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Identification of Aroma Active Compounds of Cereal Coffee Brew and Its Roasted Ingredients

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ABSTRACT: Cereal coffee is a coffee substitute made mainly from roasted cereals such as barley and rye (60−70%), chicory (15−20%), and sugar beets (6−10%). It is perceived by consumers as a healthy, caffeine free, non-irritating beverage suitable for those who cannot drink regular coffee made from coffee beans. In presented studies, typical Polish cereal coffee brew has been subjected to the key odorants analysis with the application of gas chromatography−olfactometry (GC−O) and aroma extract dilution analysis (AEDA). In the analyzed cereal coffee extract, 30 aroma-active volatiles have been identified with FD factors ranging from 16 to 4096. This approach was also used for characterization of key odorants in ingredients used for the cereal coffee production. Comparing the main odors detected in GC−O analysis of roasted cereals brew to the odor notes of cereal coffee brew, it was evident that the aroma of cereal coffee brew is mainly influenced by roasted barley. Flavor compound identification and quantitation has been performed with application of comprehensive multidimentional gas chromatography and time-of-flight mass spectrometry (GCxGC-ToFMS). The results of the quantitative measurements followed by calculation of the odor activity values (OAV) revealed 17 aroma active compounds of the cereal coffee brew with OAV ranging from 12.5 and 2000. The most potent odorant was 2-furfurylthiol followed by the 3-mercapto-3-methylbutyl formate, 3-isobutyl-2-methoxypyrazine and 2-ethyl-3,5-dimethylpyrazine, 2-thenylthiol, 2,3-butanedione, 2-methoxy phenol and 2-methoxy-4-vinyl phenol, 3(*sec*-butyl)-2-methoxypyrazine, 2-acetyl-1-pyrroline, 3-(methylthio)-propanal, 2,3-pentanedione, 4-hydroxy-2,5-dimethyl-3-(2*H*)-furanone, (*E*,*E*)-2,4-decadienal, (*Z*)-4-heptenal, phenylacetaldehyde, and 1-octen-3-one.

KEYWORDS: *cereal coffee brew, 2-furfurylthiol, aroma active compounds, roasting, AEDA, OAV, GCxGC*

INTRODUCTION

Coffee infusion popularity is still increasing all over the world. It is reported to be among the most widely consumed beverage appreciated for its distinct aroma, which is obtained as a result of raw coffee beans processing, especially roasting. Coffee volatile profile is estimated to be over 1000 compounds of different chemical classes and character.[1] However, as the list of volatiles increased, the question arose whether all of them contribute to the aroma of the product. Grosch[2] using gas chromatography−olfactometry (GC−O) and odor activity value (OAV) revealed that only 27 compounds are able to generate an odor similar to that of roasted Arabica coffee, when present in certain concentrations.

Excessive consumption of coffee beverages is reported to cause several negative side-effects, mainly because of the presence of the alkaloid caffeine, including increase in blood pressure and heart beat, plasma renin activity, urine production, and gastric acid secretion.[3,4] To the contrary, recent studies reported by Gunter et al.[5] suggest that the risk of some type of gynecological cancer is reduced in regular coffee drinkers as well as in those drinking decaffeinated coffee. Therefore, some studies have been carried out for the preparation of coffee substitutes, free from caffeine, but with the similar sensory attributes. The proposed substitutes were composed of different roasted products such as chicory roots, malt, barley, and rye or blends of them[6,7] or prepared by extrusion of wheat germ and chicory roots.[8] During roasting as well as extrusion of biological materials (cereals, potato granules), different chemical reactions take place, including Maillard reaction, leading to the formation of a wide range of volatiles such as pyrazines, furans, ketones, and aldehydes.[9−11] Holsher[6] and Fadel et al.[8] reported that most of the identified compounds in coffee substitute are also presented in real coffee, except for 3-methyl-2-butene-1-thiol and 3-isobutyl-2-methoxypyrazine, which were characteristic only for roasted coffee. Among the identified compounds were 2-furfural, which was reported to be the predominant volatile in the headspace of roasted chicory roots.[12] However, to define which compound contributes to overall aroma, and could be considered as a key odorant, GC−O analysis should be used. Holsher et al.[13] and Blank et al.[14] applied aroma extract dilution analysis (AEDA) to ground coffee and brew to screen for the most potent odorants. AEDA analysis revealed 13 compounds as important contributors to the aroma of coffee powder, among which were mainly pyrazines, thiols, and furans formed during roasting.[14] To indicate which of the volatile compounds revealed by the dilution experiments might be involved in the aroma, quantitation of the potent odorants and calculation of odor activity value is needed.[15,16]

To the best of our knowledge, there is no information in the literature on the key odorants of cereal coffee brew and little information on its ingredients. Therefore, the aim of this study was to identify the main volatile compounds present in roasted

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ingredients brew such as barley, rye, chicory roots, and sugar beets and characterize these compounds that are key odorants and influence the cereal coffee brew aroma using gas chromatography−olfactometry.

MATERIALS AND METHODS

Cereal Coffee Brew. Cereal coffee samples as well as its roasted ingredients—chicory, sugar beets, rye, and barley—were obtained directly from the producer of analyzed coffee Kujawianka, located in Włocławek, Poland. The analyzed cereal coffee brew consisted of 40% barley, 25% rye, 25% chicory, and 10% sugar beets. The specific roasting conditions are know-how of the company however they are much about 180−200 °C for barley and rye, 140−160 °C for chicory and sugar beets. All samples were stored in sealed plastic bags until analyzed but no longer than 1 month.

Chemical Standards. Solvents, such as diethyl ether, methylene chloride, and sodium sulfate, were obtained from Sigma-Aldrich (Poznań, Poland). The following reference aroma compounds were purchased from Sigma-Aldrich (Poznań, Poland): methanethiol, 2,3-butanedione, 3-methyl butanal, 2,3-pentanedione, hexanal, 2(3)-methyl butanoic acid, 2-methyl-3-furanthiol, (*Z*)-4-heptenal, 3-(methylthio)-propanal, 2-furfurylthiol, dimethyl trisulfide, 1-octen-3-one, phenylacetaldehyde, 4-hydroxy-2,5-dimethyl 3(2*H*)-furanone, 2-methoxy phenol, 2-ethyl-3,5-dimethylpyrazine, 3(*sec*-butyl)-2-methoxypyrazine, (*E*,*Z*)-2,6-nonadienal, 2-methyl-[3-methyldithio]-furan, 3-isobutyl-2-methoxypyrazine, 2-methoxy 4-methyl phenol, (*E*,*E*)-2,4-decadienal, *β*-damascenone. The following compounds were purchased from Aroma LAB (Freising, Germany): 2-acetyl-1-pyrroline, [$^{13}C_4$]-2,3-butanedione, [$^{13}C_4$]-2,3-pentanedione, [$^{2}H_3$]-3-(methylthio)-propanal, [$^{2}H_2$]-2-furfurylthiol, [$^{13}C_2$]-2-acetyl-1-pyrroline, [$^{2}H_3$]-1-octen-3-one, [$^{13}C_2$]-phenylacetaldehyde, [$^{2}H_3$]-2-methoxy phenol, [$^{2}H_5$]-2-ethyl-3,5-dimethylpyrazine, [$^{2}H_2$]-3-isobutyl-2-methoxypyrazine, and [$^{2}H_3$]-2-methoxy-4-vinylphenol. The purity of solvents and reference standards was no less than 99 and 97%, respectively. 2-Thenylthiol and 3-mercapto-3-methylbutyl formate were synthesized in our laboratory according to the methods given in refs 17 and 18.

Isolation Method. The cereal coffee brew was prepared from 35 g of cereal coffee boiled during 5 min in 1 L of water. After cooling, the cereal coffee brew was filtered into an Erlenmeyer flask and extracted at ambient temperature separately with two solvents of different polarities, diethyl ether (300 mL) and methylene chloride (300 mL), for 2 h each by shaking it in the horizontal shaker. Both fractions were filtered and combined prior to distillation, which was performed using a solvent-assisted flavor evaporation method SAFE described by Engel et al.[19] During this procedure, the temperature of the water bath was held at 40 °C, and the pressure was reduced using an Edwards RV5 rotary vane pump (<300 mTorr). Distillate with volatile flavor compounds was collected in a flask cooled with liquid nitrogen. After distillation, the solution was dried over anhydrous Na_2SO_4, and the fraction was concentrated to about 300 μL using a Kuderna Danish concentrator. The same amount of sample and the same procedure has been used for roasted chicory, rye, barley, and sugar beets.

Isolation of Thiols. For selective preconcentration of thiols, 200 mL of cereal coffee brew prepared as described above was separated by trapping on SPE cartridge using *p*-hydroxymercurybenzoate described by Mateo-Vivaracho et al.[20] with small modification, as the cereal coffee brew extract was mixed with *p*-hydroxymercurybenzoate prior to SPE separation and percolated through the cartridge as a mixture.

Fractionation of Volatiles by Column Chromatography. For the identification of thiols and pyrazines, 5 L of cereal coffee brew was prepared from 175 g of cereal coffee boiled during 5 min. After filtration, the cereal coffee brew has been extracted with methylene chloride (1.2 L) for 2 h. The organic layer was separated and distilled using a SAFE apparatus, as described above performing five different distillations. Obtained distillates were combined, concentrated to about 1.5 mL, and separated by flash column chromatography using a glass column filled with silica gel (75 g). Elution was performed using four pentane/diethyl ether mixtures (4 × 450 mL) of increasing polarity (100:0; 90:10; 70:30; and 50:50) obtaining fractions A−D. Fractions containing thiols and pyrazines (B and C) have been concentrated to 300 μL and analyzed by GCxGC-ToFMS.

Gas Chromatography−Olfactometry (GC−O). GC−O was performed on an HP 5890 chromatograph using the following capillary columns: SPB-5 (30 m × 0.53 mm × 1.5 μm,) and Supelcowax 10 (30 m × 0.53 mm × 1 μm); Supelco, Bellefonte, PA. The GC was equipped with a Y splitter dividing effluent between olfactometry port with humidified air as a makeup flow, and a flame ionization detector. The operating conditions were as follows for the SPB-5 column: initial oven temperature 40 °C (1 min), raised at 6 °C/min to 180 °C and at 20 °C/min to 280 °C. Operating conditions for the Supelcowax 10 column were as follows: initial oven temperature, 40 °C (2 min), raised to 240 °C at 8 °C/min rate, held for 2 min isothermally. For all peaks and flavor notes, retention indices were calculated to compare results obtained by GC/MS with literature data. Retention indices were calculated for each compound using a homologous series of C_7−C_{24} *n*-alkanes.

Gas Chromatography/Mass Spectrometry. Compound identification was performed using two instruments: a Hewlett-Packard HP 7890A GC coupled to a 5975C MS (Agilent Technologies) with a Supelcowax-10 column (30 m × 0.25 mm × 0.25 μm) or an SLB-5MS (25m × 0.2 mm × 0.33 μm) column. Operating conditions for GC/MS were as follows: helium flow, 32.2 cm/s; oven conditions were 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. Additionally, to confirm the identities of the compounds, samples were run on a multidimensional gas chromatograph GCxGC-ToFMS (Pegasus IV, LECO). The GC was equipped with a DB-5 column (25 m × 0.2 mm × 0.33 μm) and Supelcowax 10 (1.2 m × 0.1 mm × 0.1 μm) as a second column. For two-dimensional analysis, the modulation time was optimized and set at 5 s and mass spectra were collected at a rate of 150 scans/s. Identification of volatiles was performed in two ways, depending on the 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 the reference standard of the investigated compound was available. In the case of 1,5-octadiene-3-one, the MS signal of the analyte was too weak to facilitate mass spectra comparison. In this case, a standard was also not available; therefore, tentative identification was performed on the basis of the comparison of RI with that available in the literature. Also, the odor characteristics for an analyzed compound were 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 AEDA.[21] 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 then stepwise diluted by a factor of 2 by addition of diethyl ether, and each sample of the dilution series was analyzed until no odor was perceivable at the sniffing port. Retention data of the compounds were expressed as RI on both columns.

Quantitation by Stable Isotope Dilution Assays (SIDA) and Standard Addition (SA) Method. For quantitation of 11 compounds, stock internal standards of the respective labeled isotopes were prepared in diethyl ether and added to the cereal coffee brew sample in the concentration similar to that of the relevant analyte present. The suspension was stirred, and volatiles were isolated as described before. Distillates were analyzed by GCxGC-ToFMS, monitoring the intensities of the respective ions given in Table 1. For all compounds, response factors were calculated in the standard mixture of labeled and unlabeled compound in known concentration. The concentrations in the sample were calculated from the peak area of the analyte and its corresponding internal labeled standard obtained for selected ions.[22] For the remaining six compounds, the standard addition method has been used.[23] Linearity for the standards curves was calculated as the regression coefficient and presented in Table 1. The calculation for both quantitation methods was done using Chroma TOF software (version 3.34).

Table 1. Method of Quantitation, Quantitation Ions, Response Factor, and Regression Coefficient of Calibration Curves Used for Concentration Calculations of 17 Key Odorants Present in the Cereal Coffee Brew

compound	method of quantitation[a]	quant ions UL/L[b]	Rf/r^2 [c]
2,3-butanedione	SIDA/$^{13}C_4$	86/90	0.9
2,3-pentanedione	SIDA/$^{13}C_4$	100/104	1.2
(*Z*)-4-heptenal	SA	84	r^2 = 0.983
3-(methylthio)-propanal	SIDA/$^{2}H_3$	104/107	0.8
2-furfurylthiol	SIDA/$^{2}H_2$	114/116	0.9
2-acetyl-1-pyrroline	SIDA/$^{13}C_2$	111/113	0.9
1-octen-3-one	SIDA/$^{2}H_3$	70/73	1.1
3-mercapto-3-methylbutyl formate	SA	102	r^2 = 0.979
phenylacetaldehyde	SIDA/$^{13}C_2$	91/93	0.8
4-hydroxy-2,5-dimethyl-3-(2*H*)-furanone	SA	128	r^2 = 0.985
2-methoxy phenol	SIDA/$^{2}H_3$	124/127	0.9
2-ethyl-3,5-dimethylpyrazine	SIDA/$^{2}H_5$	135/140	1.3
2-thenylthiol	SA	130	r^2 = 0.981
3(*sec*-butyl)-2-methoxypyrazine	SA	138	r^2 = 0.992
3-isobutyl-2-methoxypyrazine	SIDA/$^{2}H_2$	124/126	1.1
2-methoxy-4-vinyl phenol	SIDA/$^{2}H_3$	150/153	0.9
(*E*,*E*)-2,4-decadienal	SA	81	r^2 = 0.979

[a]SIDA, stable isotope dilution assay; SA, standard addition. [b]UL, unlabeled; L, labeled. [c]Rf, response factor between analyzed compound and its internal standard (labeled isotope); r^2, regression coefficient

Sensory Evaluation. Sensory analyses were performed by a 12-member panel experienced in descriptive analysis. Aroma profile analyses were performed by orthonasally scoring 10 odor qualities on a 5 cm linear scale anchored on either side for the intensity of attributes as "none" and "very strong". The odor descriptors were determined in preliminary tests performed for cereal coffee brew as well as for brews made from separate ingredients. The 100 mL of freshly brewed cereal coffee or its roasted ingredients were presented to the panelists in 200 mL glass containers. The results from linear scale were converted into numerical values for data analysis.

RESULTS AND DISCUSSION

Identification of Key Aroma Compounds in Cereal Coffee Brew and Its Roasted Ingredients by the Means of GCO AEDA Analysis. The sensory analysis had revealed a considerable difference between the aroma profile of cereal coffee brew and its ingredients. The biggest difference was observed for roasted rye and sugar beet samples. Panelists scored much lower on the odor notes such as coffee-like, popcorn-like, nutty, and smoked in sugar beet, Figure 1. On the other hand, the aroma of roasted barley brew resembled the aroma of cereal coffee brew in the best way, with a similar intensity pattern of all attributes. This can be explained by the cereal coffee compositions, which mostly consist of barley (45%). For coffee-like odor, note rye scored similar to cereal coffee brew and barley. Chicory had a similar intensity of smoky note as cereal coffee brew and barley but being more intense in a buttery note. Coffee-like, popcorn-like, nutty, and smoky were the most intense attributes in coffee brew, as well as in its main ingredients.

Coffee is one of the most complex food products in terms of the number of volatile compounds. Cereal coffee, being the

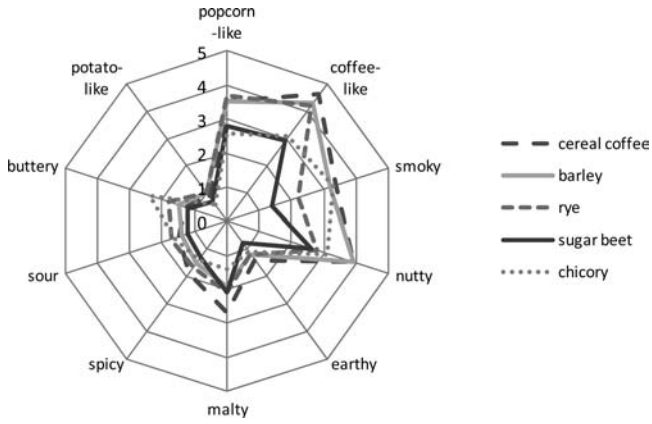

Figure 1. Sensory aroma profiles of cereal coffee brew and brews obtained from its roasted ingredients: barley, chicory, rye, and sugar beet.

product of roasting barley, chicory, sugar beets, and rye, is a product of a comparable degree of complexity. The number of volatile compounds depends on the method of sampling, separation, and detection used, but using comprehensive gas chromatography (GCxGC), the chromatograms can be extremely complex and the number of peaks can easily exceed 1000 compounds. Mondello et al.[24] by investigating volatiles of coffee beans showed great separation power of two-dimensional gas chromatography. Additionally, the separation and detection power of GCxGCMS systems (quadrupole and time-of-flight) were compared, where authors focused on identification of the volatile compounds in Arabica and Robusta and in their blend evaluating 44 main volatiles.[25] As the number of volatile compounds is vast, a sensory guided approach has to be used to select compounds playing a crucial role in formation of cereal coffee flavor.

To evaluate which compounds are responsible for the aroma of cereal coffee brew, AEDA was applied to the aroma concentrates obtained using the SAFE method. This approach was also used for characterization of key odorants in a brew made from ingredients used for the cereal coffee production. For cereal coffee brew, olfactometry analysis revealed 30 compounds having FD factors not less than 16, Table 2. The compounds with the highest FD were the following: 2-furfurylthiol with coffee-like aroma (FD 4096), 3-(methylthio)-propanal (boiled potatato, FD 2048), 2-thenylthiol (coffee, FD 2048), 2,3-butanedione (buttery, FD 2048), phenylacetaldehyde (honey, FD 1024), 2-ethyl-3,5-dimethylpyrazine (roasted, earthy, FD 1024), 3(*sec*-butyl)-2-methoxy pyrazine (earthy, pepper-like, FD 512), and 2-methoxy phenol (phenolic, meat FD 512). Comparing ingredients used for cereal coffee brew production, the weakest overall FD values were noted for roasted sugar beet. The phenolic, meat note was the most abundant (FD 512), followed by buttery, roasted, and coffee-like. Also, roasted chicory, although showing many sensory important compounds, had no compounds which FD exceeding 128. The highest values were noted for the phenolic, meaty note and buttery one. Brew made from roasted cereal grains resulted in a more abundant profile of sensory active compounds. Roasted rye, and especially roasted barley, exhibited many highly odor active compounds and the overall profile of odorants was similar, with differences being quantitative, not qualitative. The most pronounced notes were buttery, boiled potatoes, coffee, roasted, honey, coffee/

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Table 2. Key Aroma Compounds Identified in Cereal Coffee Brew and Brews Made from Its Roasted Ingredients: Barley, Rye, Chicory, and Sugar Beet

				FD[a]				
odor[b]	compound[c]	RI[d] SPB5	Supelcowax-10	cereal coffee brew	roasted barley brew	roasted rye brew	roasted sugar beet brew	roasted chicory brew
burnt	methanethiol	<500	<500	64	32	2	64	16
buttery	2,3-butanedione	580	990	2048	1024	512	128	128
malt	3-methylbutanal	650	940	16	16	4		8
buttery	2,3-pentanedione	697	1060	256	64	16	4	16
fresh, green grass	hexanal	803	1075	64	64	4	4	8
cabbage	unknown	840		32	64	4	4	32
cheesy, old socks	2(3)-methyl butanoic acid	860	1660	32	32	4	8	16
meat	2-methyl-3-furanthiol	870	1309	32	32			16
rancid	(*Z*)-4-heptenal	901	1240	128	128	256	16	32
boiled potatoes	3-(methylthio)-propanal	906	1455	2048	1024	256	4	16
coffee	2-furfurylthiol	911	1432	4096	1024	512	16	64
popcorn	2-acetyl-1-pyrroline	922	1327	256	128	256	4	16
garlic	dimethyl trisulfide	965	1367	32	64	2	4	16
mushroom	1-octen-3-one	978	1298	256	64	4	64	32
geranium	1,5-octadien-3-one[e]	981	1373	64	32	4	4	16
roasted	unknown	1018		32	32	4		4
roasted, catty	3-mercapto-3-methylbutyl formate	1025	1603	512	512	16	16	32
honey	phenylacetaldehyde	1048	1642	1024	256	16	16	64
cotton candy	4-hydroxy-2,5-dimethyl-3-(2*H*)-furanone	1065	2035	128	64	16	4	16
phenolic, meat	2-methoxy phenol	1089	1850	512	1024	1024	512	128
roasted, earthy	2-ethyl-3,5-dimethylpyrazine	1090	1480	1024	512	256	128	64
coffee	2-thenylthiol	1099	1685	2048	1024	256	128	64
earthy, pepper-like	3(*sec*-butyl)-2-methoxypyrazine	1159	1495	512	128	4	4	32
cucumber	(*E*,*Z*)-2,6-nonadienal	1161	1583	32	8	16	4	4
boiled meat	2-methyl-[3-methyldithio]-furan	1172	1655	64	32	4		16
coffee, chili pepper	3-isobutyl-2-methoxypyrazine	1212	1500	256	128	4		16
earthy	unknown	1220		64	64	16	4	4
smoky	2-methoxy-4-vinyl phenol	1315	2193	128	64	4		8
fatty, oily	(*E*,*E*)-2,4-decadienal	1321	1755	256	64	4	4	16
boiled fruits	*β*-damascenone	1500	1815	16	32	4		4

[a]Flavor dilution factor on DB-5 column. [b]Odor perceived at the sniffing port. [c]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. [d]Retention indices. [e]Tentatively identified. The MS signal was too weak for an unequivocal interpretation. The compound was identified on the basis of RI and odor note compared to that available in the literature.

chili pepper. Comparing the main odors detected in GC−O analysis of brew made from roasted cereals to the odor notes of cereal coffee brew, it is evident that the aroma of cereal coffee is mainly influenced by its cereal ingredients, in this case barley. As barley is a basic ingredient in analyzed cereal coffee (45%), this relation has been clear observing data in Table 2.

Compounds responsible for the particular odor notes were identified using both single dimension as well as two-dimensional gas chromatography with mass spectrometry (GC/MS and GCxGC-ToFMS, respectively). The identification was performed using retention data on two columns of different polarity, comparing its mass spectrum and odor notes with authentic standards, Table 2. Of 30 sensory active compounds, 26 were identified using the criteria described above, one (1,5-octadien-3-one) was identified without MS identification, and three remained unidentified.

The most odor active compounds (comparing FD values) in cereal coffee brew were 2-furfurylthiol (FD 4096), 2,3-butanedione, 3-(methylthio)-propanal, 2-thenylthiol (FD 2048), phenylacetaldehyde, 2-ethyl-3,5-dimethylpyrazine (FD 1024), 3(*sec*-butyl)-2-methoxypyrazine, 3-mercapto-3-methylbutyl formate, and 2-methoxy phenol (FD 512). Coffee odor notes were associated with two thiols: 2-furfurylthiol and 2-thenylthiol. Coffee/chili pepper note was associated with a presence of 3-isobutyl-2-methoxypyrazine; however, its impact for the overall aroma was smaller.

The literature data on odor-active compounds of cereal coffee or its ingredients is scarce. Comprehensive two-dimensional chromatography−time-of-flight mass spectrometry (GCxGC-ToFMS) was used to identify volatiles of roasted barley but without an insight into the sensory importance of the compounds.[9] On the basis of the highest probability, the similarities and reverse matches of 64 compounds were identified. The detected compounds represented aromatic hydrocarbons, thiophenes, benzofurans, phenols, esters, alcohols, and furans. Although authors detected numerous

pyrazines, no methoxy pyrazines were detected. Additionally, no thiols described in Table 2 were identified in roasted barley in a work of Bianchi et al.[9] Moreover, Baek and Cadwallader[12] identified 1-octene-3-one and 2-ethyl-3,5-dimethylpyrazine as the most odor active compounds of roasted chicory using dynamic headspace, and additionally 3-methylbutanal and 2,3-butanedione using simultaneous steam-distillation−solvent extraction.

Important odorants in cereal coffee brew resemble that of Arabica or Robusta coffee when literature data is compared. Several very important papers on the aroma active compounds in coffee have been written by Grosch and co-workers[14,26−29] or others.[30,31]

In a work of Sanz and co-workers,[30] the predominant compounds regarding their FD values were (*E*)-*β*-damascenone, 2-methoxyphenol, 4-vinyl-2-methoxyphenol, and abhexone (FD > 4096), followed by methional, 3-mercapto-3-methylbutyl formate, 4-ethyl-2-methoxyphenol, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanoneethylfuraneol, and vanillin (FD 1048). The earlier studies of Semmelroch and Grosch[29] showed, on the basis of high FD factors, that 2,3-butanedione, 2,3-pentanedione, 3-methyl-2-butenthiol, methional, 2-furfurylthiol, and 3-mercapto-3-methylbutylformate were the key odorants of the powders of both analyzed coffees: Arabica and Robusta. Furthermore, comparison of the potent odorants of the coffee powder with the coffee brew revealed a distinct difference in the decrease of thiol FD factors in the coffee brew. Authors showed that only two compounds of coffee brew agreed with those of the potent odorants of the coffee powder: 2,3-butanedione and 2,3-pentanedione.[29]

Compounds that were identified and presented in this research paper as the most important for the cereal coffee brew flavor, based on their FD values, were also presented in coffee, although their FD values and proportions differed. The potent buttery note in cereal coffee brew, represented by 2,3-butanedione (FD 2048) as well as a compound with coffee-like flavor, 2-furfurylthiol (FD 4096), have been reported to reach much lower FD in a coffee powder and coffee brew.[14,30] A similar situation was observed for honey/flowery like compounds: phenylacetaldehyde and 3(*sec*-butyl)-2-methoxypyrazine with an earthy odor note. On the other hand, phenols such as 2-metoxyphenol or 4-ethyl-2-methoxyphenol had a higher FD in freshly filtered coffee brew than in cereal coffee brew.[29] However, one should remember that this comparison of FD values is very subtle, as FD is highly dependent on extraction and concentration steps of sample preparation.

Techniques such as AEDA, which ranks the odor activities of a single odorant on the basis of their odor threshold in air, do not reflect on the odorant/food matrix interaction. Therefore, the odor activity value (OAV) concept[32] was applied in this study to the odorants of cereal coffee brew. It involves calculation of the aroma active compounds concentration and relating it to the odor threshold in the matrix resembling analyzed product. For that reason, two quantitation methods have been applied—stable isotope dilution analysis (SIDA) and standard addition (SA)—to assess the concentration of 17 key odorants of cereal coffee with their FD factors at least 128. Both methods have been described in detail in the Materials and Methods section of this article. Odor threshold values (in water) for each odorant were obtained from the literature.[33]

The results of the calculation of the odor activity values for 17 compounds present in the cereal coffee brew have been revealed in Table 3. The most significant according to OAV

Table 3. Concentration, Odor Thresholds, and Odor Activity Values of Aroma Active Compounds of Cereal Coffee Brew

	compound	OT[a] (μg/L water)	concentration[b] (μg/L brew)	OAV[c]
1.	2-furfurylthiol	0.012	24	2000
2.	3-mercapto-3-methylbutyl formate	0.003	2.5	833
3.	3-isobutyl-2-methoxypyrazine	0.002	1.2	600
4.	2-ethyl-3,5-dimethylpyrazine	0.04	17	425
5.	2-thenylthiol	1	151	151
6.	2,3-butanedione	15	2240	149
7.	2-methoxy phenol	1	137	137
8.	2-methoxy-4-vinyl phenol	5	520	104
9.	3(*sec*-butyl)-2-methoxypyrazine	0.001	0.1	100
10.	2-acetyl-1-pyrroline	0.1	8	80
11.	3-(methylthio)-propanal	0.2	11	55
12.	2,3-pentanedione	30	1550	52
13.	4-hydroxy-2,5-dimethyl-3-(2*H*)-furanone	30	1450	48
14.	(*E*,*E*)-2,4-decadienal	0.16	6.6	41
15.	(*Z*)-4-heptenal	0.06	1.1	18
16.	phenylacetaldehyde	4	65	16
17.	1-octen-3-one	0.04	0.5	12.5

[a]Odor thresholds in water.[40] [b]Mean values based on three replicates with RSD value ≤12%. [c]Odor activity values calculated by dividing the concentration of analyte by its odor threshold value.

odorant was, without a doubt, 2-furfurylthiol with the OAV of 2000 followed by the 3-mercapto-3-methylbutyl formate (833), 3-isobutyl-2-methoxypyrazine (600), and 2-ethyl-3,5-dimethylpyrazine (425). Their concentration altogether did not exceed 45 μg/L of cereal coffee brew; however, they still obtained very high values of OAV which was a consequence of their very low odor thresholds, below 0.012 μg/L. 2-Fururylthiol with the coffee-like scent and 3-mercapto-3-methylbutyl formate with roasted odor notes have also been determined as some of the most important aroma active compounds of coffee brew. However, the concentration of 3-mercapto-3-methylbutyl formate was 2 times lower in cereal coffee brew than in natural coffee brew.[2,27] In addition, it has been noted that very similar amounts of 3-isobutyl-2-methoxypyrazine and 2-ethyl-3,5-dimethylpyrazine were obtained for cereal coffee brew as compared for that reported by Mayer et al. in coffee brew.[27] On the other hand, the second coffee-like smelling compound, 2-thenylthiol, has not been reported as an aroma active compound of coffee, but it has been found in roasted hazelnut paste in a concentration of 9.3 μg/kg, which is 16 times less than in analyzed cereal coffee brew.[34] Among all detected potent odorants of cereal coffee brew, 2,3-butanedione and 2,3-pentanedione were presented in the highest amounts (2240 and 1550 μg/L, respectively) which corresponds to the natural coffee brew. In contrast to the results obtained for regular coffee,[27] our quantitative results have shown a large variation in the concentration of 4-hydroxy-2,5-dimethyl-3-(2*H*)-furanone which was almost 5 times less in a cereal coffee brew than in regular coffee made from coffee beans. The amounts of both phenolic compounds, 2-methoxy phenol and 2-methoxy-4-vinyl phenol, did not differ significantly from regular coffee brew. Although the subsequent six aroma active compounds of cereal coffee brew such as 3(*sec*-butyl)-2-methoxypyrazine, 2-acetyl-1-

pyrroline, (*E*,*E*)-2,4-decadienal, (*Z*)-4-heptenal, phenylacetaldehyde, and 1-octen-3-one are not revealed in research on potent odorants of coffee powder or brew, they are mentioned in several scientific articles on the aroma of roasted or thermally treated foods such as french fries,[35] baguettes,[36,37] extruded potato snacks,[38] roasted shrimps,[39] peanut meal,[40,41] cocoa powder,[42] or hazelnut paste.[34]

The obtained results showed that all quantitated 17 compounds were present at a concentration higher than their threshold value (OAV > 1); therefore, they all potentially contribute to the overall aroma of cereal coffee brew, though the definitive answer would be provided if the omission experiment had been carried out. The majority of those compounds were previously reported as aromatic compounds either of regular coffee or other roasted food stuffs, forming the group of thermally produced compounds following the pathways of generally called Maillard reactions. This can lead to the conclusion that the difference in the overall aroma of those specific roasted products or their derivatives is more in their concentration amount rather than in their composition.

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Notes

The authors declare no competing financial interest.

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