol/L acetate buffer (pH 3.6) and 10 mmol/L TPTZ solution in 40 mmol/L HCl in the proportions 1:10:1 (v/v/v). An appropriate concentration of spice extract was allowed to react with 3 mL of FRAP solution for 10 min at room temperature and the absorbance was measured at 593 nm. Results were expressed as mmol Trolox equivalents per gram of dry matter of extract. A standard curve was prepared using Trolox in the range 80–500 µmol/l.

Antimicrobial activity

Antimicrobial activity was carried out using the agar diffusion method (Bonev, Hooper, & Parisot, 2008). Lactobacilli were revived in MRS broth at either 30 or 37°C for 18 h. Petri dishes were loaded with 0.5 mL of the culture broth cultured to a concentration of about 10⁶ CFU mL⁻¹, which was then covered with MRS agar medium at 45°C. After solidification, the wells were cut to a diameter of 6 mm and each was loaded with 20 µL of spice extracts dissolved in dimethyl sulfoxide (DMSO). The Petri dishes were incubated at either 30 or 37°C under anaerobic conditions for 72 h and the respective zone of inhibition was measured. The inhibition tests were done in quadruplicate. The wells containing DMSO but no spice extracts were used as a negative control.

Statistical analysis

All results are expressed as mean \pm standard deviation (SD). Statistical evaluations were performed using Statgraphics Plus version 4 software. The data were subjected to one-way analysis of variance and the significant differences between means were calculated by Tukey's test. Differences at $P \leq 0.05$ were considered significant.

Results and discussion

Total polyphenol content

Total phenols determined in the spice extracts are shown in Figure 1. The highest content was recorded in lemon balm extract (239 mg/g of extract as gallic acid equivalents), followed by hyssop and rosemary (198 and 190 mg/g, respectively), with nettle extract the lowest (48 mg/g). A similar TPC value for rosemary leaves was obtained by Dorman, Peltoketo, Hiltunen and Tikkanen (2003). However, Gramza-Michałowska, Sidor and Hes (2011) found a lower TPC value for rosemary extract (161.26 mg/g). Gallego, Gordon, Segovia, Skowryra and Almajano (2013) reported a value of 219 mg/g for lyophilized rosemary leaf extract, a value higher than that found in the present study (Figure 1). A slightly higher TPC value in lemon balm extract was recorded by Marcinčák, Popelka, and Šoltysová (2008). The TPC value for cumin seed extract determined in the present study is different to that determined by Mariod, Ibrahim, Ismail, and Ismail (2009), because they estimated TPC among different fractions of black cumin seedcake. However, El-Ghorab, Nauman, Anjum, Hussain, and Nadeem (2010) reported that in regard to the extraction of polyphenols from cumin, methanol proved a better solvent than hexane. The solvent polarity used

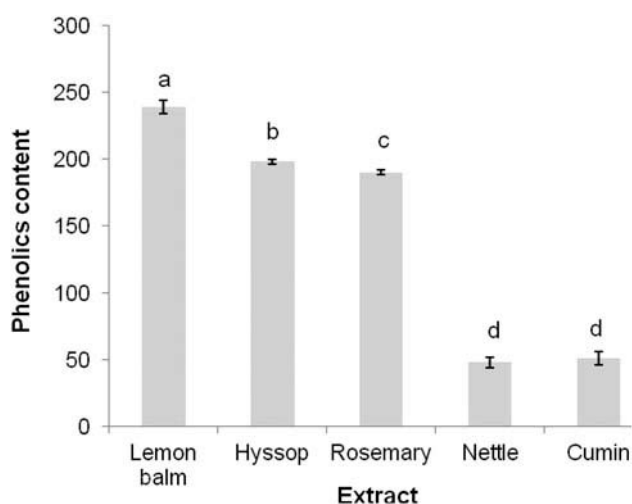


Figure 1. Total phenolic content of spice extracts (mg/g of extract as gallic acid equivalent).

Figura 1. Contenido de fenoles totales en extractos de especias (mg/g de extracto como equivalente ácido gálico).

Notes: Mean of three replications \pm standard deviation (SD). Different superscript letters are significantly different ($P \leq 0.05$). Data of total phenolic content expressed as mg gallic acid equivalents per gram of extract. (mg/g of extract as gallic acid equivalents)

Notas: Promedio de tres réplicas \pm desviación estándar (DS). Letras diferentes sobre las barras indican diferencias estadísticamente significativas ($P \leq 0.05$). Los datos del contenido total de fenoles están expresados como mg equivalentes de ácido gálico por g de extracto (mg equivalentes de ácido gálico/g de extracto)

for extraction and method of extraction may affect variation in TPC of plant extracts and thus their antioxidant activity.

Antioxidant capacity

The results of antioxidant activity measured by different assays are shown in Table 1. In the ABTS, DPPH and FRAP assays, lemon balm extract demonstrated the highest antioxidant capacity followed by hyssop and rosemary extracts, while nettle and cumin extracts exhibited the lowest activity. Among the five tested spices, lemon balm, hyssop and rosemary belong to the Lamiaceae family, cumin to the Apiaceae family and nettle to the Urticaceae family. Herbs from the Lamiaceae family are well known for their antioxidant activity because they are a rich source of biologically active substances, especially phenolic compounds classified into three groups: phenolic diterpenes, flavonoids and phenolic acids (Shan, Cai, Sun, & Corke, 2005). Zgórk and Główniak (2001) found that among plant extracts from the Lamiaceae family, hyssop extract contained the highest content of chlorogenic, ferulic and protocatechuic acids that are implicated as natural antioxidants. Babovic, Djilas, Jadranin, Vajs, Ivanovic, Petrovic, and Zizovic (2010) reported that rosemary, sage and thyme extracts obtained by fluid extraction with carbon dioxide demonstrated high DPPH radical scavenging activity in comparison to hyssop extract. Spiridon, Bodirlau and Teaca (2011) showed that extracts from *Origanum vulgare*, *Lavandula angustifolia* and *Melissa officinalis* demonstrated the highest antioxidant activity of all plants tested in their study. The presence of phenolic compounds, especially flavonoids and caffeic acid derivatives, in nettle extract was found to influence its antioxidant effect although significantly less so than plants from the Lamiaceae family (Pinelli et al., 2008). In our study, the extract from cumin seeds presented the lowest value of antioxidant activity in all assays, with the exception of the FRAP assay (0.024 mM/g dm of extract; Table 1). Seed extracts of cumin (Altrooz, 2013) were able to neutralize free radicals over a period of 60 min in a DPPH assay. The ability of ethanol cumin extract to scavenge DPPH radicals and changes in the FRAP of the tested cumin extracts were observed by Kim et al. (2009). Various authors have reported a correlation between antioxidant activity and TPC in selected plants. Our results also indicate that TPC significantly correlated with DPPH ($r^2 = 0.97$), ABTS ($r^2 = 0.99$) and FRAP

Table 1. Antioxidant activity of spice extracts determined by DPPH, ABTS and FRAP assays (mM/g dm of extract).

Tabla 1. Actividad antioxidante de extractos de especias determinada mediante los métodos DPPH, ABTS y FRAP (mM/g dm de extracto).

Spice extract	ABTS	DPPH	FRAP
Lemon balm	0.142 ^a \pm 0.014	0.133 ^a \pm 0.008	0.146 ^a \pm 0.011
Hyssop	0.115 ^b \pm 0.009	0.099 ^b \pm 0.009	0.115 ^b \pm 0.007
Rosemary	0.112 ^b \pm 0.012	0.103 ^b \pm 0.011	0.113 ^b \pm 0.009
Nettle	0.021 ^c \pm 0.002	0.019 ^c \pm 0.002	0.023 ^c \pm 0.001
Cumin	0.018 ^c \pm 0.003	0.018 ^c \pm 0.001	0.024 ^c \pm 0.001

Notes: Mean of three replications \pm standard deviation (SD). Means in the same vertical columns followed by the same superscript letter do not differ significantly ($P \leq 0.05$). Data of total antioxidant activity expressed as mmol Trolox equivalents per gram of dry matter of extract (mM/g dm of extract).

Notas: Promedio de tres réplicas \pm desviación estándar (DS). Promedios en las mismas columnas seguidos por la misma letra no difieren significativamente ($P \leq 0.05$). Los datos de actividad antioxidante están expresados como mmol Trolox equivalentes por g de materia seca de extracto (mM/g ms de extracto).

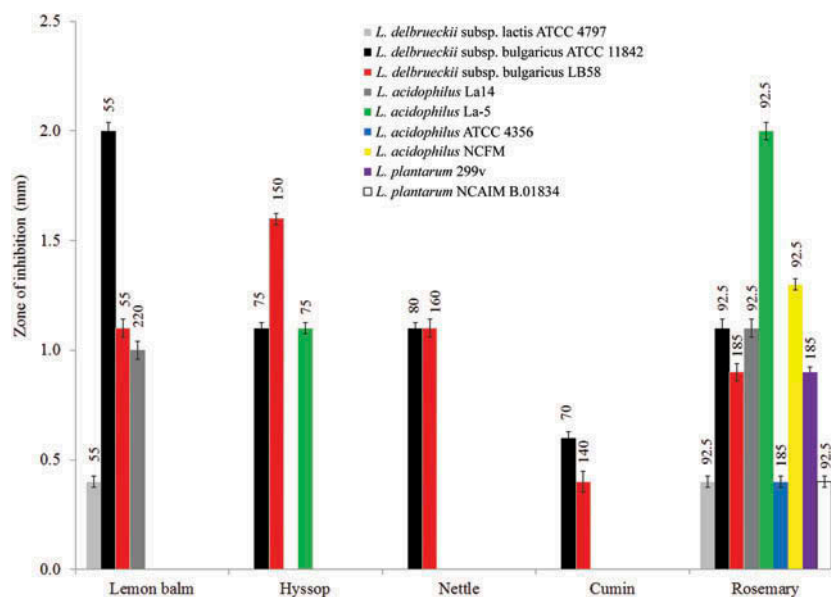


Figure 2. Antimicrobial activity of spice extracts (zone inhibition).

Figura 2. Actividad antimicrobiana de extractos de especias (zona de inhibición).

Notes: Mean of three replications \pm standard deviation (SD). Numbers above the bars indicate the concentration of spice extract (mg/L) at which the zone of inhibition of growth of lactic acid bacteria was observed.

Notas: Promedio de tres réplicas \pm desviación estándar (DS). Números sobre las barras indican las concentraciones de los extractos de especias (mg/L) en las cuales se observó la zona de inhibición del crecimiento de bacterias ácido-lácticas.

($r^2 = 0.99$). It can therefore be concluded that the antioxidant capacity of the spice extracts tested is mainly due to the presence of phenolic compounds.

Antimicrobial activity

The spice extracts exhibited an inhibitory effect towards 9 of 17 LAB strains tested. Nettle and cumin seed extracts were active against only two strains of *L. delbrueckii* subsp. *bulgaricus* (ATCC 11842 and LB58) (Figure 2). These bacterial strains were also susceptible to hyssop and lemon balm extracts. Additionally, hyssop extract in the concentrations used was active against *L. acidophilus* La-5 while lemon balm extract inhibited the growth of *L. acidophilus* La14 and *L. delbrueckii* subsp. *lactis* ATCC 4797. Rosemary extract showed the strongest inhibitory effect towards all strains of *L. acidophilus* and *L. delbrueckii*, as well as two strains of *L. plantarum*. Zaika, Kissinger and Wasserman (1983) investigated the effect of herbs on the growth and acid production of *L. plantarum* and *Pediococcus acidilactici*. They found that increasing concentrations of oregano and rosemary gradually delayed the growth of these two microorganisms, especially *P. acidilactici*. However, Saguibo and Elegado (2012) observed the resistance of the probiotic *L. plantarum* BS against all plant part extracts including methanol extracts of avocado (*Persea americana* Mill.) and malunggay (*Moringa oleifera* Lam.) leaves. Other workers investigating LAB (*Pediococcus acidilactici* 4E5, *P. acidilactici* AA5a, *P. acidilactici* 3G3, *P. acidilactici* K3A2-2, *P. pentosaceus* K2A2-3, *L. plantarum* BS) showed selective sensitivity to various herb and vegetable extracts. In general, methanol plant extracts exhibited greater inhibitory effect than aqueous and ethanol extracts. Methanol avocado seed extract was found to be a very good growth inhibitor of *P. acidilactici* 4E5. All LAB tested with guava leaf extract were sensitive, apart from *P. acidilactici*, and malunggay leaf extract had an inhibitory effect towards *P. acidilactici* 4E5 and *P. acidilactici* AA5A (Saguibo & Elegado,

2012). Verluyten, Leroy, and Vuyst (2004) studied the effect of different spices applied in fermented sausage manufacture on the growth of *L. curvatus* LTH 1174. They found that pepper, nutmeg, rosemary and garlic decreased the maximum specific growth rate of *L. curvatus* while paprika was the only spice that increased its growth. Essential oils obtained from coriander, angelica and parsley were not effective against *L. plantarum* (Elgayyar, Draughon, Golden, & Mount, 2001), while the essential oils of five different varieties of basil possessed mild antimicrobial activity (Lachowicz et al., 1998).

Conclusions

Plant extracts used in the present study had an impact on the growth of 9 of 17 LAB tested. LAB are frequently often used as starter cultures in fermented foods, playing an important role in maintaining and promoting human health. Many spices and herbs have multiple biological effects including antioxidant activity, especially when these contain polyphenolic compounds. In the present study, no relationship between phenolic content in spice extracts and their capacity to inhibit LAB growth was observed. Therefore, plant extracts may be successfully used as a functional additive in fermented foods.

Disclosure statement

No potential conflict of interest was reported by the authors.

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