Chemometric fatty acidomics to distinguish between yeast and sourdough breads from Serbia and Turkey

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Summary

A targeted gas chromatography-flame ionization detection profiling method was used to study the distribution of fatty acids amongst yeast and sourdough breads, as a basis for developing a novel strategy for classification into relevant groups employing chemometric algorithms. Authentic sourdough bread samples were produced by spontaneous sourdough fermentation. Common yeast-leavened breads, made from refined and wholegrain flours, were obtained from commercially sources. Retail samples were collected from Serbian and Turkish markets. Bread crumbs, crusts and whole slices were analysed separately. C18:2n6c, C18:1n9c, C16:0, C18:0, and C18:3n3 were the most abundant fatty acids in all bread samples. Short-chain and odd-chain fatty acids were detected, however, in minor quantities. Principal component analysis was used to explore differences between bread types and gave an insight into the potential of a fatty-acidomic approach for authentication purposes. The similarity percetage (SIMPER) test allowed the selection of fatty acids with a high discrimination potential, which were further employed as matrices for construction of a discriminant analysis classification model. Fatty acids proved to be excellent chemical descriptors for classification of bread crumbs, resulting in 100% correct classification obtained for two groups, namely, spontaneous sourdough versus yeast fermentation and wholegrain versus refined wheat flour content.

Keywords

sourdough bread; yeast bread; wholegrain flour; refined flour; gas chromatography; fatty acidomics; chemometrics

The development of chromatographic technologies has enabled the study and understanding of lipid biochemistry and the role that lipids play in the pathology of many diseases [1]. An increasing body of evidence indicates that long-, medium- and short-chain free fatty acids serve, not only as energy sources, but are also intertwining metabolism and immunity in multiple ways, such as via inflammation regulation and secretion of peptide hormones [2]. Many studies prove that mono-unsaturated, poly-unsaturated fatty acids of the *n*-6 and *n*-3 series, and short-chain fatty acids, even in small quantities, are associated with a reduced risk for various health disorders, such as coronary heart diseases [1, 3–7].

Nowadays, sourdough bread and other sourdough-based bakery products (e. g. biscuits, crackers, pastry, pizza or pasta) are enjoying an increasing popularity as convenient, nutritious, stable, natural, low processed and health-promoting foods. The use of sourdough in bakery industry has received a great attention worldwide in recent years [8]. It needs to be pointed out that no national legislation anywhere in the world regulates and protects traditional or typical sourdough breads. However, the use of sourdough has to be guaranteed to meet both bakery and consumer expectations, and to fulfil legal requirements [9]. In contradiction, there are only a few papers published in 2000s, reporting procedures

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for bread authentication. BRESCIA et al. [10, 11] suggested the application of nuclear magnetic resonance (NMR) and inductively-coupled plasma mass spectrometry (ICP-MS) methods to differentiate between sourdough breads obtained from common and durum wheat flours, and to determine geographical origin of breads from southern Italy. BIANCHI et al. [12] reported a gas chromatography-mass spectrometry (GC-MS) method for authentication of Protected designation of origin (PDO) Italian durum sourdough 'Altamura' bread, using volatile compounds as characteristic markers. Some studies demonstrated the potential of alkylresorcinol levels as markers for determining wholegrain and refined wheat and/or rye content in bread using gas chromatography [13, 14]. Other investigations employed GC-MS [15], high-performance liquid chromatography (HPLC) combined with single nucleotide polymorphism analysis [16], near-infrared spectroscopy (NIRS) with hyperspectral imaging [17] or liquid chromatograpy-tandem mass spectrometry (LC-MS/MS) [18] to determine the type of flour in bread, reveal the substitution of more expensive with cheaper grains or trace specific durum wheat cultivars.

Whithin the frame of bread authentication, most published research articles employed polymerase chain reaction (PCR) [19–21]. Research closely linked to sourdough bread authentication applied a quantitative PCR to detect lactic acid bacteria in bread and, in this way, to discriminate between breads made with and without sourdough fermentation [9].

An improvement in the sensorial, nutritional, textural, and shelf-life properties of baked goods containing sourdough is now well documented [8]. To date, authentication of sourdough breads solely relies on food labels, self-declared by manufacturers, and the determination of various parameters, such as pH value or content of lactic acid and acetic acid [9]. Thus, there is evidently a lack of analytical methods for authentication of breads, especially more valuable sourdough breads. In this regard, there is a strong obligation for the scientific community to work on developing accurate, reliable and robust methods for verifying authenticity of sourdough breads and other bakery products, using sophisticated analytical instrumentation linked with modern statistical and data processing tools. Targeted analytical methods or profiling strategies, in which specific chemical compounds are monitored, are frequently employed for these purposes [22].

Considering higher prices of sourdough and wholegrain breads, the main purpose of this study was to employ chemometric data-processing techniques on gas chromatography-flame ionization detection (GC-FID) data in order to (i) establish fatty acid profiles in breads obtained from Serbia and Turkey, leavened either through a traditional sourdough, or through industrialized yeast fermentation, using both refined and wholegrain flours; as well as to (ii) explore possible correlations and select chemical markers that will have the potential to authenticate fermentation method and/or type of flour used in the formulation. Bread crumbs, crusts and whole slices were investigated separately. According to the authors' knowledge, this is the first paper employing chemical descriptors for the authentication of sourdough bread, as a more valuable alternative to common bread.

MATERIALS AND METHODS

Bread samples collection

Commercial bread samples were collected in Novi Sad (Serbia) and Istanbul (Turkey) from bakeries, small shops and supermarkets. According to the product label and information from the bakers, collected breads have been produced using refined white wheat or wholegrain wheat flour, some of them containing an addition of wholegrain rye flour. Besides yeast-leavened common bread, sample collection encompassed breads leavened through a sourdough fermentation, but also those produced by the addition of a certain amount of sourdough to common yeast bread. Control samples were baked from wholegrain wheat flour in home conditions, exclusively by sourdough fermentation. The 30 collected and analysed bread samples are presented in Tab. 1, along with the label descriptions, and the pH values measured using a pH-meter for solid samples M150 (Milwaukee Electronics, Milwaukee, Wisconsin, USA). Considering different thermal effects on different parts of bread loafs, surface and inside, fatty acids were separately analysed in bread crumbs, crusts and whole bread slices.

Extraction and instrumentation parameters

A crust, crumb and whole slice (5 g) of each bread sample was weighed on an analytical balance. Hexane (20 ml, 95 %, Sigma-Aldrich, St. Louis, Missouri, USA) was added to each sample and left for 2 h to extract lipids This process will apparently extract only a fraction of lipids [23]. The mixture was filtered using a paper filter and 10 ml of the hexane extract was poured into test tubes and evaporated under nitrogen flow. 2 $mol \cdot l^{-1}$ KOH solution in methanol (200 μl)

Tab. 1. Description of the analysed samples of bread from Serbia and Turkey.

No.	Country	Label	Description	Leavening method	Flour type	pH
1	Serbia	White toast bread	White wheat flour with wheat sourdough	M	W	5.08
2	Serbia	Mixed rye bread	Mixed wheat and rye flour with rye sourdough	M	X	5.06
3	Serbia	Wholegrain bread	Mixed whole-wheat and rye flour with wheat sourdough	M	WG	5.13
4	Serbia	Black bread	Dark wheat flour with wheat sourdough	M	WG	5.31
5	Serbia	Classic toast bread	White wheat flour with wheat sourdough	M	W	5.27
6	Turkey	Toast bread	White wheat flour	Y	W	5.10
7	Turkey	Toast bread	White wheat flour	Y	W	4.81
8	Turkey	Village bread	Whole grain wheat and white wheat flour	Y	WG	4.95
9	Turkey	Multigrain and einkorn bread	Whole grain wheat, other types of flour and seeds	Y	X	4.93
10	Turkey	Village bread	White wheat flour	Y	W	4.94
11	Turkey	Authentic tava bread	White wheat flour with wheat sourdough	M	W	4.90
12	Turkey	Whole rye bread	Whole grain wheat and rye flour with sourdough	M	WG	4.91
13	Turkey	Organic whole wheat bread	Whole grain wheat flour with organic sourdough	M	WG	4.09
14	Turkey	Somun bread	White wheat flour	Y	W	5.09
15	Turkey	Sourdough bread	Whole grain wheat flour with sourdough	M	WG	5.15
16	Turkey	Wood oven bread	White wheat flour	Y	W	5.08
17	Turkey	White bread	White wheat flour	Y	W	4.90
18	Turkey	Sourdough bread	White wheat flour with sourdough	M	W	5.07
19	Turkey	Homemade sourdough bread (authentic) - control	White wheat sourdough	SD	W	4.39
20	Turkey	Homemade sourdough bread (authentic) - control	White wheat sourdough	SD	W	4.70
21	Turkey	Wood oven bread	White wheat flour	Y	W	5.08
22	Turkey	Homemade sourdough bread (authentic) - control	Whole grain wheat sourdough	SD	WG	4.70
23	Turkey	Homemade sourdough bread (authentic) - control	Whole grain wheat sourdough	SD	WG	4.56
24	Turkey	Grm ottoman bread	White wheat flour	Y	W	5.42
25	Turkey	Organic whole wheat bread	Whole grain wheat sourdough	SD	WG	3.96
26	Turkey	Bread with germ	Whole grain wheat flour	Y	WG	5.47
27	Turkey	Traditional village bread	Whole grain wheat flour	Y	WG	5.20
28	Turkey	Sourdough bread with whole wheat	Whole grain wheat flour	SD	WG	4.40
29	Turkey	Whole wheat bread	Whole grain wheat flour	Y	WG	5.32
30	Turkey	White wheat bread	White wheat flour	Y	W	5.41

Authentic homemade sourdough breads were used as control samples (samples 19, 20, 22, 23).
Leavening method: M – yeast leavening with the addition of a certain amount of sourdough, Y – common yeast-leavening method, SD – sourdough fermentation.
Flour type: W – refined white wheat flour, X – mixture of white wheat, wholegrain wheat and rye flour, WG – wholegrain flour.

was added in order to perform derivatization of fatty acids into corresponding volatile methyl esters (FAME). A volume of 2 ml of hexane was added and the mixure was centrifuged for 3 min at 1 300 $\times g$. Sample extract (1 μl) was injected to 6850 series gas chromatograph with a flame ionization detector, equipped with an automatic sample injector (Agilent Technologies, Santa Clara, California, USA). The instrument was controlled by the Agilent Chemstation software (Agilent Technologies). Fatty acid methyl esters from C4 to C24 were separated, including: saturated, *cis*- and *trans*-monounsaturated, and *cis*- and *trans*-polyunsaturated FAME. The standard mixture of 37 FAME (Sigma-Aldrich) was used for identification purposes [24]. Helium was used as a carrier gas, at a flow of 1 ml·min^{-1}. Column and temperature program were modified to improve separation and shorten the analysis time, compared to those suggested by the standard ISO 12966 [24]. A HP-88 capillary column (100 m × 0.25 mm × 0.20 μm, Agilent Technologies) was used with the following temperature program: hold for 2 min at 100 °C, 100–140 °C at 10 °C·min^{-1}, 140–190 °C at 3 °C·min^{-1}, 190–260 °C at 30 °C·min^{-1}, with a final hold for 2 min at 260 °C. The total analysis time was 27 min. The injector and detector temperatures were 250 °C.

Chemometric data processing

The GC-FID fatty acid profiles were used as chemical descriptors of each bread sample to assess their characterization and classification using chemometric data analysis tools. The fatty acid content was expressed as area percentage for each fatty acid present. Raw data matrix was processed as such, but various pre-processing methods were also investigated, namely, mean-centering, autoscaling and Pareto-scaling [25]. To explore differences or similarities between the analysed bread samples and to investigate the authentication potential of the applied targeted fatty-acidomic approach, principal component analysis (PCA) was employed. PCA finds hypothetical variables (components) representing linear combinations of the original variables, accounting for as much as possible of the variance in GC data [26]. The multivariate datasets were reduced to two dimensions - first two principal components, for plotting purposes. All samples were grouped in different ways, according to (i) leavening method: yeast-leavened (Y), sourdough-fermented (SD), mixture of the two (M) and (ii) the flour type used in formulation: refined white wheat flour (W), wholegrain flour (WG), mixture of the two (X). In order to select variables primarily responsible for inter-group differences between the analysed samples, a similarity percentage algorithm (SIMPER test) was used [27]. Euclidean similarity measure was employed to pool all samples and perform one overall multi-group SIMPER. After variables selection, discriminant analysis (DA) classification algorithm was applied, giving a scatter plot of samples along the first two canonical axes, producing maximal and second to maximal separation between all groups. The group assignment was cross-validated by a leave-one-out cross-validation procedure. An open-source PAST 4.06 software (University of Oslo, Oslo, Norway) was used for all calculations [26, 28].

RESULTS AND DISCUSSION

Targeted profiling chromatographic strategies are frequently used in the classification and authentication of food products. In previous works, the authors described the application of fatty acids as chemical descriptors, used in discrimination of various types of flours (cereal versus pseudocereal, small grain versus maize), edible vegetable oils and bread types (wheat flour breads versus blended wheat and buckwheat breads) [15, 27]. The present study evaluated the potential of GC-FID profiles as sample chemical descriptors in the authentication of (i) sourdough versus yeast breads and (ii) wholegrain flour breads versus refined white wheat breads. Regarding the pH values, which are given in Tab. 1, it could be concluded that lactic acid-containing sourdough breads were characterized by lower pH values (pH 3.96–4.70), along with one mixed bread sample (sample 13) produced using common leavening method with the addition of sourdough (pH 4.09).

Principal component analysis

Correlations between fatty acid profiles established by GC-FID were firstly evaluated using an unsupervised exploratory analysis tool – PCA. Its employment enables dimensionality reduction and helps with an enhanced interpretation of the obtained data. Convex hulls in the scatter plots show the areas occupied by points belonging to different groups. The convex hull is the smallest convex polygon containing all points [25]. Fig. 1 and Fig. 2 depict the convex hulls correlation matrix PCA biplots, as PC1 versus PC2, with detected fatty acids as correlation variables. Fatty acids not present in any of the analysed bread samples were, naturally, omitted from the chemometric data treatment: C11:0, C13:0, C18:1n9t, C18:2n6t, C20:3n6, C20:3n3, C22:2, C22:6n9 – from bread crumbs; C11:0, C13:0, C18:1n9t, C18:2n6t, C22:2, C22:6n3 – from bread crusts; and C11:0, C13:0, C18:1n9t, C18:2n6t, C20:3n3, C22:2, C22:6n3 – from whole bread slices. Mean-centering, autoscaling or Pareto-scaling did not change the grouping patterns compared to raw fatty acid data, expressed as peak area percentage.

Evaluation of breads according to the leavening method

Fig. 1 shows groupings of the crumb, crust and whole slice samples of sourdough-fermented breads, yeast-leavened breads and mixed bread produced by the addition of sourdough to common yeast bread. The sourdough bread samples were grouped on the left side of the PCA diagrams,

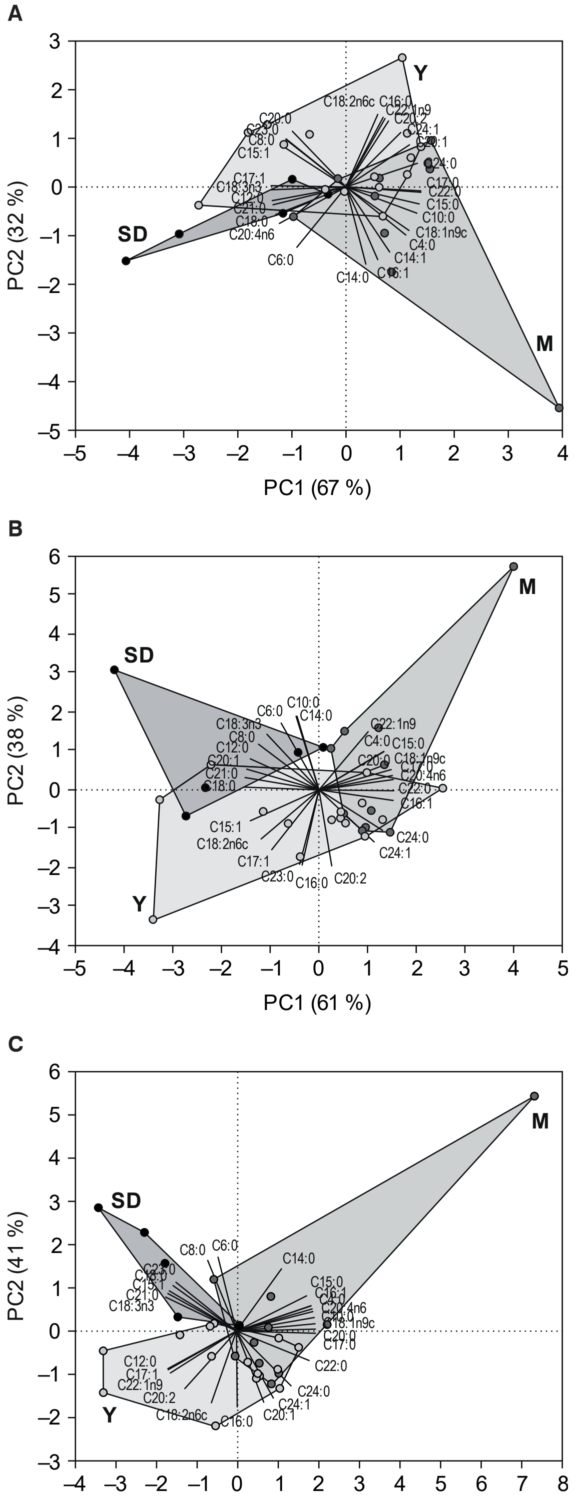

Fig. 1. Principal component analysis convex-hulls correlation matrix bi-plots of fatty acid profiles of bread samples according to the leavening method.

A – bread crumbs, B – crusts, C – whole bread slices.
M – bread samples produced using yeast leavening with the addion of a certain amount of sourdough, Y – bread samples produced using a common yeast-leavening method, SD – bread samples produced using sourdough fermentation.

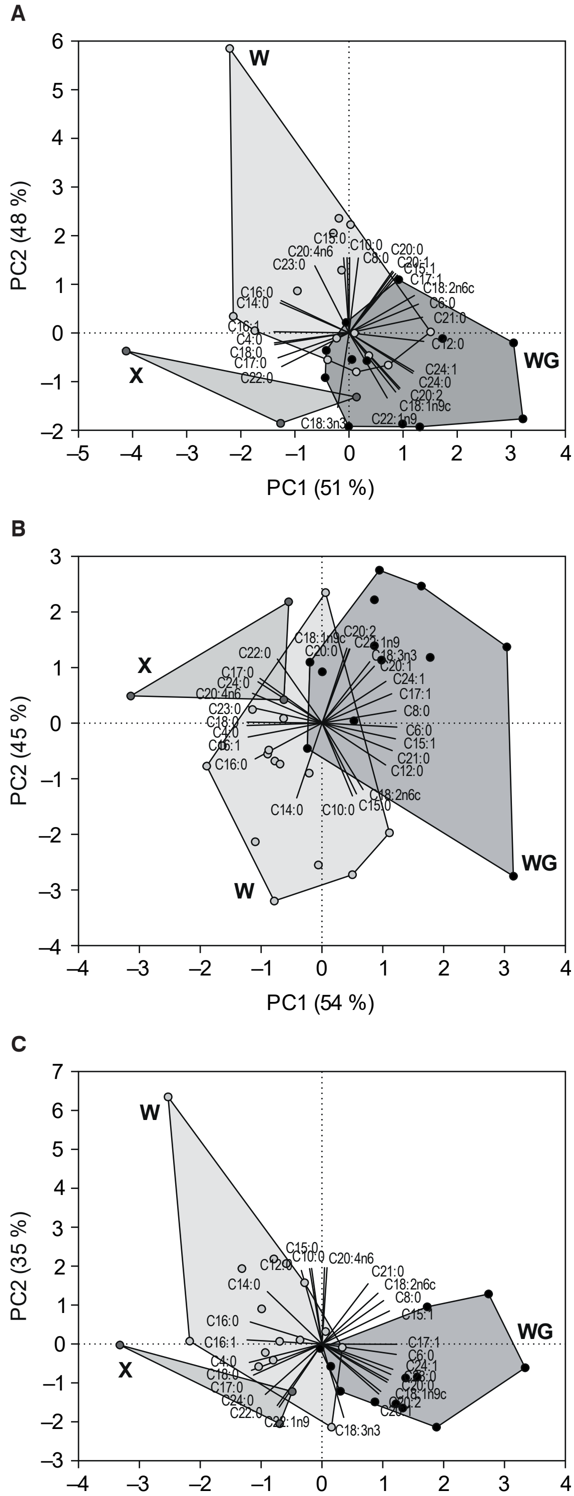

Fig. 2. Principal component analysis convex-hulls correlation matrix bi-plots of fatty acid profiles of bread samples according to the flour type.

A – bread crumbs, B – crusts, C – whole bread slices.
W – bread samples produced using refined white wheat flour, X – bread samples produced using a mixture of white wheat, wholegrain wheat and rye flour, WG – bread samples produced using wholegrain flour.

thereby exhibiting negative scores on PC1 and, thus, a clear discrimination from other groups. Yeast-leavened breads formed groups in the central and left side, while mixed bread samples on the right side of the obtained PCA plots. All three plots were not able to fully separate samples of yeast-leavened and breads obtained with the addition of sourdough in unknown amounts. As represented by the biplot, the whole fatty acid profile could be considered to have a certain effect on different grouping patterns of bread crumbs, crusts and whole bread slices. The first two principal components accounted for 99 % (PC1 67 % and PC2 32 %), 99 % (PC1 61 % and PC2 38 %) and also 99 % (PC1 58 % and PC2 41 %) of the overall variance in the obtained datasets, respectively.

Evaluation of breads according to the flour type

Fig. 2 shows groupings of the samples of crumb, crust and whole slice of wholegrain flour breads, refined white wheat breads, and a blend of the two. In all three PCA plots, representing bread crumbs, crusts and whole bread slices, wholegrain bread samples were gouped in the middle right part of the PCA diagram, exhibiting negative or slight positive scores on PC1, showing a slight overlap with the samples of refined white wheat breads. White wheat breads formed a group in the central part of the obtained PCA plots, while bread samples made of blended flour were positioned on the left side for bread crusts, crumbs and also whole bread slices. Bread samples containing wholegrain rye flour were grouped within the wholegrain group, as expected. As represented by the biplot, the whole fatty acid profile could be considered to have a certain effect on different grouping patterns in cases of bread crumbs, crusts and whole bread slices. The first two principal components accounted for 99 % (PC1 51 % and PC2 48 %), 99 % (PC1 54 % and PC2 45 %) and also 99 % (PC1 64 % and PC2 35 %) of the overall variance in the obtained datasets, respectively.

Discrimination marker selection using a SIMPER test

In order to extract meaningful information from complete fatty acid datasets, i. e. to select fatty acid variables with a maximum contribution to inter-group separations, a SIMPER test was applied. The results are shown in Tab. 2–4 of the supplementary material, representing samples of bread crumbs, crusts and whole bread slices, respectively. The tables encompass information about following groups: (i) sourdough breads, yeast-leavened breads and a mixture of the two leavening methods; and (ii) wholegrain flour breads, white wheat flour breads and mixed breads obtained by blending the two. Based on the results given in Tab. 1S, 2S and 3S, the most abundant fatty acids in crumbs, crusts and whole slices of all bread samples were C18:2n6c (*cis*,*cis*-linoleic acid), C18:1n9c (*cis*-oleic acid), C16:0 (palmitic acid), C18:0 (stearic acid), and C18:3n3 (*cis*,*cis*,*cis*-linolenic acid), with an evidently higher level of C12:0 (lauric acid) present only in crusts of sourdough breads. Short-chain (C4:0 and C6:0) and odd-chain (C21:0, C15:1, C15:0, C17:1, C17:0 and C23) fatty acids were detected in minor quantities, exhibiting a smaller influence to the discrimination procedure. Similar results were obtained in a study by Carocho et al. [29], who determined a high amount of saturated fatty acids present in bread samples analysed, among which palmitic was the most abundant. However, the contents of polyunsaturated fatty acids were even higher, with linoleic being the most abundant. Fatty acids giving a cumulative contribution of 100 % to the inter-group discriminations that were not taken into account for further data evaluation are shown in italics at the bottom of the presented tables.

Discriminant analysis

A SIMPER test was able to select fatty acids, which had a high discrimination potential, thereby identifying discrimination markers for further use in DA model construction. DA enabled a supervised inter-group classification of the analysed bread samples.

Breads classification according to the leavening method

Fig. 3 shows a 95% ellipse classification DA score-plots between groups of yeast-leavened breads, breads obtained by sourdough fermentation and mixed breads obtained by the addition of sourdough to common bread, in crumbs, crusts, and whole slices of the analysed breads. A 100% correct classification was obtained in the case of bread crumbs, while 93.3 % and 96.7 % of correct classifications were obtained for crusts and whole bread slices, respectively. Samples of sourdough breads exhibited positive correlations with Axis 1 in all three cases, while other bread samples tended to have negative or slightly positive scorses on Axis 1. Thus, DA score plots showed an excellent discrimination of the bread samples produced by sourdough fermentation. Tab. 5 in supplementary material contains the confusion matrices produced by the obtained DA model.

Tab. 2. Results of the SIMPER test performed on area percent fatty acid profiles of bread crumbs.

Variable	Leavening methods					Types of flour				
	Contribution [%]	Cumulative [%]	Mean [%]			Contribution [%]	Cumulative [%]	Mean [%]		
			M	Y	SD			W	X	WG
C18:2n6c	42.5	42.4	40.9	42.3	39.9	42.3	42.3	42.2	38.2	41.2
C18:1n9c	28.8	71.2	28.9	25.4	25.1	29.3	71.7	24.8	26.4	29
C16:0	15.1	86.4	14.9	16.8	14.1	15.9	87.6	16.9	17.1	13.6
C18:0	12.4	98.7	9.2	9.4	13.8	11.2	98.8	10	11.7	9.7
C18:3n3	0.4	99.2	2.2	2.3	2.5	0.5	99.3	1.9	3	2.7
C14:0	0.4	99.5	0.9	0.6	0.8	0.2	99.6	0.8	0.8	0.5
C12:0	0.1	99.6	0.4	0.5	0.7	0.1	99.7	0.5	0.2	0.5
C21:0	0.1	99.8	0.1	0.3	0.9	0	99.8	0.3	0.2	0.4
C20:1	0	99.8	0.5	0.5	0.4	0	99.8	0.4	0.5	0.6
C17:1	0	99.8	0	0.1	0.3	0	99.8	0.2	0.5	0.2
C22:0	0	99.8	0.3	0.2	0.1	0	99.8	0.1	0	0.1
C16:1	0	99.9	0.2	0.1	0.2	0	99.9	0.2	0.3	0.1
C6:0	0	99.9	0.1	0.1	0.1	0	99.9	0.1	0	0.1
C10:0	0	99.9	0.1	0	0	0	99.9	0	0	0
C15:1	0	99.9	0	0.1	0.1	0	99.9	0.1	0	0.1
C20:2	0	99.9	0	0	0	0	99.9	0	0	0.1
C20:0	0	99.9	0.3	0.3	0.3	0	99.9	0.3	0.2	0.3
C4:0	0	99.9	0	0	0	0	99.9	0	0	0
C8:0	0	99.9	0	0	0	0	99.9	0	0	0
C15:0	0	99.9	0	0	0	0	99.9	0	0	0
C20:5n3	0	99.9	0	0	0	0	99.9	0	0	0.1
C24:1	0	99.9	0	0	0	0	99.9	0	0	0
C24:0	0	99.9	0	0	0	0	99.9	0	0	0
C17:0	0	99.9	0	0	0	0	100	0	0.1	0
C14:1	0	100	0	0	0	0	100	0	0	0
C20:4n6	0	100	0	0	0	0	100	0	0	0
C23:0	0	100	0	0	0	0	100	0	0	0
C22:1n9	0	100	0	0	0	0	100	0	0	0
C18:3n6	0	100	0	0	0	0	100	0	0	0
C20:3n3	0	100	0	0	0	0	100	0	0	0
C20:3n6	0	100	0	0	0	0	100	0	0	0
C13:0	0	100	0	0	0	0	100	0	0	0
C11:0	0	100	0	0	0	0	100	0	0	0
C18:2n6t	0	100	0	0	0	0	100	0	0	0
C22:6n3	0	100	0	0	0	0	100	0	0	0
C18:1n9t	0	100	0	0	0	0	100	0	0	0
C22:2	0	100	0	0	0	0	100	0	0	0

M – bread samples produced using yeast leavening with the addition of a certain amount of sourdough, Y – bread samples produced using a common yeast-leavening method, SD – bread samples produced using sourdough fermentation.
W – bread samples produced using refined white wheat flour, X – bread samples produced using a mixture of white wheat, wholegrain wheat and rye flour, WG – bread samples produced using wholegrain flour.

Tab. 3. Results of the SIMPER test performed on area percent fatty acid profiles of bread crusts.

Variable	Leavening methods					Types of flour				
	Contribution [%]	Cumulative [%]	Mean [%]			Contribution [%]	Cumulative [%]	Mean [%]		
			M	Y	SD			W	X	WG
C18:2n6c	42.2	42.2	40.2	43.5	42.9	41.4	41.4	44.5	37.6	40.3
C18:1n9c	37.9	80.1	33.8	27.7	26.3	38.1	79.5	26.3	31.4	33.6
C16:0	9.8	90	13.2	15.1	13.6	10.2	89.8	15.5	15.4	12.1
C18:0	8.3	98.3	6.6	7.9	9.4	8.5	98.4	8.1	9.5	6.8
C18:3n3	0.6	98.9	2.5	2.4	3	0.7	99.1	1.9	2.9	3.3
C12:0	0.4	99.4	0.2	0.3	1.3	0.3	99.5	0.4	0.1	0.5
C14:0	0.3	99.8	0.6	0.6	0.7	0.3	99.8	0.9	0.4	0.3
C21:0	0	99.8	0.1	0.2	0.4	0	99.8	0.4	0.6	0.8
C20:1	0	99.8	0.6	0.6	0.6	0	99.9	0.2	0.1	0.2
C8:0	0	99.9	0	0	0.2	0	99.9	0	0	0.1
C22:0	0	99.9	0.4	0.3	0.2	0	99.9	0.3	0.4	0.3
C10:0	0	99.9	0.1	0	0.1	0	99.9	0.1	0	0
C16:1	0	99.9	0.2	0.2	0.1	0	99.9	0.2	0.3	0.1
C6:0	0	99.9	0.1	0	0.1	0	99.9	0.3	0.4	0.4
C20:0	0	99.9	0.4	0.3	0.3	0	99.9	0.1	0	0.1
C17:1	0	99.9	0.1	0.1	0.1	0	99.9	0.1	0.1	0.1
C15:1	0	99.9	0	0.1	0.1	0	99.9	0	0.1	0.1
C20:2	0	99.9	0	0.1	0	0	99.9	0.1	0	0.1
C17:0	0	99.9	0.1	0.1	0	0	99.9	0.1	0.2	0.1
C15:0	0	99.9	0.1	0	0	0	99.9	0.1	0.2	0.1
C24:0	0	99.9	0.1	0.1	0	0	99.9	0	0	0
C24:1	0	100	0.1	0.1	0	0	100	0.1	0.1	0.1
C20:5n3	0	100	0	0	0	0	100	0	0	0
C4:0	0	100	0	0	0	0	100	0	0	0
C23:0	0	100	0	0	0	0	100	0	0	0
C22:1n9	0	100	0	0	0	0	100	0	0	0
C14:1	0	100	0	0	0	0	100	0	0	0
C20:3n6	0	100	0	0	0	0	100	0	0	0
C20:4n6	0	100	0	0	0	0	100	0	0	0
C18:3n6	0	100	0	0	0	0	100	0	0	0
C20:3n3	0	100	0	0	0	0	100	0	0	0
C13:0	0	100	0	0	0	0	100	0	0	0
C11:0	0	100	0	0	0	0	100	0	0	0
C18:2n6t	0	100	0	0	0	0	100	0	0	0
C22:6n3	0	100	0	0	0	0	100	0	0	0
C18:1n9t	0	100	0	0	0	0	100	0	0	0
C22:2	0	100	0	0	0	0	100	0	0	0

M – bread samples produced using yeast leavening with the addition of a certain amount of sourdough, Y – bread samples produced using a common yeast-leavening method, SD – bread samples produced using sourdough fermentation.
W – bread samples produced using refined white wheat flour, X – bread samples produced using a mixture of white wheat, wholegrain wheat and rye flour, WG – bread samples produced using wholegrain flour.

Tab. 4. Results of the SIMPER test performed on area percent fatty acid profiles of whole bread slices.

Variable	Leavening methods					Types of flour				
	Contribution [%]	Cumulative [%]	Mean [%]			Contribution [%]	Cumulative [%]	Mean [%]		
			M	Y	SD			W	X	WG
C18:2n6c	41.2	41.2	39.9	42.7	40.3	40.4	40.4	42.5	37.7	40.7
C18:1n9c	33.9	75.1	31.3	27.7	26.5	34.2	74.6	26.2	28.4	32.4
C18:0	12.4	87.5	8	8.4	12.8	12.3	87.1	16.4	16.5	12.4
C16:0	11.1	98.6	14.6	15.6	13.5	11.6	98.7	9.1	11.1	8.2
C18:3n3	0.5	99.1	2.3	2.4	2.9	0.6	99.3	1.9	2.9	3
C14:0	0.4	99.6	0.9	0.4	0.7	0.3	99.6	0.8	0.6	0.2
C12:0	0.1	99.7	0.2	0.3	0.3	0.1	99.8	0.4	0.2	0.1
C21:0	0.1	99.8	0.1	0.2	0.7	0.1	99.8	0.2	0.2	0.2
C20:1	0	99.8	0.5	0.6	0.5	0	99.8	0.4	0.5	0.7
C22:0	0	99.9	0.3	0.3	0.1	0	99.9	0.3	0.4	0.2
C10:0	0	99.9	0.1	0	0	0	99.9	0.3	0.3	0.1
C6:0	0	99.9	0.1	0.1	0.2	0	99.9	0.1	0	0.1
C16:1	0	99.9	0.2	0.2	0.2	0	99.9	0.1	0	0
C17:1	0	99.9	0	0.1	0.1	0	99.9	0.1	0	0.1
C20:0	0	99.9	0.4	0.3	0.3	0	99.9	0	0.1	0.1
C20:2	0	99.9	0	0.1	0.1	0	99.9	0.3	0.3	0.4
C15:0	0	99.9	0.1	0	0	0	99.9	0.1	0	0
C23:0	0	99.9	0	0	0.2	0	99.9	0	0	0.1
C17:0	0	99.9	0.1	0	0	0	99.9	0.1	0.1	0
C24:0	0	99.9	0.1	0.1	0.1	0	99.9	0.1	0	0.1
C8:0	0	99.9	0	0	0.1	0	99.9	0.1	0.1	0.1
C15:1	0	99.9	0	0.1	0.1	0	99.9	0	0	0
C24:1	0	99.9	0.1	0.1	0	0	99.9	0.1	0	0.1
C20:5n3	0	100	0	0	0	0	100	0	0	0
C4:0	0	100	0.1	0	0	0	100	0	0	0
C14:1	0	100	0	0	0	0	100	0	0	0
C22:1n9	0	100	0	0	0	0	100	0	0	0
C20:3n6	0	100	0	0	0	0	100	0	0	0
C18:3n6	0	100	0	0	0	0	100	0	0	0
C20:4n6	0	100	0	0	0	0.1	100	0	0	0
C20:3n3	0	100	0	0	0	0	100	0	0	0
C13:0	0	100	0	0	0	0	100	0	0	0
C11:0	0	100	0	0	0	0	100	0	0	0
C18:2n6t	0	100	0	0	0	0	100	0	0	0
C22:6n3	0	100	0	0	0	0	100	0	0	0
C18:1n9t	0	100	0	0	0	0	100	0	0	0
C22:2	0	100	0	0	0	0	100	0	0	0

M – bread samples produced using yeast leavening with the addition of a certain amount of sourdough, Y – bread samples produced using a common yeast-leavening method, SD – bread samples produced using sourdough fermentation.
W – bread samples produced using refined white wheat flour, X – bread samples produced using a mixture of white wheat, wholegrain wheat and rye flour, WG – bread samples produced using wholegrain flour.

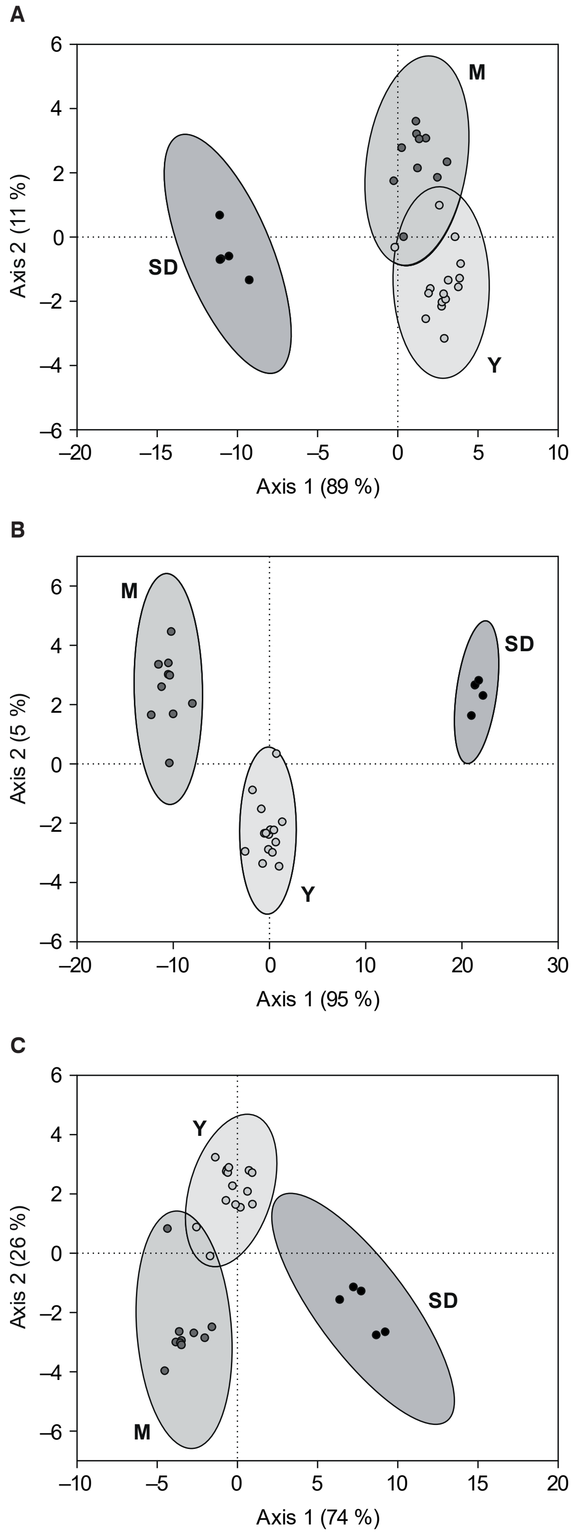

Fig. 3. Discriminant analysis 95% ellipse score-plots classifying bread samples according to the leavening method.

A – bread crumbs, B – crusts, C – whole bread slices.
M – bread samples produced using yeast leavening with the addition of a certain amount of sourdough, Y – bread samples produced using a common yeast-leavening method, SD – bread samples produced using sourdough fermentation.

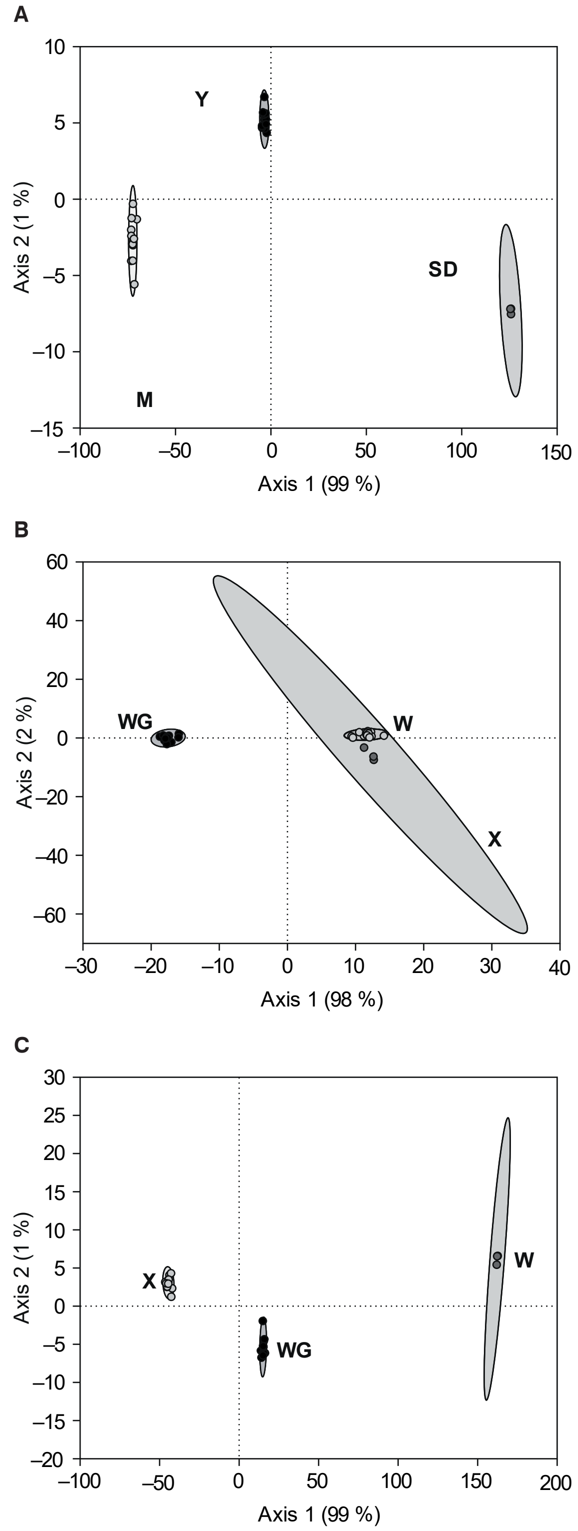

Fig. 4. Discriminant analysis 95% ellipse score-plots classifying bread samples according to the flour type.

A – bread crumbs, B – crusts, C – whole bread slices.
W – bread samples produced using refined white wheat flour, X – bread samples produced using a mixture of white wheat, wholegrain wheat and rye flour, WG – bread samples produced using wholegrain flour.

Tab. 5. Confusion matrices of discriminant analysis classification of breads according to the leavening method.

Leavening method	M	Y	SD	Total
Bread crumbs				
M	10	0	0	10
Y	0	15	0	15
SD	0	0	5	5
Total	9	15	5	30
Bread crusts				
M	9	1	0	10
Y	1	14	0	15
SD	0	0	5	5
Total	9	15	5	30
Whole bread slices				
M	10	0	0	10
Y	1	14	0	15
SD	0	0	5	5
Total	11	14	5	30

M – bread samples produced using yeast leavening with the addition of a certain amount of sourdough, Y – bread samples produced using a common yeast-leavening method, SD – bread samples produced using sourdough fermentation.

Tab. 6. Confusion matrices of discriminant analysis classification of breads according to the flour type.

Flour type	W	X	WG	Total
Bread crumbs				
W	2	6	4	12
X	1	1	1	3
WG	4	0	1	5
Total	7	7	6	30
Bread crusts				
W	16	0	0	16
X	0	3	0	3
WG	1	0	10	11
Total	17	3	10	30
Whole bread slices				
W	16	0	0	16
X	0	3	0	3
WG	0	0	11	11
Total	16	3	11	30

W – bread samples produced using refined white wheat flour, X – bread samples produced using a mixture of white wheat, wholegrain wheat and rye flour, WG – bread samples produced using wholegrain flour.

Breads classification according to the flour type

Fig. 4 shows a 95% ellipse classification DA score-plots between groups of refined white wheat flour breads, wholegrain flour breads and mixed breads obtained by blending the two, in crumbs, crusts and whole slices of the analysed breads. A 100% correct classification was obtained in the case of bread crumbs and whole bread slices, while 96.7 % of correct classification was obtained for bread crusts. Tab. 6 in supplementary material contains the confusion matrices produced by the obtained DA model.

CONCLUSIONS

This study evaluated the distribution of saturated, mono- unsaturated and poly-unsaturated fatty acids of short, medium and long chains in bread samples made of wholegrain, refined wheat and blended flours, produced by various fermentation methods – sourdough, yeast and by blending the two, purchased from the commercial markets in Serbia and Turkey. Fatty acids C18:2n6c, C18:1n9c, C16:0, C18:0, and C18:3n3 showed to be most abundant in crumbs, crusts and whole slices of all bread samples analysed. Short-chain and odd-chain fatty acids were detected in minor quantities. The fatty acidomic approach proved to be able to extract important discrimination markers from the fatty acid dataset, thus enabling classification according to the bread fermentation method and the type of flour used in bread formulation. While unsupervised PCA was able to confirm the potential of the fatty acidomic approach for this purpose, SIMPER test enabled the selection of inter-group descriptors, which were further utilized for model contruction with supervised DA employing a leave-one-out cross-validation. A 100% classification accuracy was obtained in authenticating sourdough fermented breads in the case of analysis of bread crumb analysis but also in authenticating wholegrain flour breads in the case of bread crumbs and whole bread slices. The targeted GC-FID chromatographic profiling strategy was relatively simple and cost-effective, addressing authentication of sourdough and wholegrain flour breads. However, the analysis of a large number of control and commercial samples belonging to each of the given groups would be required to assess the capabilities of the proposed authentication methodology with a higher level of confidence.

Acknowledgements

The presented research was funded by the Scientific and Technological Research Council of Turkey (TÜBITAK) and the Ministry of Education, Science and Technological Development of the Republic of Serbia (Programs no. 451-03-68/2022-14/200134, 451-03-68/2022-14/200222). Furthermore, Dr. Kristian Pastor acknowledges the support from COST Action 18101 – Sourdough biotechnology network towards novel, healthier and sustainable food and bioprocesses *(Sourdomics).*

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Received 11 April 2022; 1st revised 1 July 2022; accepted 2 September 2022; published online 18 October 2022.