Impact of spices addition on 3-monochloropropane-1,2-diol formation in biscuit and cracker model systems

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Summary
The effect of the addition of ten selected spice extracts (caraway seeds, bell pepper, black pepper, garlic, Herbes de Provence, cinnamon, ginger, gingerbread spice, star anise, vanilla) on the 3-monochloropropane-1,2-diol (3-MCPD) formation in biscuit (sweet) and cracker (salty) model systems was investigated. 3-MCPD levels were analysed in the relation to the spices' antioxidant capacity, total polyphenol and protein content, and the lipase activity increase with added extracts. The experiment revealed that, although no correlations between antioxidant capacity or total polyphenol content and the 3-MCPD levels were found, the presence of spices influenced the 3-MCPD formation. In case of the cracker model system, the addition of spices rich in proteins, such as caraway seeds, resulted in a lower 3-MCPD formation, while the presence of garlic led to a higher 3-MCPD content compared to control samples. For the biscuit model system, a positive influence of residual lipase content or the compounds increasing the lipase activity on 3-MCPD formation was observed. The experiment also showed that heating foods containing garlic, ginger, star anise or vanilla extracts in the presence of salt might contribute to the increase of the amount of 3-MCPD generated even if no fat is added.

Keywords
3-monochloropropane-1,2-diol; spices; cereal model systems; lipase activity

Sweet biscuits and salty crackers belong to the most popular snacks all over the world. They are based mainly on fats, sugar, flour and salt, with the addition of flavourings or spices, for improving the taste and flavour. Apart from its potential health benefits, spices also contain many important components including proteins, antioxidants and essential oils [1–3]. However, baking of cereal products at temperatures above 160 °C can lead to the formation of various undesirable compounds such as 3-monochloropropane-1,2-diol (3-MCPD). 3-MCPD is a food-processing contaminant formed by heat as a reaction product of triacylglycerols, phospholipids or glycerol and hydrochloric acid. Low pH level (below 6) and/or low water content (less than 15 %) are the other factors contributing to 3-MCPD generation [4]. 3-MCPD may occur as a free substance, but also in the form of an ester with fatty acids. In this case, free 3-MCPD is released by lipase-catalysed hydrolysis during processing and storage. The presence of lipase can also contribute to acceleration of fat decomposition with the release of free glycerol, which is a main precursor of 3-MCPD [4–6].

The main target organ for 3-MCPD toxicity is the kidney, with chronic oral exposure resulting in nephropathy and tubular hyperplasia and adenomas. Therefore, International Agency for Research on Cancer (IARC) classified 3-MCPD as a "possible human carcinogen (group 2B)" and Scientific Committee on Food (SCF) established a tolerable daily intake (*TDI*) for 3-MCPD at 2 $\mu g \cdot kg^{-1}$ body weight (bw) [6].

Considering the possible routes of 3-MCPD formation, its general mitigation strategies in food should involve most of all: (1) raising pH of high-moisture foods; (2) lowering the maximum processing temperature and salt content of the food; (3) avoiding low-water and high-temperature treatments; (4) limiting the amount of glycerol in the food; (5) avoiding exposure to lipases [7].

The composition of biscuits and crackers, also

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including all additives used in its preparation, plays a crucial role in 3-MCPD generation. 3-MCPD in these products is formed mostly during reactions of sodium chloride with glycerol, lipids and carbohydrates [8–10]. It was also reported that the sugar content has not a strong influence on 3-MCPD formation but it might contribute to its generation [10]. 3-MCPD formation from glycerol was also studied in the presence of some amino acids, such as glutathione and/or cysteine, and with the addition of yeasts that could release amino acids. The results showed that amino acids have the potential to decompose 3-MCPD or to inhibit its generation [11]. Therefore, the high content of proteins could be another factor reducing the formation of 3-MCPD in foods.

3-MCPD can also be generated from 3-MCPD esters that are present in fats, oils or margarine used for biscuits and crackers production. Hence, in the case of foods containing high levels of lipids, such as aforementioned products, prevention of degradation of lipids could be effective means to decrease glycerol levels. It is commonly known that the addition of certain spices can minimize degradation of lipids, thereby inhibiting the release of free glycerol [3, 12, 13]. Thus, the addition of these spices could be an effective strategy of lowering 3-MCPD levels. However, the promotion of lipase activity by some spices has been also observed by other researchers [3, 14]. Thus, in this case, the addition of spices to cereal fat-based products should not be recommended. Additionally, it also has to be considered that spice components can also affect the formation 3-MCPD during baking.

Nonetheless, data on the impact of spices on 3-MCPD formation are limited and remain unclear. Furthermore, up to now no studies have been conducted on the influence of spices and their composition on the formation of 3-MCPD during heat treatment of cereal products. Since biscuits and crackers have become an element of the human diet, this topic should be thoroughly investigated.

Therefore, the aim of this research was to deepen the knowledge on the safety of baked cereal products with added spices through the investigation of 3-MCPD formation in cracker (salty) and biscuit (sweet) model systems. The protein content, total polyphenols content (TPC), antioxidants capacity and lipase inhibition/promotion by tested spice extracts were also investigated. The correlation between these factors and 3-MCPD content were estimated using the tools of statistical analysis.

MATERIALS AND METHODS

Chemicals and materials

Hexane, acetone and acetonitrile, grade for liquid chromatography LiChrosolv, were purchased from Merck (Darmstadt, Germany). Ethanol (98%), sodium chloride, sodium carbonate, glucose and liquid paraffin were purchased from Chempur (Piekary Śląskie, Poland). Primary secondary amine (PSA), octadecyl (C_{18}) solid phase extraction (SPE) bulk sorbent was obtained from Agilent Technologies (Santa Clara, California, USA). 2,2-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Foline-Ciocalteu reagent, *p*-nitrophenyl acetate, potassium persulfonate, gallic acid, glyceryl trioleate (triolein), Tween 80, lipase from porcine pancreas type II, 3-monochloropropane-1,2-diol (3-MCPD), 3-monochloropropane-1,2-diol-d_5 (3-MCPD-d_5) (surrogate standard), 3-monobromochloropropane-1,2-diol (3-MBPD; syringe standard) and phenylboronic acid (PBA; derivatization agent) were obtained from Sigma-Aldrich (Saint Louis, Missouri, USA). All reagents were of at least analytical purity.

A sodium chloride solution of 200 $g \cdot l^{-1}$ was prepared in deionized water. Intermediate and working standard solutions of chloropropanols at a concentration of 2 $\mu g \cdot ml^{-1}$ were prepared in a 200 $g \cdot l^{-1}$ NaCl solution. A PBA solution was prepared by dissolving 5 g of PBA in a 20 ml mixture of acetone and water (19 : 1, v/v). An ABTS solution (7 $mmol \cdot l^{-1}$) was prepared in water with the addition of a potassium persulfonate solution. Gallic acid (5 $mg \cdot ml^{-1}$) and Trolox (2 $mmol \cdot l^{-1}$) solutions were prepared in a mixture of ethanol and water (1 : 1, v/v). The solution of *p*-nitrophenyl acetate (10 $mmol \cdot l^{-1}$) was prepared in acetonitrile. Lipase was dissolved in phosphate buffer (pH 7.2) at 10 $mg \cdot ml^{-1}$, and then centrifuged for 5 min at 8 700 $\times g$.

The spices used in the study were selected according to their common application: caraway seeds (*Carum carvi)*, bell pepper (*Capsicum annuum*), black pepper (*Piper nigrum),* garlic (*Allium sativum*), Herbes de Provence mixture for the cracker model system and cinnamon (*Cinnamomum zeylanicum*), ginger (*Zingiber officinale*), gingerbread spice, star anise (*Illicium verum*) and vanilla (*Vanilla planifolia*) for the biscuit model system. Herbes de Provence and gingerbread spice were prepared in the laboratory, according to traditional recipes.

The Herbs of Provence mix was composed of thyme (*Thymus vulgaris*), oregano (*Origanum vul-*

gare), basil (*Ocimum basilicum*), rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*), savory (*Satureja hortensis*) and marjoram (*Origanum majorana*) in the proportion of 2:2:2:2:1:1:1 (w/w). The gingerbread spice was composed of cinnamon, ginger, nutmeg (*Myristica fragrans*), allspice (*Pimenta dioica*), clove (*Syzygium aromaticum*), cardamom (*Elettaria cardamomum*), coriander (*Coriandrum sativum*) and black pepper, in the proportion of 30:10:5:5:5:5:1:1 (w/w). All spices were obtained dried from Prymat (Jastrzębie-Zdrój, Poland).

Instrumentation

A gas chromatograph coupled to mass spectrometer (GC-MS) Varian 4000 GC-MS (Agilent Technologies) was used for the determination of 3-MCPD after its derivatization with phenylboronic acid. The injector was a CP-1177 Split/Splitless capillary injector, used at a temperature of 180 °C and an injection volume of 1.0 μl. Each sample was analysed in triplicate. Chromatographic separations were conducted using a DB-5MS column (30 m × 0.25 mm × 0.25 μm; Agilent Technologies). The GC oven was operated with the following temperature program: initial temperature 60 °C (1.0 min) – 6 °C·min^{-1} – 190 °C (1.0 min) – 30 °C·min^{-1} – 280 °C (6.0 min). The analyses were carried out with a solvent delay of 8.0 min. Helium 5.0 (Linde Group, Munich, Germany) was used as the carrier gas at a flow rate of 1.0 ml·min^{-1}. The ion trap mass spectrometer was operated in the internal ionization mode, scan of *m/z* from 45 to 500. The emission current of the ionization filament was set at 10 μA. The trap and the transfer line temperatures were set at 180 °C and 230 °C, respectively. All analyses were conducted in the selected ion monitoring mode (SIM) based on the use of one quantitative ion of PBA derivatives (147 for 3-MCPD and 3-MBPD, 150 for 3-MCPD-d_5), qualitative ions (196 for 3-MCPD, 201 for 3-MCPD-d_5 and 241 for 3-MBPD) and retention times (17.07 min, 17.14 min and 18.29 min for 3-MCPD-d_5, 3-MCPD and 3-MBPD, respectively).

MS1 Minishaker (IKA, Königswinter, Germany) and 350 R centrifuge (MPW, Warsaw, Poland) were employed at sample preparation. Accublock (Labnet, Edison, New Jersey, USA) with nitrogen 5.0 (Linde Group) was used to evaporate the solvent, concentrate the extracts and also to incubate the samples. Spectrophotometric assays were performed using a Cary UV-Vis 50 spectrophotometer (Agilent Technologies). The nitrogen content was determined using TruSpec N (LECO, St. Joseph, Missouri, USA).

Preparation of spice extracts

An amount of 2 g of dried and thoroughly homogenized spice was placed in a 50 ml polypropylene (PP) tube, 10 ml of an extraction solvent (a mixture of ethanol/water, 1:1, v/v) was added, the mixture was vortexed for 1 min and then extracted in an ultrasonic bath for 10 min. The mixture was centrifuged for 15 min at 8 700 ×*g*. After that, the supernatant was transferred to another PP tube, and the spice residues were re-suspended in another portion of the fresh extraction solvent. This step was repeated four times, finally giving 50 ml for each spice extract. Three independent extractions were prepared for each spice. The extracts were kept at –20 °C prior to further use.

Determination of total phenolic content

The content of total phenolic compounds was determined according to Przygodzka et al. [15]. A volume of 0.125 ml of the extract was mixed with 4.125 ml of water and 0.5 ml of Folin-Ciocalteu reagent (previously diluted with water, 1:1, v/v). The mixture was incubated at room temperature for 25 min. Then, 0.5 ml of saturated sodium carbonate (Na_2CO_3) was added to the mixture and it was left to stand for 30 min. After centrifugation (15 min at 10 000 ×*g*), absorbance of the supernatant was measured at 725 nm using a spectrophotometer. Total phenolic content (*TPC*) was standardized against gallic acid and expressed as grams of gallic acid equivalents (GAE) per kilogram of sample. The range of the calibration curve was 0–0.5 g GAE per a litre of the standard solution (r = 0.99). All samples were analysed in triplicate.

Determination of antioxidant capacity

The antioxidant capacity was estimated using a modified method described by Przygodzka et al. [15]. The stock ABTS solution was diluted with the extraction solvent to the absorbance level of 0.72 at 734 nm. A volume of 10 μl of an extract (diluted previously with the extraction solvent, 1:4, v/v) or Trolox solution were mixed with 2.99 ml of the ABTS solution, and the mixture was incubated at 30 °C for 6 min. After that, the absorbance of the solutions was measured at 734 nm. The calibration curve (r = 0.99) was plotted based on the length of the lag phase versus Trolox concentrations within the range of 0–0.5 mmol·l^{-1} of Trolox standard solutions. The antioxidant capacity was expressed as moles of Trolox per kilogram. All samples were analysed in triplicate.

Determination of lipase activity

Determination of the lipase activity of spice extracts was performed according to the proce-

Tab. 1. Composition of model systems.

	Crackers	Biscuits
General composition of cereal products		
Fat [g·kg^{-1}]	250	250
Monosaccharides [g·kg^{-1}]	70	280
Salt [g·kg^{-1}]	24	8
Water [g·kg^{-1}]	35	35
Other ingredients (proteins, starch, fibre) [g·kg^{-1}]	621	427
Simplified composition of cereal models		
Triolein [g·kg^{-1}]	250	250
Glucose [g·kg^{-1}]	70	280
Salt [g·kg^{-1}]	24	8
Water [g·kg^{-1}]	35	35
Paraffin [g·kg^{-1}]	521	327
Tween 80 [g·kg^{-1}]	100	100

dure described by PODSĘDEK et al. [16] with slight modifications. A volume of 200 μl of the spice extract (diluted previously with water to approx. 200 μg·ml^{-1}) was added to 200 μl of the porcine pancreas lipase (PPL) solution, and the mixture was incubated at 37 °C for 15 min. Then, 200 μl of 10 mmol·l^{-1} *p*-nitrophenyl acetate solution was added and the mixture was incubated at 37 °C for further 15 min. Thereafter, 2.4 ml of 0.1 mol·l^{-1} phosphate buffer (pH 7.2) was added to the sample (marked as A_s) and its absorbance at 405 nm was measured immediately. The positive control sample (marked as A_{c1}) was prepared by the addition of 200 μl of the extractant solvent (diluted similarly as the spice extracts) to 200 μl of the lipase solution. In the first negative control sample (marked as A_{c2}), the PPL solution was replaced with a phosphate buffer solution, while in the second negative control sample (marked as A_{c3}), the PPL solution was replaced with a phosphate buffer solution and the extract was replaced with 200 μl of the diluted extractant solvent. The results were expressed as a percentage of inhibition (I) of the lipase activity according to the following formula:

$$I = \left[1 - \frac{(A_s - A_{c2})}{(A_{c1} - A_{c3})}\right] \times 100 \quad (1)$$

where A_s, A_{c1}, A_{c2} and A_{c3} were absorbances of individual solutions.

Protein content

Determination of nitrogen was done by the Dumas method (PN-EN ISO 16634-1:2008E, [17]) and, from this value, protein content was calculated (using a coefficient of 5.8).

Scheme of the experiment

Six groups of samples were prepared with 5 replicates of each, for both biscuit and cracker model system, with the addition of appropriate spice extracts. The controls have remained without the addition of spice extracts.

The composition of the model systems was based on typical ingredients of biscuits and crackers. Fat was replaced with triolein, sugar with glucose and the other ingredients, which usually do not substantially participate in the formation of 3-MCPD, were replaced with liquid paraffin (Tab. 1). An inert emulsifier was added to the ingredients to make a dispersed system of these immiscible substances.

A volume of 625 μl of spice extract (which represented 2.5%, w/w of the total sample) was placed in a 7 ml glass vial and evaporated to dryness under a stream of nitrogen. Then, an appropriate amount of other reagents (Tab. 1) was added to the vial. The vials were sealed with screw caps, vortexed for 1 min and heated (without screw caps) in an oven at 200 °C for 10 min. After heating, the vials were capped and cooled to room temperature.

3-MCPD determination

A volume of 100 μl of the surrogate standard solution was added to each vial. Afterwards, 3.34 ml of acetonitrile was added to the vial, the content was vortexed for 1 min and the vial content was transferred to a 50 ml PP tube. The vial was rinsed again with two portions of acetonitrile, 3.34 ml each and additionally with two portions (2.5 ml each) of water. The whole acetonitrile-water extract was collected in a 50 ml PP tube. Next steps of the analytical process, including clean-up step, derivatization with PBA and the final analysis by GC-MS, were performed according to the procedure that was previously developed and validated in our laboratory [18].

Statistical analysis

The effect of the spice extract addition on 3-MCPD formation was evaluated by a one way variance analysis (ANOVA). P values lower than or equal to 0.05 were considered significant. Intergroup differences were defined by the multiple comparison method of Tukey. All statistical analyses were performed using the Statistica 12.0 software (StatSoft, Tulsa, Oklahoma, USA). The correlation analysis between antioxidants capacity, *TPC*, lipase activity and the 3-MCPD content

was performed by calculation of the determination coefficient R^2 (square of the Pearson correlation coefficient).

RESULTS AND DISCUSSION

The main goal of this research was to verify whether the bioactive compounds present in spice extracts entailed the increase or decrease of the 3-MCPD content. Another question was if the spice extracts could have the ability to inhibit or promote the lipase activity and thus contribute to 3-MCPD formation. Hence, *TPC*, antioxidant capacity and lipase inhibition/activation by tested spice extracts were investigated. The correlation between these factors and 3-MCPD content were estimated using the tools of statistical analysis.

Characterization of spices

All extracts, except for black pepper, were characterized by high content of polyphenols, above 180 g·kg^{-1} (expressed as GAE), while the antioxidant capacity (expressed as moles of Trolox) was more diversified (Tab. 2). The highest value was found for cinnamon and gingerbread spice extracts (21.5 mol·kg^{-1} and 24.0 mol·kg^{-1}, respectively), and the lowest one for the garlic extract – 1.5 mol·kg^{-1}. All investigated spice extracts, with the exception of cinnamon, demonstrated the ability to increase the lipase activity. Among them, the extracts of garlic, black pepper and Herbes de Provence showed the greatest activation of lipase.

3-MCPD formation in heat-processed spice extracts

To verify if 3-MCPD could be formed in spice extracts without the addition of other components, 625 μl of each spice extract was heated at 200 °C for 10 min. The results showed that, for most spices, 3-MCPD was not formed during heating, with the exception of garlic, ginger, star anise and gingerbread spice extracts (Tab. 3). Nonetheless, the level of 3-MCPD was relatively low and ranged from 10.7 μg·kg^{-1} to 12.5 μg·kg^{-1}. As for garlic, 3-MCPD was synthesized probably from allyl alcohol (prop-2-en-1-ol), a product of thermal decomposition of alliin, (S)-allyl-l-cystein sulphoxide, a cysteine amino acid present in garlic, and from the traces of chloride ions present e.g. in water [19]. In case of three other spices (ginger, star anise and gingerbread spice), the formation of 3-MCPD during heat treatment might be further explained by the presence therein of trace amounts of fat derived from residual spice oils that could also react with the chloride ions present in water or in spices.

In another experiment, the spice extracts were heated with the addition of NaCl (24 mg in case of the cracker model system and 8 mg in case of the biscuit model system), to confirm if the substances present in the spice extracts would react with chloride ions. 3-MCPD was detected in garlic, ginger, star anise, gingerbread spice and vanilla extracts (Tab. 3). In case of ginger and star anise, the results were comparable with those obtained for the spice extracts that had been heated without

Tab. 2. Total polyphenol content, antioxidant capacity and lipase inhibition capacity of spices.

Spices	Total polyphenol content [g·kg^{-1}]	Antioxidant capacity [mol·kg^{-1}]	Lipase inhibition [%]
Cracker model system			
Caraway seeds	227 ± 6	2.2 ± 0.2	–17.2 ± 0.7
Bell pepper	343 ± 17	3.8 ± 0.4	–9.2 ± 0.5
Black pepper	84 ± 7	7.3 ± 0.5	–20.5 ± 0.3
Garlic	184 ± 6	1.5 ± 0.5	–23.5 ± 0.1
Herbs of Provence	334 ± 9	19.3 ± 1.1	–20.1 ± 0.7
Biscuit model system			
Cinnamon	285 ± 7	21.5 ± 1.8	2.1 ± 0.2
Ginger	264 ± 17	8.8 ± 0.7	–4.1 ± 0.1
Gingerbread spice	356 ± 11	24.0 ± 1.1	–6.5 ± 0.2
Star anise	343 ± 14	18.4 ± 0.9	–8.9 ± 0.4
Vanilla	297 ± 9	9.6 ± 0.9	–3.4 ± 0.2

Results are mean ± standard deviation of 3 determinations. Total polyphenol content is expressed as grams of gallic acid equivalents. Antioxidant capacity is expressed as moles of Trolox. Negative values of lipase inhibition indicate increase in lipase activity.

the NaCl addition. Hence, for these spices, the addition of NaCl had no effect on formation of 3-MCPD. The highest contents of 3-MCPD were found for the garlic and gingerbread spice extracts (27.8 $\mu g \cdot kg^{-1}$ and 22.5 $\mu g \cdot kg^{-1}$, respectively), which were two times higher than in the extracts heated without NaCl. Based on this, it can be assumed that the addition of salt is a significant factor contributing to 3-MCPD formation in foods containing garlic and should be strictly controlled. The higher level of 3-MCPD formed in gingerbread spice extract heated in the presence of chloride ions arose probably from the components of this spice mixture. As cinnamon did not influence 3-MCPD formation and the addition of NaCl had no effect on formation of 3-MCPD in star anise and ginger, it can be supposed that some other compounds present in the rest of spice mixture components (e.g. nutmeg, allspice or clove) reacted with chloride ions leading to the increase of 3-MCPD level.

3-MCPD formation in cereal model systems

The content of 3-MCPD formed during heating of samples is presented in Tab. 3. The results obtained in the experiment varied and depended on the content of salt and on the spices added to the samples. 3-MCPD content in the control group of the cracker model system reached 118.1 $\mu g \cdot kg^{-1}$. In the samples with the addition of bell pepper, black pepper and Herbes de Provence, the content of 3-MCPD remained at a level similar to the control group. In the samples with garlic addition, the 3-MCPD level was by about 17% higher than the control one, whereas the samples with the caraway seeds addition had the lowest 3-MCPD content (101.6 $\mu g \cdot kg^{-1}$). One-way analysis of variance and Tukey's post-hoc test showed significant intergroup differences between sample 4 (with garlic addition) and the rest of samples, and between sample 1 (control) and sample 3 (with caraway seeds).

In case of the biscuit model system, the 3-MCPD amounts formed during heating were generally lower than those observed for crackers. 3-MCPD content in the control samples was found to be 65.7 $\mu g \cdot kg^{-1}$. A similar value was observed only for the samples with cinnamon extract addition (63.3 $\mu g \cdot kg^{-1}$), while for the rest of the samples, 3-MCPD content was significantly higher, in particular in the samples containing star anise and gingerbread spice extracts (108.1 $\mu g \cdot kg^{-1}$ and 100.5 $\mu g \cdot kg^{-1}$, respectively). The ANOVA analysis with Tukey's post-hoc test confirmed these observations, the significant intergroup differences ($p < 0.05$) being observed for almost all samples except for the differences between samples 7, 8 (control, cinnamon), 10, 11 (gingerbread spice, star anise) and 9, 12 (ginger, vanilla).

The findings showed that amounts of 3-MCPD formed during heat treatment depended on the composition of model systems, mostly on the con-

Tab. 3. 3-Monochloropropane-1,2-diol content in model systems after heat treatment.

Model systems No.	Spice added	3-MCPD content in samples [$\mu g \cdot kg^{-1}$]		
		Spice extracts	Spice extracts with NaCl addition	Models with spice extract addition
Cracker model system				
1	Control	nd	nd	118.1 ± 9.3
2	Bell pepper	nd	nd	112.1 ± 8.2
3	Black pepper	nd	nd	116.8 ± 9.2
4	Caraway seeds	nd	nd	101.6 ± 7.6
5	Garlic	12.5 ± 0.3	27.8 ± 1.8	140.5 ± 9.6
6	Herbs of Provence	nd	nd	110.6 ± 6.1
Biscuit model system				
7	Control	nd	nd	65.7 ± 2.6
8	Cinnamon	nd	nd	63.3 ± 3.9
9	Ginger	11.4 ± 0.7	17.5 ± 1.2	88.2 ± 5.0
10	Gingerbread spice	12.4 ± 0.8	22.4 ± 1.4	100.5 ± 6.1
11	Star anise	10.7 ± 0.8	11.2 ± 1.2	108.1 ± 9.9
12	Vanilla	nd	10.5 ± 0.9	78.0 ± 5.2

Results are mean ± standard deviation of 3 determinations. 3-MCPD – 3-monochloropropane-1,2-diol, nd – not detected.

Tab. 4. Correlations between 3-monochloropropane-1,2-diol content and total polyphenol content, antioxidant capacity and lipase inhibition capacity of spices.

	Coefficient of determination R^2		
	3-MCPD / Total polyphenol content	3-MCPD / Antioxidant capacity	3-MCPD / Lipase inhibition capacity
Cracker model system	0.36	0.08	0.06
Biscuit model system	0.32	0.20	0.96

3-MCPD – 3-monochloropropane-1,2-diol.

tent of salt (chloride ions) and on the added spice extracts. Addition of a greater quantity of NaCl generated a greater amount of 3-MCPD, which is in concordance with data previously reported by other authors [8, 19].

Influence of spices on 3-MCPD formation in cereal models

In order to estimate the potential impact of *TPC*, antioxidant capacity and lipase activity on the amount of 3-MCPD formed, coefficient of determination (R^2) was calculated for each pair of factors.

Biscuit model system

In case of the biscuit system, a strong correlation (R^2 = 0.96) between the 3-MCPD level and the ability to activate lipase was observed. This means that the 96% increase in the 3-MCPD content resulted probably from the addition of those spice extracts, which activated lipase. This phenomenon can be explained as follows: the lipase-activating extracts enhanced the lipase activity to decompose triolein, leading to release of a higher amount of glycerol, which is a main precursor of 3-MCPD. The increase in lipase activity by star anise and ginger extracts and no effect of cinnamon extract had been already reported by other authors [3, 14, 20]. The lipase activity increase has presumably arisen from the active compounds present in the spices, e.g. 6-gingerol or 6-shoagol [1] but it could also be explained by the presence of natural lipase in certain spices. For instance, according to Halpern and Weverka [21], ginger could contain lipase. Therefore, it is thought that other spices with observed lipase activation could also contain some amounts of lipases, as this enzyme is often found in plants with higher levels of oils [22–24]. Nonetheless, these suggestions have not been confirmed by other authors yet.

Another interesting phenomenon discovered in this experiment was that water-ethanol extracts still contained residual lipase or showed an increase of the lipase activity (Tab. 2). This observation contradicts the results of previous research [25] that had recommended the use of spice extracts, instead of native spices, to avoid lipase activity. This observation is extremely important especially for processing or storage of foods with the addition of spice extracts, because the residual lipase may cause an increase of 3-MCPD levels and also deteriorate the quality of these products.

Cracker model system

No correlations between lipase activity and 3-MCPD content were observed for the cracker model system (Tab. 4). Hence, the increase in the content of 3-MCPD in the samples with the garlic extract addition, as well as a lower 3-MCPD amount in the samples with the caraway seeds extract, were caused by other factors, probably by the substances present in the spices. Thus, investigation of the composition of spices with special attention to the protein content was performed. The higher level of 3-MCPD in the model system with the addition of garlic extracts was the effect of allyl alcohol, which has been already discussed. Caraway seeds, compared to other spices used in this study, contained the highest content of proteins, 186 $g \cdot kg^{-1}$ of fresh product, on the contrary to other savoury spices (garlic – 143 $g \cdot kg^{-1}$; Herbes de Provence – 148 $g \cdot kg^{-1}$; bell pepper – 143 $g \cdot kg^{-1}$; black pepper – 103 $g \cdot kg^{-1}$). It appeared that ammonia, produced by decomposition of proteins, probably reacted with 3-MCPD already formed or prevented its formation. The determination coefficient calculated for the protein content and the content of 3-MCPD was 0.06 but, when garlic was excluded, it increased to 0.68. This indicates that the higher content of proteins might inhibit formation of 3-MCPD in the salty model system.

CONCLUSIONS

The relationship between the spice extract addition and 3-MCPD formation in cereal model systems during their thermal processing has been in-

vestigated. In the salty model system, the presence of amino acids in added spices was a crucial factor affecting 3-MCPD generation. Consequently, the addition of protein-rich additives to bakery products prior to its heat treatment may possibly prevent the generation of 3-MCPD. On the contrary, the study showed that the addition of garlic may lead to the increase of 3-MCPD level. This suggests that heating of garlic or its extract may be another source of 3-MCPD in the diet. In case of the sweet model system, the increase of lipase activity by almost all spices (except cinnamon) was the most important parameter increasing 3-MCPD levels. Hence, the addition of some spices, such as ginger or star anise, to bakery products may probably result in the increase of the 3-MCPD amount generated under thermal food processing. The study also showed that the preparation of water-ethanol spice extracts and its subsequent heat treatment were probably not effective enough to inhibit the lipase activity. This phenomenon appears to be highly relevant in case of thermal processing or storage of products containing extracts of spices, because this may contribute to an increased 3-MCPD generation and also may deteriorate the product quality through degradation of lipids.

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