Mitigation role of erythritol and xylitol in the formation of 3-monochloropropane-1,2-diol and its esters in glycerol and shortbread model systems

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Abstract The effect of the addition of six sweeteners (erythritol, xylitol, maltitol, sucrose, steviol sweetener, and stevia leaves) on the formation of 3-monochloropropane-1,2-diol (3-MCPD) and 3-MCPD esters in glycerol and shortbread model systems subjected to heat treatment was investigated. The highest level of free 3-MCPD was reached for the glycerol model system with the addition of steviol sweetener ($114.8 \mu\text{g kg}^{-1}$), in contrast to the model with stevia leaves ($51.9 \mu\text{g kg}^{-1}$). The level of free 3-MCPD in the shortbread model was roughly similar, whilst the levels of esterified 3-MCPD were about 12 times higher. The maximum content was obtained in the model with sucrose ($1112 \mu\text{g kg}^{-1}$) and the lowest with erythritol ($676.4 \mu\text{g kg}^{-1}$). In the glycerol model system, 3-MCPD esters were not detected at all. The experiment revealed that acidic conditions, generated mainly by thermal decomposition of glucose, were a key factor promoting the formation of free and bound 3-MCPD, while in the case of the application of polyols, especially erythritol or xylitol, the chloropropanol production was significantly lower. The experiment also showed that antioxidant capacity of products formed under heat treatment ($0.53\text{--}1.03 \text{ mol Trolox kg}^{-1}$) might have some significance in 3-MCPD formation. The findings of the study suggest that erythritol and xylitol added instead of sugar to shortbread can be effective agents in the mitigation of free and bound 3-MCPD formation. However, we also revealed that the application of sweeteners based on maltodextrin or pure stevia extract

did not show any inhibitory effect, and in some cases led to the increase in the 3-MCPD generation, so their use in the bakery industry should be reconsidered.

Keywords 3-monochloropropane-1,2-diol · 3-MCPD esters · Thermal processing · pH · Antioxidants · Sweeteners

Introduction

Because of their sensory qualities, pastry goods such as shortbread are very popular all over the world and are an important segment of the global confectionery market. The basic ingredients of standard shortbread are flour, fat (up to 25–30%), sugar, eggs, and flavourings [1, 2]. During baking at a temperature of at least 200 °C, all these ingredients interact with each other to give the essential attributes of shortbread, such as surface colour, texture, and flavour, which are the main features that influence a consumer's preference for bakery products [3]. The chemical reactions that are responsible for these features are complex and interrelated, but the most important are: lipid decomposition (hydrolysis and oxidation), Maillard reaction, and caramelisation. Lipid hydrolysis often requires the presence of specific enzymes such lipase, which is present, e.g., in flour; lipid oxidation is a radical chain reaction leading to the decomposition of fat. Both processes occur in a wide range of temperatures. The Maillard reaction usually occurs at 140–160 °C between carbonyl groups of reducing sugars and the $-\text{NH}_2$ functions of amino acids, peptides, and proteins; caramelisation involves direct degradation of sugars and takes place at higher temperatures (above 170 °C) [4–6]. However, some of these processes can also result in the formation of harmful components such as furans,

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acrylamide, polycyclic aromatic hydrocarbons, and finally, chloropropanols, mainly 3-monochloropropane-1,2-diol (3-MCPD) and its esters [3, 7–9].

In shortbread, free 3-MCPD is formed mostly upon reactions of sodium chloride (present naturally or added; up to $1.3 \text{ g } 100 \text{ g}^{-1}$ [2]) with glycerol, lipids, and carbohydrates [10, 11]. Apart from the influence of the amount of NaCl, sugar is also considered to promote the production of 3-MCPD through lowering the pH level by organic acids formed upon thermal decomposition of glucose [12, 13]. 3-MCPD can occur also in bound form with higher fatty acids (3-chloropropane-1,2-dipalmitate, 1,3-chloropropanediol-1,2-dipalmitate, 2-chloropropanediol-1,3-distearate, and 3-chloropropane-1,2-dioleate). The content of 3-MCPD esters usually significantly exceeds that of free 3-MCPD; in shortbread, the level of free 3-MCPD is about $70 \text{ } \mu\text{g kg}^{-1}$, while 3-MCPD esters can reach even $632 \text{ } \mu\text{g kg}^{-1}$ [14]. 3-MCPD esters can be present in fats, oils, or margarine used for shortcrust pastry production [15–18], but they might also be generated upon heat treatment as the effect of the reaction between triacylglycerols (TAG)/diacylglycerols (DAG)/monoacylglycerols (MAG) and glycerol and chloride ions originating from NaCl added or residual (natural) salt present in raw materials [18]. Although a detailed mechanism of these reactions has not been yet clearly explained, five routes of 3-MCPD ester formation have already been proposed. Two of them involved a chlorine anion directly substituted to either a hydroxyl group or a fatty acid ester group at a glycerol carbon atom. Another involved the formation of an epoxide ring along with nucleophilic attack by a chloride anion to open the ring. The others postulated the formation of an intermediate acyloxonium cation followed by a cation ring opened by the nucleophilic attack of a chloride anion. It has been demonstrated that all these mechanisms require an acidic environment to perform 3-MCPD ester formation. Another proposed mechanism included the formation of a cyclic acyloxonium free radical intermediate, followed by its reaction with a chlorine radical or chlorinated compound [19]. However, all routes and the factors affecting the generation of 3-MCPD esters were postulated only in the case of 3-MCPD ester formation during deodorization of refining oils.

Free 3-MCPD and its esters, which can release free 3-MCPD in the body, are considered as probably or potentially carcinogenic to humans [17], so great attention is paid to ways of eliminating them from food. Therefore, an urgent area of interest is the possibility of inhibiting the formation of these compounds through the modification of shortbread dough ingredients. This can be achieved, e.g., by sugar replacement with other sweeteners. This approach is also highly anticipated, since consumers have become much more concerned about health

issues and demand natural food products that confer health benefits, such as low sugar and calories. In addition, the use of sweeteners instead of sugar in bakery products is the only solution for people with diabetes.

Sugar substitutes involve two groups: energy-free high intensity artificial sweetening agents and natural carbohydrate-based reduced energy sweeteners, such as polyols (sugar alcohols), or stevia leaves [20, 21]. Polyols are naturally present in smaller quantities in fruits and in certain kinds of vegetables or mushrooms; they are regulated as generally recognised as safe, or as food additives. Among polyols, erythritol ((2S,3R)-butane-1,2,3,4-tetrol), xylitol (2S,4R)-pentane-1,2,3,4,5-pentol), and maltitol ((2S,3R,4R,5R)-4-[(2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxyhexane-1,2,3,5,6-pentol) are the most recognised polyols used in bakery industry [21, 22]. They provide good stability during baking with acceptable textural and sensory properties, low glycemic index [23, 24], and their sweetness is roughly comparable with this obtained from sugar (erythritol: 70%, xylitol: 100%, maltitol: 90% of the sucrose sweetness [24]). Stevia (*Stevia rebaudiana*) is a small, herbaceous shrub of the *Asteraceae* family. Stevia leaves contain a complex mixture of sweet diterpene glycosides, including stevioside, steviolbiosides, rebaudiosides (A, B, C, D, E, and F), and dulcoside A [25]. Dry leaves of stevia are sweeter approximately 10–15 times than sucrose [26]. The use of sweeteners containing stevia or steviol glycosides is recommended for diabetics and obese persons, as they are non-toxic and non-addictive, and can be cooked or baked [27]. However, very little attention has been directed towards the safety of these sweeteners in bakery products and their role in the formation of heat-induced compounds. It has been reported that substituting sucrose with stevia decreased the acrylamide level eight times. Replacing reducing saccharides with polyols in the dough formulation led to a decrease in the extent of browning reactions, because the formation of HMF was limited during the baking process. Similar behaviour has been described for acrylamide [28, 29]. Meanwhile, there are no data in recent literature on the influence of sugar replacers on the formation of 3-MCPD and its esters. For this reason, the main goal of this work was the assessment of the impact of natural sugar substitutes on the formation of 3-MCPD and its esters in model systems simulating shortbread dough, composed of triolein, wheat flour, sweetener (erythritol/xylitol/maltitol/sucrose/steviol sweetener/stevia leaves), water, and salt. In addition, to investigate the changes in the level of free and bound 3-MCPD, pH values and antioxidants capacity of model systems after its heat treatment were determined. The correlations between these factors and free and bound 3-MCPD content were estimated using statistical analysis

tools. The impact of various sweeteners on 3-MCPD ester formation is discussed and some explanatory hypotheses are considered.

Materials and methods

Chemicals and materials

Hexane and acetonitrile were purchased from Merck (Germany). Methanol, tetrahydrofuran, acetone, sulphuric acid (98%), sodium chloride, and sodium hydrogen carbonate were purchased from Chempur (Poland). Solid phase extraction (SPE) bulk sorbents: primary secondary amine (PSA) and octadecyl (C_{18}) were purchased from Agilent Technologies (USA). 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), potassium persulfonate, glyceryl trioleate (triolein), Tween 80, 3-monochloropropane-1,2-diol (3-MCPD), 3-monochloropropane-1,2-diol- d_5 (3-MCPD- d_5) (surrogate standard), and phenylboronic acid (PBA) (derivatisation agent) were obtained from Sigma-Aldrich (USA). Leco Dry (infusorial soil) was from Leco (USA). All reagents were at least of analytical purity. Commercially available sugar alternatives erythritol (99%), xylitol (99.5%), maltitol (98%), and dried stevia leaves (100%) were supplied by Intenson, Poland; steviol sweetener (maltodextrin–97.7%, steviol glycosides 2.3%) was purchased from Domos, Poland. According to the manufacturers, all sweeteners were suitable for baking. Sucrose and wheat flour (type 550) were obtained from local market. Sodium chloride solution of 200 mg mL^{-1} (20%) and saturated solution of sodium hydrogen carbonate were prepared in deionised water. Intermediate and working standard solutions of chloropropanols at a concentration of 2 $\mu g mL^{-1}$ were prepared in a 20% 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).

Instrumentation

Gas chromatography coupled to mass spectrometry (GC–MS) Varian 4000 GC–MS (Agilent Technologies) was applied for the determination of 3-MCPD after its derivatisation with phenylboronic acid. The injector was a CP-1177 Split/Splitless Capillary Injector with a temperature of 180 °C and an injection volume of 1.0 μL (splitless mode). Each injection was performed in triplicate. Chromatographic separations were conducted using a DB-5MS column (30 m $\times$ 0.25 mm $\times$ 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, Germany) was used as the GC carrier gas at a flow rate of 1.0 $mL min^{-1}$. The ion trap mass spectrometer was operated in internal ionisation mode, scanning from m/z 45 to 500. The emission current of the ionisation filament was set at 10 μA . The trap and the transfer line temperatures were set at 180 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 150 for 3-MCPD- d_5), qualitative ions (196 for 3-MCPD, 201 for 3-MCPD- d_5), and retention times (17.07 min for 3-MCPD- d_5 and 17.14 min for 3-MCPD, respectively).

MS1 Minishaker (IKA, Germany) and MPW 350 R Centrifuge (MPW Med. Instruments, Poland) were employed during the sample preparation. Accublock (Labnet, USA) with nitrogen 5.0 (Linde Group) was used to evaporate the solvent, concentrate the extracts, and to incubate the samples. Spectrophotometric assays were performed using a Cary UV–Vis 50 spectrophotometer (Agilent Technologies). Fat extraction and determination was conducted with the use of TFE 2000 (Leco) using CO_2 with a purity of 4.5. The temperature of the sample was 100 °C, CO_2 pressure of about 62 MPa, flow rate of 2 $L min^{-1}$ (calculated on CO_2 after decompression), and the static and dynamic extraction times were 15 and 35 min.

Fat extraction

Extraction of fat was performed according to the procedure developed in our laboratory [30]. Briefly, 0.5 g of sample was mixed with about 1 g of Leco Dry and placed in a metal tube for extraction (12 cm length and 10 mm diameter) between two layers of clean Leco Dry. The tube was placed in the TFE 2000 apparatus and fat was extracted using CO_2 . Extracted fat was collected in Eppendorf vials.

Schema of the experiment

Seven groups of model systems were prepared with four replicates of each, with the use of appropriate ingredients (Table 1).

The composition of the shortbread model systems was based on typical ingredients of shortbread, according to a standard shortbread recipe [1] and a chemical shortbread composition [2]. However, to simplify the models, margarine was replaced with triolein, and the other ingredients which usually do not substantially participate in the formation of 3-MCPD (e.g., eggs) were omitted. In sweetener model systems, sucrose was replaced by an appropriate sweetener; control did not contain sweeteners. In case of

Table 1 Composition of model systems

Components	Glycerol model system (mg g ⁻¹)	Shortbread model system (mg g ⁻¹)
Flour	300	300
Triolein	–	250
Glycerol	250	–
Sweetener (erythritol/ maltitol/steviol sweetener/sucrose/xylitol/)	280	280
or Stevia leaves	30	30
Salt	8	8
Water	35	35
Tween 80	–	100

the use of stevia leaves, its amount (Table 1) was adjusted to obtain a comparable sweetness as sugar [26], while for the rest of the sweeteners, their amount was the same as the amount of sucrose. An inert emulsifier (Tween 80) was added to the ingredients to make a dispersed system of these immiscible substances. The mixture also contained water and salt in amounts comparable to real products [2].

To explore the impact of sweetener on the 3-MCPD formation from the basic precursors (glycerol and chloride ions), another model system was incorporated that excluded flour, triolein, and emulsifier, and was composed only of glycerol, sweetener, water, and salt (Table 1). All ingredients were thoroughly mixed in glass vials that were sealed with screws, vortexed for 1 min, and heated in open vials (without screws) in an oven at 200 °C for 10 min. After heating, the vials were cooled to room temperature.

Free 3-MCPD determination

100 µL of the surrogate standard solution was added to each vial. 5 mL of acetonitrile was then added to the vial; the contents were vortexed for 1 min and transferred to a 50 mL PP tube. The vial was rinsed again with another 5 mL of acetonitrile, and additionally with two portions (2.5 mL each) of water. All the acetonitrile–water extract was collected in a 50 mL PP tube. The next steps of the analytical process, including the clean-up step, derivatisation with PBA, and the final determination of GC–MS were performed according to a procedure that had previously been developed and validated in our laboratory [31]. Briefly, 1 g of NaCl was added, and the sample was shaken vigorously and centrifuged. The supernatant was transferred into a 15 mL glass vial and the extracts were kept in freezer for overnight to freeze out the fat. Thereafter, the extract was immediately filtrated to the volume of 6 mL and transferred to the 15 mL PP tube containing 150 mg of PSA and 300 mg of C₁₈ sorbents. The tubes were shaken and centrifuged. A 2 mL amount of the extract was evaporated at

ambient temperature under a stream of N₂ to dryness, the residues were dissolved in 100 µL of 20% NaCl aqueous solution, and 25 µL of PBA solution was added. The mixture was heated at 90 °C for 20 min. After cooling, 0.5 mL of hexane was added, the mixture was shaken vigorously, and 200 µL of upper hexane layer was transferred into an insert in an autosampler vial. The extracts were then analysed by GC–MS.

3-MCPD esters determination

Determination of 3-MCPD esters via acid transesterification was performed according to Ermacora and Hrncirik [32] with some slight modification. Extracted fat was dissolved in two portions of tetrahydrofuran, 0.5 mL each, transferred to a 4 mL glass vial, and 1.8 mL of sulphuric acid solution in methanol (1.8%, v/v) was added to the sample. The mixture was incubated at 40 °C for 20 h. The reaction was stopped by addition of 0.5 mL saturated sodium hydrogen carbonate solution and the organic solvents were evaporated under a nitrogen stream. Fatty acid methyl esters were separated from the sample by addition of 2 mL of aqueous sodium chloride solution (20%, w/v) followed by liquid–liquid extraction with hexane (4 × 1 mL). The released 3-MCPD present in the extract was then derivatised as previously and analysed by GC–MS.

Preparation of sample for the determination of pH value and antioxidant capacity

pH value and antioxidant capacity were assayed in raw materials (without heat treatment) as well as in samples after heat treatment.

Raw materials: 0.4 g of dried and thoroughly milled dried stevia leaves was placed in a 15 mL polypropylene (PP) tube, 2 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 10,000 rpm. The supernatant was then transferred to another PP tube and the residues were re-suspended in another portion of the fresh extraction solvent. This step was repeated four times, finally giving 10 mL of the extract. For the polyols, 0.4 g of each sweetener was dissolved in 5 mL of water and 5 mL of ethanol was added. Three independent extractions/solutions were prepared for each sweetener. The extract of stevia was kept at –20 °C prior to determination of pH and antioxidant capacity.

Samples were subjected to thermal processing: 280 mg of appropriate sweetener (glycerol models systems) or 280 mg of appropriate sweetener with the addition of 300 mg of flour (shortbread model systems) were mixed and heated in 200 °C for 10 min. After cooling, 10 mL of

deionised water was added; the samples were vortexed for 2 min and filtered with a filter paper.

Determination of antioxidant capacity

The antioxidant capacity in prepared extracts was estimated using ABTS. radical cation decolourisation assay that was modified in our laboratory and previously described [33]. The antioxidant capacity was expressed as moles of Trolox per kilogram. All samples were assayed in triplicate.

Statistical analysis

The effect of the use of various sweeteners on 3-MCPD formation was determined by a one-way variance analysis (ANOVA). *P* values of ≤ 0.05 were considered significant. Intergroup differences were defined by Tukey's multiple comparison method. All analyses were performed using Statistica 12.0 software (StatSoft, USA).

The correlation analysis between 3-MCPD content was performed by calculating the determination coefficient R^2 (square of the Pearson correlation coefficient).

Results and discussion

Level of free 3-MCPD and 3-MCPD esters in glycerol model systems

The level of free 3-MCPD in glycerol model systems differed and was affected by the type of sweetener used (Table 2). The highest 3-MCPD level was reached for the model with the addition of steviol sweetener ($114.8 \mu\text{g kg}^{-1}$). The lowest 3-MCPD level ($51.9 \mu\text{g kg}^{-1}$) was found in the model with stevia leaves. The model with the addition of sucrose also demonstrated a high level of

free 3-MCPD ($99.6 \mu\text{g kg}^{-1}$), while its level in the models with the use of polyols was noticeably lower, from $57.3 \mu\text{g kg}^{-1}$ for erythritol to $77.4 \mu\text{g kg}^{-1}$ for maltitol. A post hoc test revealed three homogeneous subsets: (1) stevia leaves, control, erythritol, and xylitol; (2) maltitol; (3) sucrose and steviol sweetener. 3-MCPD esters were not detected in any of the models, due to the absence of added fat.

Level of free 3-MCPD and 3-MCPD esters in shortbread model systems

The level of free 3-MCPD in the model with the addition of triolein was roughly comparable to those received for the glycerol models (the same order of magnitude, Table 2), while for the models containing steviol sweetener and sucrose, the presence of fat resulted in a decrease of free 3-MCPD content of about 40%. The lowest level of free 3-MCPD was observed for control samples ($55.5 \mu\text{g kg}^{-1}$), but the model system with the addition of erythritol still exhibited the lowest content of free 3-MCPD among sweetener model systems ($62.4 \mu\text{g kg}^{-1}$), whereas the addition of stevia leaves induced the highest result ($75.3 \mu\text{g kg}^{-1}$). The latter result was significantly different from the rest of the models. Significant differences were observed between the models of control and sucrose, and control and stevia leaves. The level of 3-MCPD was comparable in the model systems with the addition of xylitol, and maltitol. For the other samples, statistical analysis did not show any significant differences.

The levels of 3-MCPD esters in the shortbread model system expressed as 3-MCPD were substantially higher (10–16 times) compared to free 3-MCPD. The maximum content was obtained in the model containing sucrose ($1112.4 \mu\text{g kg}^{-1}$) and in the model with steviol sweetener ($1036.2 \mu\text{g kg}^{-1}$). The lowest ester content was discovered

Table 2 Content of free 3-MCPD and 3-MCPD esters (expressed as free 3-MCPD) in investigated model systems

Sweetener	Glycerol model system		Shortbread model system	
	3-MCPD content ($\mu\text{g kg}^{-1}$)	3-MCPD esters content ($\mu\text{g kg}^{-1}$)	3-MCPD content ($\mu\text{g kg}^{-1}$)	3-MCPD esters content ($\mu\text{g kg}^{-1}$)
Control	54.8 ± 3.1^a	n.d.	55.5 ± 2.8^a	552 ± 13^a
Erythritol	57.3 ± 1.8^a	n.d.	$62.4 \pm 1.9^{a,b}$	676 ± 12^b
Maltitol	77.4 ± 2.1^b	n.d.	$65.5 \pm 2.3^{a,b}$	$859 \pm 35^{b,c}$
Steviol sweetener	114.8 ± 4.7^c	n.d.	$70.5 \pm 2.6^{a,b}$	1036 ± 17^d
Stevia leaves	51.9 ± 0.9^a	n.d.	75.3 ± 3.2^b	$789 \pm 36^{b,c}$
Sucrose	99.6 ± 3.3^c	n.d.	72.9 ± 1.4^b	1112 ± 13^d
Xylitol	63.0 ± 1.1^a	n.d.	$65.0 \pm 1.7^{a,b}$	$725 \pm 41^{b,c}$

Mean values with different letters in a column differ significantly ($P < 0.05$)

n.d. not detected; (free 3-MCPD: limit of detection, LOD = $2.8 \mu\text{g kg}^{-1}$, limit of quantification, LOQ = $8.4 \mu\text{g kg}^{-1}$; bound 3-MCPD: LOD = $6 \mu\text{g kg}^{-1}$ expressed in fat basis, LOQ = $18 \mu\text{g kg}^{-1}$ expressed in fat basis)

once again in the control model and the model containing erythritol (552 and 676.4 $\mu\text{g kg}^{-1}$, respectively). Models including xylitol, maltitol, and stevia leaves behaved similarly and did not differ significantly (post hoc Tukey test results).

Effect of sweetener on free 3-MCPD content

Comparing the results obtained for both glycerol and shortbread systems, it seemed that some of the sweeteners influenced greatly the level of free 3-MCPD. This phenomenon was particularly noticeable for the model involving sucrose and polyols, especially erythritol. Other polyols, xylitol and maltitol, also had an impact on 3-MCPD formation; however, the effect was not as noticeable as for erythritol. An interesting occurrence was observed for steviol sweetener and stevia leaves, which, in some cases, enhanced 3-MCPD level in the investigated model systems.

In general, a possible explanation for the impact of sweetener on free or bound 3-MCPD formation arose from the mechanism of 3-MCPD formation. The higher level of 3-MCPD in glycerol model systems with the addition of sucrose was consistent with the previous studies [12, 13] and was simply caused by the decomposition of sugar with the generation of organic acids (lactic acid, levulinic acid, and formic acid [34]) that lowered pH. Low pH values are a valuable factor contributing to formation of 3-MCPD from chloride ions and glycerol. However, a comparable

high content of 3-MCPD was also detected in models with the use of steviol sweetener. For this reason, the pH value of the investigated sweeteners was measured after thermal processing (Fig. 1a). As can be observed, the lowest pH values were obtained for steviol sweetener (4.88), while the highest was for erythritol (5.95). The rest of the sweeteners were characterised by pH values in the range 5.49–5.89. Among polyols, pH values decreased with the increase of the molecular mass and the number of carbon atoms. The only exception in the pH values was the stevia leaves; however, the amount of stevia leaves used in the model systems was lower.

In the case of the shortbread model systems, a similar correlation was observed, including the outliers for stevia leaves (Fig. 1b). For both systems, when excluding the values for stevia leaves, the coefficient of determination between 3-MCPD content and pH of sweeteners was equal to 92 and 85% for the glycerol and shortbread models, respectively. This confirmed that pH value had a strong impact on free 3-MCPD generation in the model systems.

Higher pH values were noticed for polyols, which can be attributed to their thermal stability. Polyols, in contrast to sucrose, do not decompose at higher temperatures and do not produce organic acids. Erythritol has excellent thermal stability even above 180 °C; xylitol is stable at temperatures up to its boiling point (216 °C) [35]. Heating of maltitol, which is hydrogenated disaccharide, leads to its cleavage into sorbitol and glucose [24], which can be

Fig. 1 **a** Influence of pH values on the content of free 3-MCPD in glycerol model systems; **b** Influence of pH values on the content of free 3-MCPD in shortbread model systems; **c** Influence of pH values on the content of esterified 3-MCPD in shortbread model systems; **d** Influence of antioxidant capacity on the content of esterified 3-MCPD in shortbread model systems

further decomposed, producing organic acids. Therefore, a lowered pH value is achieved that is in line with our observations. pH of steviol sweetener resulted from the presence of maltodextrin (polysaccharide), which was a basic ingredient of this sweetener. Excessive heating of such starch-derived sweeteners gives rise to a partial release a free glucose, anhydrosugars, and fructose-containing sugars [36] that can be further decomposed with the production of organic acids and lowering the pH level.

An interesting occurrence is a high level of 3-MCPD obtained in the shortbread model system incorporating stevia leaves. Data in the literature on the thermal stability of stevia during baking are generally conflicting and unclear. On one hand, it has been reported that the leaves, as well as the pure stevioside extracts, could be used in their natural state or cooked, and are thermostable at temperatures up to 200 °C. On the other hand, at temperatures exceeding 140 °C, forced decomposition was seen which resulted in total decomposition at 200 °C. Degradation products of stevioside included, e.g., traces of steviolbioside and glucose, which could be attributed to the rupture of the C19 ester bond in stevioside [37, 38]. Hence, it can be assumed that the traces of glucose could be attributed to the 3-MCPD formation through the lower pH level. Nonetheless, the amount of stevia leaves extract added to the model system was low, so the amount of glucose that could be released upon heating would be low as well. Furthermore, pH values of the stevia leaf extract were similar to maltitol; however, the corresponding free 3-MCPD levels varied.

Another question was whether the polyols could interact with other shortbread batter components, such as amino acids contained in flour. It is commonly known that reducing sugars or monomers that are generated from polymetric sugars can react with amino compounds in the Maillard reaction. This mechanism can also explain why glucose promotes 3-MCPD generation, i.e., via the removal of amino acids by the Maillard reaction. Polyols do not act in the Maillard reaction [35, 36]. When glucose or starch-derived sweeteners are absent, amino compounds can inhibit 3-MCPD formation [18]. This also explains the decrease of 3-MCPD formation in the models with the addition of polyols.

Effect of sweetener on esterified 3-MCPD content

Regarding 3-MCPD bound in the form of esters, its formation from triglycerides and chloride ions is significantly more complex. As has been mentioned in the Introduction, most proposed mechanisms of 3-MCPD ester formation take place in acidic media; hence, it was expected that the formation of 3-MCPD esters depended on pH value. Another mechanism, suggested by Zhang et al. [19], involves reactions with free radicals.

Therefore, in this case, 3-MCPD ester formation should be related to radical scavengers present in the model system. In conclusion, two hypotheses explaining the influence of sweetener on 3-MCPD ester formation can be assumed: (1) the impact of pH generated during thermal processing of sweeteners; (2) the effect of sweeteners as antioxidants that can inhibit lipid decomposition.

To examine a hypothetical effect of pH resulting from the use of various sweeteners on 3-MCPD esters formation, a mixture of sweetener and flour was heated in the same conditions as model systems and then dissolved in water. Received values of pH for each sweetener are presented in Fig. 1c. As can be seen, the lowest pH values were obtained for sucrose (5.12), while the highest was for erythritol (5.98). The high determination coefficient (93%) suggested that pH value altered the 3-MCPD generation in model systems. As pH decreased, more 3-MCPD ester was formed, which can be seen as proof that an acidic environment promotes the reaction between lipids and chloride ions.

The second hypothesis explaining the inhibitory effect of polyols on the formation of 3-MCPD esters is based on the potential radical mechanism of 3-MCPD ester generation that was proposed by Zhang et al. [19]. The authors observed that four antioxidants (L-ascorbyl palmitate, α -tocopherol, lipophilic tea polyphenols, and rosemary extract) could decrease 3-MCPD esters in palm oil during deodorization. Consequently, we hypothesised that compounds with antioxidant capacity should contribute to the scavenging of free radicals and the inhibition of 3-MCPD ester generation.

In the case of polyols, their antioxidant properties have already been reported by Faraji et al. [39], who revealed that polyols could function as antioxidants in fish oil. Hartog et al. [40] demonstrated that in vitro erythritol was an excellent radical scavenger. Nevertheless, our study did not confirm these findings: none of the six examined sweeteners, either raw or after thermal processing, demonstrated any antioxidant properties (data not shown, due to lack of any significant results).

The only source of compounds with antioxidant properties could, therefore, be compounds formed during the Maillard reaction. It was previously confirmed that reducing tones and melanoidins formed in browning reactions present antioxidative activity based on reducing power and metal chelating capability [4]. Nonetheless, as has been mentioned earlier, heating of sugar alcohols does not lead to the browning process that occurs when sugars are heated. Therefore, they should not form compounds with antioxidant potential. However, to explore this relation, we decided to assess the antioxidant capacity of a mixture of flour and sweeteners that had previously been subjected to heat treatment at 200 °C.

Figure 1d shows the relationship between the antioxidant capacities of compounds produced during heating and the amount of esters formed. As can be seen from the figure, the highest potential antioxidant capacity was obtained for the model incorporating stevia leaves ($1.03 \text{ mol Trolox kg}^{-1}$) and sucrose ($0.88 \text{ mol Trolox kg}^{-1}$), whereas the smallest was for the control ($0.53 \text{ mol Trolox kg}^{-1}$) and the model involving erythritol ($0.59 \text{ mol Trolox kg}^{-1}$). Slightly less antioxidant potential than those obtained for sugar was found for steviol sweetener containing maltodextrin, which could also partly take part in the Maillard reaction. However, the antioxidant capacity of Maillard products was about ten times lower than, e.g., the antioxidant capacity of spices [41]. The relationship between the amount of produced esters and the antioxidant potential of model systems was, therefore, the opposite of the hypothesis that antioxidants would inhibit the formation of esters of 3-MCPD.

As has been suggested before, in the case of free MCPD, the increasing effect between the amount of produced ester and the antioxidant capacity of the samples can be explained by the capture by the Maillard reaction of amino acids that could [1] increase the pH favouring retention of forming esters and [2] inhibit the formation of 3-MCPD esters. Nonetheless, the latter hypothesis was formed in the case of free 3-MCPD, and no previous research on the relation between amino acids and 3-MCPD esters formation has been performed.

Another possible factor could be that some of the compounds of the Maillard reaction can promote fat oxidation [5]. However, it must be emphasised that the Maillard reaction usually occurs at higher temperature and requires more energy than the oxidation of fats [6], so compounds of the Maillard reactions might not have a direct inhibitory effect on lipid oxidation. In addition, this effect has not been explored enough in recent literature. Another problem is that all processes occurring upon heat treatment of fat-rich food are correlated, such that the products of each reaction can modify the other [5].

The most effective formation of 3-MCPD esters occurs at about 230°C [42]; this temperature is close to the process of caramelisation, which itself generates an acidic condition. In conclusion, pH seems to have a dominant effect over other factors contributing to 3-MCPD ester generation.

Conclusions

The findings obtained in this study indicate that the acidic environment generated by sucrose or maltodextrin added to shortbread is a key factor promoting the formation of free and bound 3-MCPD. On the other hand, the

application of erythritol or xylitol leads to inhibition of chloropropanol production due to polyols' high thermal stability and lack of acidic products of decomposition.

The results clearly demonstrated that besides some benefits arising from the use of natural sweeteners such as, e.g., low glycemic index, the addition of erythritol and xylitol instead of sugar could be a valuable factor that can prevent bakery products containing 3-MCPD and formation of its esters. However, it should be emphasised that erythritol is relatively less sweet than sugar, and a higher amount of the sweetener needs to be added to a product to maintain comparable sweetness. This may result in the increase in the content of 3-MCPD esters (up to $966 \mu\text{g kg}^{-1}$, assuming that erythritol has only 70% of the sugar sweetness). However, this is still lower than in the case of the use of sucrose.

Second, we also revealed that the application of sweeteners based on maltodextrin had an opposite effect, so their use in the bakery industry, particular in production of dietary pastry goods, should be reconsidered.

Overall, a few other meaningful conclusions can be drawn from the achieved results. One of the most important is an unambiguous but significant role of Maillard reaction products exhibiting antioxidant properties, in the 3-MCPD formation mechanism. Due to the removal of amino acids, they can give rise to the enhancement of 3-MCPD production, but their role has not been yet clearly defined. The second conclusion is the stability and the role of stevia extract in free 3-MCPD generation during baking. Therefore, this area of interest and other research gaps such as the influence of other shortbread ingredients—mainly flour—need urgent attention.

Compliance with ethical standards

Conflict of interest Anna Sadowska-Rociek, Ewa Cieřlik, and Krzysztof Sieja declare that they have no conflict of interest.

Compliance with ethics requirements This article does not contain any studies with human participants or animals performed by any of the authors.

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