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Effect of Cysteine and Cystine Addition on Sensory Profile and Potent Odorants of Extruded Potato Snacks

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Aromas generated in extruded potato snacks without and with addition of 0.25, 0.5, and 1% (w/w) of flavor precursors, cysteine and cystine, were compared and evaluated by descriptive sensory profiling. The results showed that high addition of cysteine (0.5 and 1%) resulted in the formation of undesirable odor and taste described as mercaptanic/sulfur, onion-like, and bitter; on the contrary, addition of cystine even at high concentration gave product with pleasant odor and taste, slightly changed into breadlike notes. GC/O analysis showed cysteine to be a much more reactive flavor precursor than cystine, stimulating formation of 12 compounds with garlic, sulfury, burnt, pungent/beer, cabbage/mold, meatlike, roasted, and popcorn odor notes. Further analysis performed by the AEDA technique identified 2-methyl-3-furanthiol (FD 2048) as a most potent odorant of extruded potato snacks with 1% addition of cysteine. Other identified compounds with high FD were butanal, 3-methyl-2-butenethiol, 2-methylthiazole, methional, 2-acetyl-1-pyrroline, and 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone. In the case of cystine addition (1%) the highest FD factors were calculated for butanal, 2-acetyl-1-pyrroline, benzenemethanethiol, methional, phenylacetaldehyde, dimethyltrisulfide, 1-octen-3-ol, 1,5-octadien-3-one, and 2-acetylpyrazine.

KEYWORDS: Extrusion; flavor enhancement; cystine; cysteine; GC/O; AEDA

INTRODUCTION

Extrusion cooking is nowadays used extensively in the food industry for the fast and economical production of various food items including ready-to-eat breakfast cereals and snacks. Although extrusion technology has been used in the food industry for over 70 years, the majority of the research has been done in the area of physical and chemical changes (*1*, *2*), and there are still a limited number of investigations on its influence on the formation of flavor compounds (*3*).

In terms of flavor, extrudates are perceived as less aromatic products than those obtained in a traditional way. It is mostly due to the short time of the extrusion process limiting the number and type of reactions necessary to develop a fully desirable food product aroma. Moreover, loss of flavor during extrusion is due to chemical degradation occurring inside the barrel (i.e., oxidation, hydrolysis), and volatile components flush off during expansion. To improve the structure and also flavor of extruded products, certain methods are employed after extrusion such as toasting, roasting, or deep-frying (*4*, *5*).

In order to enhance the flavor, aroma compounds are usually added to the surface of the product after extrusion; however, it is also possible to add flavor precursors to flour prior to extrusion to obtained desirable flavor. Since extrusion conditions favor the Maillard reaction, all of the studies deal with addition of substances with either amino or carbonyl groups. Ames et al. (*6*) studied the effect of pH and temperature on the formation of volatile compounds in cysteine/reducing sugar/starch mixtures during extrusion cooking, Hwang et al. (*7*) showed that addition of cysteine during extrusion of wheat flour forms a number of volatile compounds, Baek et al. (*8*) demonstrated that sulfur-containing amino acids and thiamin are important precursors in aroma formation of beeflike flavor during extrusion, and Bailey et al. (*9*) investigated the effect of whey protein concentration on the volatile compounds in an extruded corn meal product.

Cysteine is known to produce meaty flavor by pyrolysis or through Strecker degradation with dicarbonyl compounds. Cysteine not only contains an α-amino group which can react in the Maillard reaction to produce pyrazines but also contains a sulfur-containing thiol group. Although a number of publications have been reported on reaction products formed from model systems containing cysteine (*7*, *10*−*14*) only few studies show the importance of formed volatiles to the overall flavor of food product and its correlation with sensory properties. Hofmann and Schieberle (*15*−*17*) identified key aroma compounds generated from cysteine and ribose, rhamnose, glucose, or other carbohydrates by aroma extract dilution analysis. The most important volatiles with the highest FD factors were 2-furfurylthiol, 2-acetyl-thiazoline, 2-propionyl-2-thiazoline, 5-methyl-2-furfurylthiol, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, (*Z*)-2-propenyl-3,5-dimethylpyrazine, and 5-acetyl-2,3-

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dihydro-1,4-thiazine. However there have been very limited number of reports on flavor compounds formed from L-cystine.

The aim of the present study was to compare the potential of cysteine and cystine addition to enhance the aroma of extruded potato snacks using the descriptive sensory profiling method and correlate it with the formation of aroma active compounds identified by GC/O analysis and potent odorants evaluated using aroma extract dilution analysis (AEDA).

MATERIALS AND METHODS

Chemicals. L-Cysteine and L-cystine, diethyl ether, *n*-pentane, methanethiol, dimethyl sulfide, 2,3-butanedione, butanal, 2,3-pentanedione, dimethyl disulfide, heptanal, 2-methyl-3-furanthiol, dimethyltrisulfide, 1-octen-3-ol, limonene, 2-acetylpyrazine, 2-ethyl-3,5-dimethylpyrazine, benzenemethanethiol, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone, (*E*,*E*)-2,6-nonadienal, 5-methyl-2,3-diethylpyrazine and hexanal, (*Z*)-2-hexenal, methional, and phenylacetaldehyde were obtained from Sigma-Aldrich-Fluka (Poznań, Poland). 2-Acetyl-1-pyrroline was obtained as a kind gift from Prof. P. Schieberle.

Preparation of Extruded Potato Snacks. Experiments were conducted with extruded potato snack produced according to our own developed recipe and procedure described in a previous paper (*5*). Before extrusion L-cysteine and L-cystine were prepared as water solutions and added separately to other ingredients in amounts resulting in 0.25, 0.5, and 1% (w/w) concentration. After extrusion, products were cut into small pieces and heated in an oven at 85 °C for 15 min followed by 45 min roasting at 130 °C to develop proper texture and aroma.

Descriptive Sensory Analysis of Potato Snacks. Sensory analyses were performed by a 10 member panel experienced in descriptive analysis. The odor profiling analysis of all samples was run in triplicate (three sessions) preceded by an introductory session. Six odor attributes, seven taste attributes (developed in a previous study), and the general desirability of the samples were scaled on a 10 cm linear scale anchored on both sides for the intensity of attributes as "none" and "very strong", and for desirability as "not desired" and "very desired". The odor and taste descriptors were chosen according to the "Basic Flavor Descriptive Language" from Givaudan Roure Flavor Ltd. (*18*). The 20 g samples of potato snacks were presented to the panelists in 100 mL closed containers, preheated to 40 °C prior to analysis. The results from the linear scale were converted into numerical values for data analysis. Mean, variance, and standard deviation were calculated for all attributes of each sample, for each session separately and across all three sessions. Obtained data were counted from 30 replicates and after statistical interpretation by a multivariate procedure presented as a graphic projection of principal component analysis (PCA) (*19*).

Isolation of Volatiles. Extruded and dried potato snacks (20 g) were ground and mixed with water (150 mL). The volatile fraction was isolated by high-vacuum distillation using the solvent assisted flavor evaporation technique (SAFE) (*20*). During this procedure, the temperature of the water bath was held at 40 °C, and pressure was reduced using an Edwards RV5 rotary vane pump. Distillate with flavor compounds was collected in a flask cooled with liquid nitrogen. After 30 min of distillation, the solution was thawed and extracted 5 times with 10 mL of a pentane−ether mixture (1:1), and after drying over anhydrous Na_2SO_4 the fraction was concentrated to about 200 μL under a delicate stream of nitrogen.

Gas Chromatography/Olfactometry (GC/O). Gas chromatography/olfactometry was performed on an HP 5890 chromatograph using the capillary columns SPB-5 (30 m × 0.53 mm × 1.5 μm) and Supelcowax 10 (30 m × 0.25 mm × 0.25 μm, Supelco Bellefonte). The chromatograph was equipped with a Y splitter dividing effluent between olfactometry port with humidified air as a make-up and a flame ionization detector. The operating conditions were the following: initial oven temperature 40 °C (1 min), then 6 °C/min to 180 °C and 20 °C/min to 280 °C for the SPB-5 column and 40 °C (2 min), then raised to 60 °C at 40 °C/min rate, held for 2 min isothermally and then raised by 5 °C/min to 240 °C for the Supelcowax 10 column. For all peaks and flavor notes occurring at a specific retention times, retention indices were calculated to compare results with that obtained by GC/MS and data in the literature. Retention indices were calculated for each compound using the homologous series of C_5-C_{24} *n*-alkanes (*21*).

Gas Chromatography/Mass Spectrometry (GC/MS). Compound identification was performed using two instruments. A Hewlett-Packard HP 5890 gas chromatograph coupled to a 5971 MSD quadrupole mass spectrometer was equipped with a fused silica DB-5 (25 m × 0.200 mm × 0.33 μm) column and the same Supelcowax-10 column used in the GC/O experiments. Operating conditions for GC/MS were as follows: helium flow 0.8 mL/min; oven conditions the same as for GC/O. Mass spectra were recorded in an electron impact mode (70 eV) in a scan range of *m*/*z* 33−350 Da. Additionally for compound identity confirmation samples were run on a GCxGC-TOF-MS (Pegasus IV, Leco) running as a GC−MS system in the EI mode. Compounds were resolved on a DB-5 column (25 m × 0.200 mm × 0.33 μm), and a second column (BPX-50, 1.3 m × 0.1 mm × 0.1 μm) in a secondary oven was kept at a temperature 30 °C higher than the first oven, for which temperature was raised from 40 °C (1 min) at 15 °C/min to 240 °C. Mass spectra were collected at a rate of 30 scans/s with ionization energy 70 eV. Identification of volatiles was performed in two ways depending on availability of standard compounds: full identification comprising comparison of mass spectra, retention indices (RI), and odor notes on two columns of different polarities was performed when a standard of the investigated compound was available. In some cases the MS signal of analyte was too weak to facilitate comparison of mass spectra. In such cases RI and odor notes of compounds were compared to a standard. In cases when standards were not available, tentative identification was performed based on the comparison of the mass spectrum of a compound with a NIST 05 library match and comparison of retention indices with that available in literature. Also the odor characteristic for an analyzed compound was compared with literature data and used in tentative identification.

Aroma Extract Dilution Analysis (AEDA). The flavor dilution factor (FD) of each of the odorants was determined by an aroma extract dilution analysis (AEDA) (*22*). The flavor extract (2 μL) was injected into a GC column. Odor-active regions were detected by GC-effluent sniffing (GC/O), and three panelists determined the description of the volatiles. The extract was than stepwise diluted by addition of pentane−ether (1:1 v/v), and each dilution was analyzed until no odor was perceivable at the sniffing port. Retention data of the compounds were expressed as retention indices (RI).

RESULTS AND DISCUSSION

Sensory Analysis. Seven extruded potato snacks were produced: one according to the recipe described in a previous paper by the authors (*5*) without any aroma compound precursors, three series with addition of cysteine in concentrations of 0.25, 0.5, and 1% (w/w), and three series with addition of cystine in the same concentrations. Obtained samples were coded with letters from A to G and subjected to descriptive analysis. The effect on sensory profile of the addition of flavor precursors was evaluated by a 10 member sensory panel, who assessed separately odor and taste of the obtained potato snacks. Results and a description of the symbols are presented in **Figures 1** and **2**. All analyzed potato snack samples were compared with each other in relation to all used odor and taste attributes. In the first case, sensory analysis revealed significant differentiation of odor profiles of analyzed samples (**Figure 1**). The first principal component (PC1) contained almost 55% of the variance in the data, and the second principal component (PC2) contained 21%. Most of the odor descriptors were strongly detected in analyzed samples and differentiated them into three groups. The first group contained samples A and B, which are extruded potato snacks without any aroma precursors and with 0.25% addition of cysteine. Those samples were judged with strong potato, brown, and roasted notes, which gave them also a high score on the desirability scale. The second group consisted

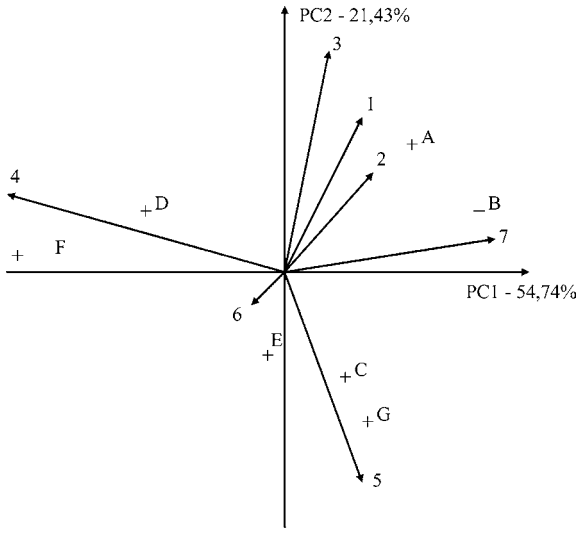

Figure 1. PCA plot of odor profile data of seven extruded potato snack samples with or without added aroma precursors. Sample codes: (A) without any precursors; (B) 0.25% of cysteine; (C) 0.25% of cystine; (D) 0.5% of cysteine; (E) 0.5% of cystine; (F) 1% of cysteine; (G) 1% of cystine. Descriptors: (1) potato; (2) brown; (3) roasted; (4) mercaptonic, sulfuric; (5) bread; (6) oxidized; (7) desirability.

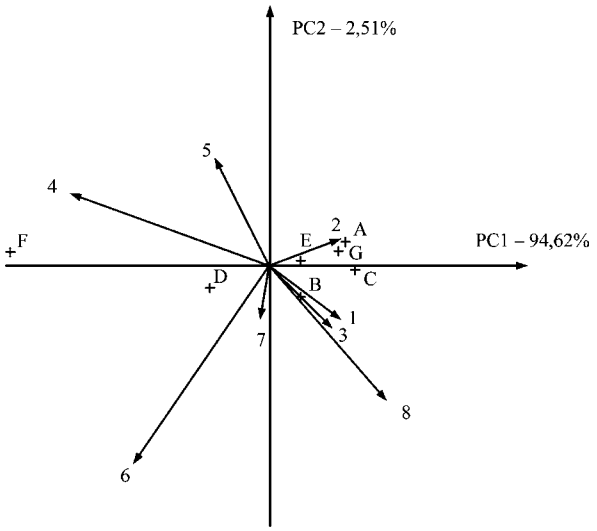

Figure 2. PCA plot of taste profile data of seven extruded potato snack samples with or without added aroma precursors. Sample codes: (A) without any precursors; (B) 0.25% of cysteine; (C) 0.25% of cystine; (D) 0.5% of cysteine; (E) 0.5% of cystine; (F) 1% of cysteine; (G) 1% of cystine. Descriptors: (1) potato; (2) brown; (3) roasted; (4) mercaptonic, sulfuric; (5) bitter; (6) onion; (7) fatty, oily; (8) desirability.

of samples C, E, and G, that is, potato snacks with the addition of 0.25, 0.5, and 1% of cystine with the most characteristic bread aroma. The last group (samples F and D) of potato snacks prepared with addition of 0.5 and 1% of cysteine was judged as very undesirable, with an intense odor described as mercaptonic and sulfuric. Oxidized odor was not strongly recognized in any of the analyzed samples.

Figure 2 shows sensory analysis of taste profile of extruded potato snacks with the first principal component (PC1) containing nearly 95% of the variance in the data and the second principal component (PC2) containing 2.5%. The difference in the type of precursor added was the most distinguishing factor in the taste analysis with PC1 separating samples A, B, C, E, and G from D and F. Samples with high addition of cysteine (0.5 and 1%) had a strong mercaptanic, sulfuric, onion, and bitter taste absolutely undesirable to consumers. On the other hand the rest of the samples, that is, with a small addition of cysteine (0.25%) and with addition of cystine at all levels (0.25, 0.5, and 1%) as well as plain potato snacks, were rated as very desirable. The highest scores were given to potato, brown, and roasted notes.

Identification of Aroma Active Compounds by the GC/O Method. After sensory analysis an aroma concentrate was prepared from each sample by solvent assisted flavor evaporation (SAFE) and then extracted with a pentane−ether solution. Concentrated extracts were analyzed for identification of odor active compounds by the gas chromatography/olfactometry method (GC/O).

GC/olfactometry performed for isolates obtained from all extrudates showed 37 odor-active compounds presented in **Table 1**. The largest number of identified compound (36) was detected from the sample produced with 1% addition of cysteine (F), whereas in sample G (1% of cystine) 28 compounds were noted. Plain extruded potato snacks were characterized by 25 compounds. The results presented demonstrate that cysteine is a much more reactive flavor precursor than cystine, stimulating formation of 12 compounds, whereas only 4 were formed from cystine. All mentioned compounds, not counting two unknowns, contain sulfur element in their structure and belong to five classes: sulfides, disulfides, thiols, thiazoles, and thiophenes. They are known as very powerful aroma compounds in food products involved in the generation of some delightful but also some irritating, unpleasant odor notes even at low concentration levels due to their very low odor thresholds down to 0.02 ng/L for bis(2-methyl-3-furyl) disulfide in water (*23*). Dimethyl sulfide and dimethyl disulfide have a garlic, cabbage type odor, and they are produced by cooking of certain vegetables, i.e., cauliflower, cabbage (*24*), and fresh garlic (*25*) as well as in thermally treated food products such as baked potato (*26*), extruded maize flour (*27*), or popcorn (*28*). They are also an indication of bacterial infection in malt production and brewing (*29*). Dimethyl sulfide can originate from disproportionation of dimethyl disulfide that is formed after methanethiol oxidation (*23*). Thiazole, 2-methylthiazole, 2-acetyl-thiazoline, and benzothiazole bringing burnt, roasty, popcornlike, and meatlike flavor to obtained potato snacks are known also as main contributors to the aroma of boiled meat (*23*), coffee (*30*), extruded oat flours (*31*), boiled potatoes (*32*), heated milk (*33*), and beer (*23*). Model experiments showed that cysteamine, formed by the decarboxylation of cysteine, and 2-oxopropanal are the precursors of 2-acetyl-2-thiazoline (*15*). Benzothiazole is produced by means of Maillard reaction and on one hand is responsible for "stale" off-flavor in heated milk but on the other it brings a pleasant meatlike aroma to cooked beef meat (*34*). Next compound 2-methyl-thiophene represents a group of compounds with a mild sulfurous odor reported in cooked meat (*35*), extruded oat flours (*31*), and extruded wheat flour with cysteine addition (*1*). Sakaguchi and Schibamoto (*36*) concluded that thiophenes were formed from the reaction of a sugar or carbohydrate with hydrogen sulfide or an amino acid. In the case of the present report, 2-methylthiophene has been formed from cysteine under extrusion and roasting conditions. There have been also two compounds with thiol group detected: 3-methyl-2-butenethiol and 2-methyl-3-furanthiol, which dimerized partially to bis(2-methyl-3-furyl) disulfide. 2-Methyl-3-furanthiol due to its extremely low odor threshold 0.0025 ng/L in air (*15*) is considered to play an important role in a flavor of meat (*37*, *38*) and coffee (*30*). Moreover, it has been identified as a high aroma value compound in chicken and bovine broths

Table 1. Aroma Active Compounds Detected with Gas Chromatography/olfactometry Method from Extruded Potato Snacks Samples[a]

no.	compound	odor description[b]	RI[c] DB-5	RI[c] Supelcowax-10	A(FD[d])	B	D	F(FD)	C	E	G(FD)
1	ethanol[e]	spirit	<500	<600	+(2)	–	–	–	–	–	–
2	methanethiol[e]	burnt, sulfury	<500	694	+(8)	+	+	+(4)	+	+	+(16)
3	dimethyl sulfide[e]	garlic	513	715	–	+	+	+(4)	–	–	–
4	2,3-butanedione[e]	buttery	593	986	+(4)	+	+	+(8)	+	+	+(8)
5	butanal[e]	rancid	598	830	+(256)	+	+	+(128)	+	+	+(256)
6	unknown	fruity	600	835	+(256)	+	+	+(128)	+	+	+(256)
7	2,3-pentanedione[e]	buttery	687	1050	+(8)	+	+	+(8)	+	+	+(8)
8	thiazole[f]	burnt	694	–	–	–	–	+(8)	–	–	–
9	2-methylthiophene[f]	sulfury	756	1082	–	+	+	+(8)	–	–	–
10	dimethyl disulfide[e]	garlic	783	1069	–	+	+	+(8)	–	–	–
11	hexanal[e]	green	801	1084	+(8)	+	+	+(4)	+	+	+(16)
12	3-methyl-2-butenethiol[f]	pungent, beer,	808	1112	–	+	+	+(32)	+	+	+(8)
13	2-methylthiazole[e]	cabbage, mold	862	–	–	+	+	+(32)	–	+	+(8)
14	2-methyl-3-furanthiol[f]	meaty, roasty	869	1320	–	+	+	+(2048)	+	+	+(8)
15	unknown	medicine	890	–	–	+	+	+(4)	+	+	+(4)
16	heptanal[e]	rancid	893	1173	+(4)	+	+	+(4)	+	+	+(4)
17	methional[e]	boiled potato	909	1456	+(512)	+	+	+(32)	+	+	+(64)
18	2-acetyl-1-pyrroline[e]	popcorn	916	1328	+(512)	+	+	+(32)	+	+	+(128)
19	dimethyltrisulfide[e]	cabbage	961	1347	+(32)	+	+	+(2)	+	+	+(32)
20	1-octen-3-ol[f]	mushroom	982	1321	+(16)	+	+	+(4)	+	+	+(32)
21	1,5-octadien-3-one[f]	geranium	984	1358	+(16)	+	+	+(8)	+	+	+(32)
22	limonene[f]	lemon	1015	1178	+(2)	+	+	+(2)	+	+	+(8)
23	unknown	pungent	1022	–	–	+	+	+(16)	–	–	–
24	2-acetylpyrazine[e]	roasty	1025	1604	+(32)	+	+	+(16)	+	+	+(32)
25	phenylacetaldehyde[e]	flowery	1053	1642	+(64)	+	+	+(16)	+	+	+(64)
26	2-ethyl-3,5-dimethylpyrazine[e]	earthy	1081	1437	+(8)	+	+	+(8)	+	+	+(8)
27	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone[f]	cotton candy	1110	2030	+(4)	+	+	+(8)	+	+	+(4)
28	2-acetyl-2-thiazoline[f]	roasty, popcorn	1119	1740	–	+	+	+(16)	–	–	–
29	benzenemethanethiol[f]	pepper-seed	1129	1615	+(512)	+	+	+(8)	+	+	+(128)
30	3-hydroxy-4,5-dimethyl-2(5*H*)-furanone[f]	spicy, seasoning-like	1159	2193	+(1)	+	+	+(32)	+	+	+(1)
31	2-methyl-3,5-diethylpyrazine[f]	parsley root	1185	1474	+(64)	+	+	+(4)	+	+	+(16)
32	2,6-nonadienal[e]	cucumber	1190	1575	+(8)	+	+	+(4)	+	+	+(8)
33	5-methyl-2,3-diethylpyrazine[e]	roasted pepper, meatlike	1212	1483	+(16)	+	+	+(4)	+	+	+(16)
34	benzothiazole[e]	roasty, meatlike	1221	–	–	–	+	+(8)	–	–	–
35	unknown	fresh pepper	1248	1498	+(64)	+	+	+(32)	+	+	+(32)
36	unknown	soap, wax	1447	1809	+(2)	+	+	+(8)	+	+	+(16)
37	bis(2-methyl-3-furyl)disulfide[f]	meatlike	1520	2151	–	–	–	+(16)	–	–	–

[a] For samples A, F, and G: dilution factors (FD) have been obtained by the AEDA method. Sample codes: (A) without any precursors; (B) 0.25% of cysteine; (C) 0.25% of cystine; (D) 0.5% of cysteine; (E) 0.5% of cystine; (F) 1% of cysteine; (G) 1% of cystine. "+", detected; "–", not detected. [b] Odor perceived at the sniffing port. [c] Retention Indices on DB-5 and Supelcowax-10 columns. [d] Flavor dilution factor. [e] Compounds identified by comparing them with reference compounds on the basis of the following criteria: retention index (RI), mass spectra obtained by MS (EI), and odor quality at the sniffing port. [f] The MS signal was too weak for an unequivocal interpretation. The compound was identified on the basis of the remaining criteria detailed in footnote *e*.

(*39*) and extruded enzyme-hydrolyzed soybean protein (*8*). A great number of investigations have been performed to explain formation of 2-methyl-3-furanthiol. Model studies have elucidated pentoses and cysteine as its precursors (*17*, *40*−*43*). The formation mechanism of 2-methyl-3-furanthiol in the Maillard reaction has been proposed to occur between a ribose Amadori product and cysteine capable of liberating hydrogen sulfide (*13*). Belitz et al. (*23*) presented norfuraneol (4-hydroxy-5-methyl-3-(2*H*)-furanone) as its precursor, which, after addition of hydrogen sulfide, leads to 4-mercapto-5-methyl-3(2*H*)-furanone following reduction, e.g., by reductones and water elimination to 2-methyl-3-furanthiol. However thermal degradation of thiamin has been discussed as an alternative reaction pathway during food processing (*44*, *45*). 3-Methyl-2-butenethiol is one of the roast odorants of coffee (*30*) and can cause off-flavor in beer (*46*).

Eight compounds such as dimethyl sulfide, thiazole, 2-methylthiophene, dimethyl disulfide, unknown with a pungent flavor, 2-acetyl-2-thiazoline, benzothiazole, and bis(2-methyl-3-furyl) disulfide were formed only in cysteine supplemented samples and not cystine. Cystine is a dimeric form of cysteine formed by linking two cysteine residues via a disulfide bond (cys-S−S-cys) between the −SH groups. This S−S bond shows to be quite strong and stable during preparation of extruded potato snacks, resulting only in the formation of four detected additional aroma active compounds with comparison to plain potato snacks: 3-methyl-2-butenethiol, 2-methylthiazole, 2-methyl-3-furanthiol, and unknown with medicine flavor. Studies by Koh et al. (*47*) and Anderson and Ng (*48*) have also shown that extrusion of protein mixtures does not affect S−S bond content. However in a recent paper Aslaksena et al. (*49*) reported that the disulfide link could be readily reduced to the corresponding thiol (−SH), for example by β-mercaptoethanol as well as by high temperatures above about 150 °C, especially at low moisture levels (below 20%). Although extrusion conditions such as high moisture material (27% of water) and temperature only up to 115 °C used in preparation of extruded potato snacks did not favor formation of flavor compound from cystine, they could be generated during the roasting process used after extrusion. As it has been also shown in a recent paper by the authors (*5*), high temperature up to 130 °C and reduced moisture to 5−10% used during the roasting process favor Maillard reaction and development of potent odorants which give the final product its characteristic aroma. As a result of Maillard reaction or oxidation the most pronounced decrease in amino

acids occurred for methionine (19%) and cysteine (13%) in the case of lupin extrudates (*50*).

The higher number of aroma active components formed in potato snacks with addition of cysteine correlates with the aroma profile presented in the PCA plot (**Figures 1** and **2**). Both charts show that samples with addition of 0.5 and 1% of cysteine differ substantially in either taste or odor profile from the rest of the samples, having strong and unpleasant mercaptonic and sulfuric smell and also bitter and onion-like taste. Compounds responsible for this reaction are probably sulfur-containing type such as dimethyl sulfide with garlic note, thiazole with burnt note, 2-methylthiophene with sulfur note, dimethyl disulfide with garlic note, 3-methyl-2-butenethiol with beer and hops note, 2-methylthiazole with cabbage and mold note, 2-methyl-3-furanthiol with meatlike and roasted note, 2-acetyl-2-thiazoline with roasty and popcorn note, benzothiazole with meatlike and roasted note, and bis(2-methyl-3-furyl) disulfide with meatlike note. On the other hand, a sample produced with addition of 0.25% of cysteine has been judged as pleasant with brown, roasted, potato-like odor and taste. Addition of cystine did not affect intensively desirability of the samples; however their odor and taste shifted more into breadlike odor instead of roasted, brown, and potato-like.

Identification of Potent Odorants by the AEDA Method. This study was performed to explain which odor-active compounds were responsible for the flavor differences between potato snacks produced with aroma precursors. For that reason the volatiles were evaluated by the AEDA technique. Three extracts were analyzed: plain sample, sample with 1% addition of cysteine, and sample with 1% addition of cystine.

In the analyzed isolates identified compounds showed FD factors in the range of 1−2048, shown in **Table 1**. The highest FD factor has been calculated in the sample with 1% addition of cysteine for a compound with meatlike and roasted note: 2-methyl-3-furanthiol. This compound has not been detected in a sample without flavor precursors addition (A) and only in a small intensity (FD 8) in a cystine added sample (G). As it has been mentioned earlier 2-methyl-3-furanthiol belongs to the most important aroma impact compounds formed in thermally produced foods such as cooked beef (*37*), roasted coffee (*51*) as well as in commercial meat flavorings (*39*). It has been also shown to be a main contributor of aroma generated by heating of model mixtures containing ribose (*15*), glucose or rhamnose (*16*) in the presence of cysteine. From the results presented it might be presumed that addition of 1% of cysteine before extrusion resulted in the formation of a product F with undesirable strong mercaptonic, sulfuric odor and onion and bitter taste caused in a major manner by 2-methyl-3-furanthiol. A similar way of thinking can be applied to sample D, produced with 0.5% addition of cysteine. Then again addition of smaller amounts of cysteine in sample B (0.25%) resulted in the formation of a pleasant aroma of potato snacks with roasted, brown, and potato-like notes. Bredie et al. (*52*) in their discussion on sensory profile of extruded wheat flour and wheat starch with or without addition of cysteine and reducing sugars noted that addition of approximately 0.5% of cysteine to starch extrudates resulted in flavor with acidic, stale cooking oil, cooked apple, and wet washing notes. On the other hand addition of cysteine and glucose resulted in pleasant popcorn, nutty/roasted, and puffed wheat aromas. Unfortunately in their work there was no comparison of sensory profiling with volatile compounds. Hwang et al. (*7*) studied the effect of cysteine addition on volatile compounds released during extrusion of wheat flour. Authors noted a high increase of sulfur-containing compounds, however did not mention its influence on sensory properties of obtained extrudates.

Although 2-methyl-3-furanthiol had the highest FD factor, other compounds formed from cysteine (no. 3, 8, 9, 10, 12, 13, 28, 34, 37) had FD factors 4−32 and they can also influence the overall flavor of analyzed product. From the PCA plot (**Figures 1** and **2**) it can be seen that both samples F and D did not show intense potato flavor, which is in correspondence with the FD factor of a potato-like smelling compound, methional. This compound, known to be formed from methionine in Strecker degradation, has been agreed to be a main contributor to typical flavor of extruded potato snacks together with 2-acetyl-1-pyrroline and benzenemethanethiol (*5*). Its FD factor in sample A was much higher (512) compared to sample F (32). In addition other compounds contributing in a significant way to the overall flavor of sample A had much lower FD factors in sample F. For example 2-acetyl-pyrroline with a pleasant popcornlike aroma had FD 512 in sample A and 32 in sample F, benzenemethanethiol with pepper-seed aroma had FD 512 in sample A and 8 in sample F, and furthermore certain pyrazines, 2-acetylpyrazine, 2-methyl-3,5-diethylpyrazine, 5-methyl-2,3-diethylpyrazine, and also phenylacetaldehyde with flowery odor, also decreased its intensity by showing lower FD factors (32, 64, 16, and 64 in sample A, and 16, 4, 4, and 16 in sample F respectively).

AEDA performed for isolates obtained from a sample with the highest addition of cystine (G) showed 29 odor-active compounds having FD factors in the range of 2−256 (**Table 1**). Among them butanal (FD 256), unknown with fruity odor (FD 256), 2-acetyl-1-pyrroline (FD 128), benzenemethanethiol (FD 128), methional (FD 64), and phenylacetaldehyde (FD 64) were considered to be main contributors to the flavor of extruded potato snacks. Other compounds, although having lower FD factors, can also contribute to overall flavor of the obtained product. As it has been mentioned earlier, four additional compounds were formed when cystine was added to potato snack samples before extrusion: 3-methyl-2-butenethiol, 2-methylthiazole, 2-methyl-3-furanthiol**,** all with 8 FD factor, and unknown with medicine odor notes with 4 FD. Although those compounds do not have high FD factors, their low odor threshold values (0.003 μg/L for 3-methyl-2-butenethiol and 0.007 for 2-methyl-3-furanthiol) can cause a great impact on the aroma profile even when they are present in small quantities. This has been seen on a PCA plot (**Figure 1**) where the odor profile of samples with addition of cystine, even with high concentration (C, E, G), changed more into breadlike odor with less distinctive potato and roasted notes yet still remaining at high desirability.

Addition of flavor precursors cysteine and cystine did affect the aroma profile of extruded potato snacks, however, in most cases, except for 0.25% cystine, not in a desirable way. The obtained flavor profile was correlated with aroma active compounds where the flavor of cysteine added snacks was more pronounced, 36 compounds, compared to cystine added, 28 compounds. 2-Methyl-3-furanthiol (FD 2048) was the most powerful volatile when cysteine was added, and the rest of the cysteine derived compounds had a FD value >8. However, a full answer on the impact of particular compounds on potato snack flavor can be gained only by estimating odor active values for all of them and performing the recombination experiments.

Cysteine was more reactive than cystine in terms of potential importance in the creation of odor active compounds. Shifts in flavor profile of snacks with addition of cystine and particularly cysteine indicate that for potential technological applications

there are perspectives for improving product flavor but aroma changes can be hard to predict.

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