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VYSOKÉ UČENÍ TECHNICKÉ V BRNĚ

VYUŽITÍ INSTRUMENTÁLNÍCH A SENZORICKÝCH METOD PRO SLEDOVÁNÍ KVALITY A BEZPEČNOSTI POTRAVIN

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Abstrakt

Tato práce shrnuje hlavní publikované výsledky výzkumu autorky zaměřené na problematiku sledování senzorické kvality potravin s hlavním důrazem na flavour a s ním spojený obsah tzv. aromaticky aktivních látek.

V jednotlivých kapitolách je nejprve charakterizována senzorická analýza jako hlavní teoretický základ této práce, zmíněny jsou instrumentální metody vhodné pro stanovení aromaticky aktivních látek. Pro senzorické hodnocení byly aplikovány metody podle platných ČSN, pro stanovení aromaticky aktivních látek byla zvolena headspace mikroextrakce pevnou fází ve spojení s plynovou chromatografií s plameno-ionizační, resp. hmotnostní detekcí (HS-SPME-GC-FID/MS). Metoda je jednoduchá, rychlá, dostatečně citlivá a přesná, vhodná i pro rutinní využití v praxi.

Další kapitoly shrnují a komentují přehled možných aplikací na vybrané typy potravin s hlavním záměrem pochopit složení flavouru a mechanismus jeho tvorby pro lepší praktickou kontrolu výroby a v konečném důsledku výrobu kvalitnějších a bezpečnějších potravin.

Klíčová slova

Senzorická analýza; flavour; aromaticky aktivní látky; SPME; GC-FID/MS

Abstract

This work summarizes the main published results of author´s research focused on sensory quality of food with the emphasis on flavour connected to aroma active compounds content.

At first sensory analysis as the main theoretical base of this work is characterised, also instrumental methods suitable for assessment of aroma compounds are mentioned. The methods according to the ISO standards were applied for sensory analysis; solid phase microextraction coupled to gas chromatography with flame ionization/mass selective detection (HS-SPME-GC-FID/MS) was used for assessment of aroma compounds. The method is simple, fast, sensitive and accurate, suitable for routine use in practice.

Finally the possible applications on selected food types are discussed to understand flavour formation and composition of single food types for better control of production such consequently produce high quality and safe foods.

Keywords

Sensory analysis; flavour; aroma active compounds; SPME; GC-FID/MS

Poděkování

Na tomto místě bych ráda poděkovala všem svým blízkým za podporu a pomoc při mé práci, tedy rodině, přátelům, kolegům i studentům.

Obsah

1 ÚVOD

Dnešní moderní trh s potravinami nabízí široké spektrum výrobků naší i zahraniční produkce, jejich kvalita je však rozdílná a variabilní.

Výroba většiny potravin je složitý proces, který je ovlivňován mnoha faktory, z nichž některé dosud neumíme ovládat, jako např. počasí, zdraví zvířat aj. Navíc bývá založena na řadě fyzikálních, chemických a biochemických změn, které často, i přes současné vědecké pokroky, neumíme přesně definovat, kvantifikovat a tedy ani ovládat. V praxi se vychází ze spojení teoretických a empirických poznatků, plynoucích z dlouholeté výrobní zkušenosti.

Abychom mohli vyrábět kvalitní potraviny, musíme jednak porozumět procesům, které v nich probíhají a také mít k dispozici dostatečně kvalitní metody pro jejich sledování a kontrolu. Výzkum v této oblasti je tedy velmi žádoucí; tato práce je zaměřena především na význam a možnosti využití senzorické analýzy a souvisejících instrumentálních metod při sledování, hodnocení a řízení kvality a bezpečnosti potravin. Získané poznatky představují důležité informace, které lze v praxi využít pro zkvalitnění a standardizaci jakosti, při vyvíjení nových výrobků aj.

Dnešní konzumenti jsou náročnější, mají více znalostí o výživě a vyžadují bezpečné, nutričně hodnotné, ale i senzoricky kvalitní potraviny; spolu s cenou, nutriční hodnotou, stupněm konvenience a designem obalu patří právě senzorická hodnota k hlavním kritériím, které spotřebitel zohledňuje při nákupu. Na základě senzorické jakosti spotřebitel usuzuje (ne vždy správně) i na kvalitu nutriční a hygienickou.

Asi nejdůležitější z hlediska spotřebitele zůstává chutnost potraviny, pro niž se dnes často používá anglický termín „flavour“, z chemického hlediska tvořený především obsahem těkavých, tzv. aromaticky aktivních látek. Bohužel právě flavour v současné době u řady potravin nedosahuje požadovaných kvalit. To je způsobeno mnoha faktory: (i) šlechtění zemědělských plodin určených pro velkokapacitní produkci je zaměřeno především na dosažení max. výtěžků a případně pouze atraktivního vzhledu; (ii) předčasná sklizeň plodů za účelem lepšího skladování, transportu a prodloužení trvanlivosti; (iii) požadavek spotřebitelů na co nejdelší trvanlivost, kdy aplikace konzervačních zásahů a dlouhodobé skladování způsobuje výrazné a nevratné ztráty flavouru; (iv) pro ČR typický požadavek spotřebitelů na nízkou cenu, výrobci jsou tak v rámci šetření nuceni vybírat levnější, často méně kvalitní a méně chutné suroviny aj.

Cílem našeho výzkumu bylo pomocí senzorických a instrumentálních metod sledovat vybrané chemické a senzorické ukazatele vhodné k posouzení flavouru potravinářských matric a jeho změn v závislosti na způsobu produkce, získávání, technologického zpracování a podmínkách následného uchovávání. Jako modelová matrice bylo použito spektrum potravin a pochutin rostlinného a živočišného původu. Práce shrnuje nejvýznamnější odborné aktivity a hlavní publikované výsledky výzkumu autorky se stručným komentářem již zveřejněných publikací, výsledky jsou doloženy vybranými publikacemi, které vznikly během téměř dvacetiletého působení na FCH VUT v Brně.

2 SENZORICKÉ A INSTRUMENTÁLNÍ HODNOCENÍ KVALITY POTRAVIN

Senzorická analýza je nejčastěji definována jako *„analytická metoda, při níž se senzorické (dříve tzv. organoleptické) vlastnosti stanoví výhradně pomocí lidských smyslů“* [1,2]. Podmínky se volí takové, aby se co nejvíce odstranily rušivé vlivy; tyto podmínky jsou určeny mezinárodními normami, které definují vybavení místnosti, způsob přípravy a předkládání vzorků, postup jednotlivých metod aj. [3,4].

Osoby, které vykonávají senzorickou analýzu, se nazývají „posuzovatelé“ [5] nebo „hodnotitelé“. V rámci prezentovaného výzkumu byli posuzovatelé vybíráni především z řad studentů doktorského studia fakulty chemické, kteří absolvovali základní senzorické zkoušky podle požadavků příslušných norem [6-8], dále byli proškoleni z principů použitých senzorických zkoušek i ze specifických vlastností a vad konkrétních typů testovaných výrobků. Jejich úroveň odpovídala definici „vybraný posuzovatel“ [5]. Druhou skupinu pak tvořili studenti bakalářského a magisterského studia; jejich názory a hodnocení odpovídají běžným spotřebitelům („laický/zasvěcený posuzovatel“ ve smyslu ČSN EN ISO 5492) [5]. Hodnocení spotřebitelů, ač velmi užitečné pro praxi, může být odlišné od hodnocení školených hodnotitelů nebo expertů [1,2,9], proto byly využívány tyto dva panely podle požadovaného účelu hodnocení, nebo byla jejich hodnocení porovnávána, podobně jako v některých publikacích [10-12].

Při stanovení senzorické kvality se u potravin většinou hodnotí: vzhled a barva, chuť (flavour) a vůně a textura (konzistence). Všechny tyto charakteristiky spolu vzájemně souvisejí a spojují se v celkový komplexní dojem, přičemž textura a flavour jsou považovány za nejdůležitější [1,2]. Vzhledem k zaměření této práce je hlavní pozornost dále věnována především flavouru.

Nevýhodou senzorické analýzy je působení subjektivních faktorů [1,2], nicméně za dodržení legislativou daných podmínek [4] je plně srovnatelná s jinými analytickými metodami. Přesto je stále některými odborníky považována za subjektivní a nepřesnou. Proto se v posledních letech řada vědeckých pracovišť, ve spojení s průmyslem, pokouší zavést do senzorické analýzy vhodné instrumentální techniky z důvodu dosažení vyšší přesnosti a spolehlivosti výsledků [2,13].

Instrumentální metody mají nesporně mnoho výhod: např. rychlost, možnost vyhodnocování velkého počtu vzorků, nízká cena, jednoduché (často automatizované) provedení, dobrá opakovatelnost, nízká chybovost, poměrně snadné zpracování výsledků [2,13]. Je však třeba si uvědomit, že zatímco instrumentální analýzou se měří podněty (tj. fyzikální nebo chemické ukazatele), senzorickou analýzou se měří počitky a vjemy (tj. informace (podněty) po zpracování v centrální nervové soustavě). Přístroj může podat informace o intenzitě, nikoli o příjemnosti dané vlastnosti; není tedy schopen hedonické analýzy [2,14]. Z uvedeného vyplývá, že není možné počítat s úplným nahrazením senzorické analytiky přístrojovou; ideální je kombinace obou za účelem potvrzení a objektivizace výsledků senzorické analýzy [13,14].

2.1 Senzorické a instrumentální hodnocení flavouru

Flavour (česky „chutnost“ nebo „komplexní chuťový vjem“) bývá nejčastěji definován jako *„senzorický vjem, zahrnující kombinaci chuti, vůně, pocitu bolesti, tepla a chladu a taktilních počitků v ústní a nosní dutině*“ [1]. Česká legislativa (ČSN EN ISO 5492) definuje flavour jako *„celkovou kombinaci čichového, chuťového a trigeminálního vjemu vnímaného během zkoušení*“ [5]. Za hlavní složku flavouru je považována vůně (aroma), která se na celkovém vjemu podílí cca ze 70–85 % [1,14,15].

Pro označení změněné, nepřirozené vůně a/nebo chuti v důsledku nejrůznějších vlivů se používá anglický termín **off-flavour** („off-odour“), případně české termíny cizí chuť a vůně [5], přípach, příchuť, pachuť apod. [1,2,16].

Charakteristický flavour daného typu výrobku je výsledkem složité rovnováhy směsi složek, z nichž těkavé tvoří aroma/vůni (interagují s čichovými receptory) a netěkavé chuť (interagují s chuťovými receptory). K nim v neposlední řadě přispívají sloučeniny tvořící „mouthfeel“ a texturu [14,15]. Stanovit flavour/off-flavour instrumentálními metodami je velmi složité, protože mechanismus vnímání není úplně znám [15]. Z chemického hlediska bývá většinou redukován na stanovení těkavých tzv. aromaticky aktivních látek (AAL), které tvoří vůni (aroma) potraviny: jsou v celkovém flavouru nejdůležitější a relativně dobře se stanovují, i když i jejich stanovení naráží na řadu problémů [13,14].

Odhaduje se, že v potravinách se vyskytuje až 10 000 těkavých látek, z nichž se dosud podařilo identifikovat cca 7000 [17]. V jednom typu potraviny se jich může vyskytovat až několik set, je tedy třeba použít výkonné dělicí metody; jednotlivé látky se nacházejí v široké škále koncentrací od $ng \cdot kg^{-1}$ do $mg \cdot kg^{-1}$, je nutné používat vysoce citlivé metody. [13]. Ne všechny sloučeniny mají stejný význam, velmi důležitý je tzv. „práh vnímání“, tj. nejnižší koncentrace sloučeniny, kterou je možné vnímat. Hodnoty se liší až v rozsahu několika řádů, příspěvek tedy nemusí být téměř v žádném vztahu ke koncentraci v potravině. Jednotlivé látky se navíc mohou vzájemně ovlivňovat (antagonismus, synergismus), případně interagovat s ostatními složkami potraviny, což není schopen podchytit žádný přístroj [17]. Dřívější snahy vědců směřovaly k identifikaci všech těkavých látek v dané potravině, v současné době se výzkum zaměřuje zejména na identifikaci sloučenin, které jsou pro flavour skutečně důležité (tzv. „klíčové“ substance), tyto se však v mnoha potravinách zatím nepodařilo zjistit [17,18]. Pro pochopení příspěvku jednotlivých AAL k flavouru je ideální kombinace senzorického hodnocení a instrumentálního měření. Výsledky se zpracují různými metodami multivariační statistiky a z naměřených hodnot se zjišťují vztahy, z nichž je možno usuzovat (i) na významnost AAL ve flavouru, (ii) zda ovlivňují flavour kladně nebo záporně [19].

Druhou možností je poměr zjištěné koncentrace dané látky v potravině a její prahové koncentrace. Tento poměr se nazývá „Aroma Value“, „Odour Unit“, „Flavour Unit“, nebo nejč. „Odour Activity Value - OAV“. Hodnota $OAV < 1$ naznačuje, že látku nelze vnímat [17,20]. I přes určité nedostatky (zanedbává synergické a antagonistické účinky), OAV se v praxi osvědčila jako vhodná míra při identifikaci důležitých AAL. Tento přístup, tedy kombinace senzorického hodnocení a instrumentálního stanovení obsahu AAL, a následné zjišťování korelací, případně stanovení OAV, byl použit v řadě publikovaných prací, na sýry např. [21,22], na ovoce a ovocné šťávy [23,24] aj., a byl aplikován i v rámci prezentovaného výzkumu autorky pro posouzení příspěvku různých těkavých látek k flavouru vybraných typů potravin. Pro zjištění souvislostí mezi výsledky senzorických a instrumentálních analýz byly použity nejprve Pearsonovy korelační koeficienty, později vícerozměrné statistické metody

(především Analýza hlavních komponent (PCA)), s využitím softwaru Unistat, v. 5.5 a Statistica, v. 12. Veškeré statistické testování bylo provedeno na hladině významnosti $\alpha = 0,05$.

2.2 Aromaticky aktivní látky

Vonné (aromaticky aktivní) jsou látky, které působí na čichové receptory a vyvolávají olfaktorické vjemy, jinak řečeno vyvolávají dojem vůně. Jsou to většinou málo polární nebo nepolární těkavé látky (Mr < 300)[20,25]. Mohou být přirozenou složkou potraviny jako produkty sekundárního metabolismu (tzv. primární AAL); řada z nich je však v potravinách přítomna ve vázané, senzoricky inaktivní formě, především jako glykosidy nebo estery. Z těchto sloučenin vznikají tzv. sekundární AAL, např. během dozrávání plodin, při zpracování a skladování potravin jako produkty enzymových a neenzymových reakcí. Fermentační pochody a tepelné zpracování (vaření, pečení apod.) jsou hlavními procesy, při kterých tyto látky vznikají, při jejich vzniku se uplatňují především autooxidační reakce, a reakce enzymového a neenzymového hnědnutí [2,25].

Vonné látky lze nalézt prakticky v každé skupině organických sloučenin. Významnými vonnými látkami jsou některé uhlovodíky, většina z nich však obsahuje v molekule kyslík (alkoholy, ethery, aldehydy, ketony, kyseliny, estery, laktony aj.), dusík (např. aminy, dusíkaté heterocykly) a síru (thioly, sulfidy, sirné heterocykly)[20,25].

Vonné látky nejsou pro člověka nezbytné, ale jsou mu prospěšné, zejména svým příznivým působením na jeho psychiku. Většina AAL je biologicky aktivní, vykazují řadu pozitivních účinků (antimikrobiální, antioxidační, analgetické, protizánětlivé, spasmolytické aj.), pro něž nalezly použití jako léčiva nebo složky farmaceutických výrobků. Pro své vonné, případně chuťové vlastnosti se přidávají do potravin, kosmetických a jiných produktů jako aditiva; takto jsou průmyslově využívány stovky až tisíce vonných látek, ukazuje se však, že řada z nich může naopak vykazovat negativní biologické účinky, v současné době jsou diskutovány především možné **alergenní účinky** [26,27]. Tento problém se nejprve objevil v souvislosti s kosmetickými výrobky, a také většina publikovaných prací je zaměřena na kosmetické produkty. Bylo prokázáno, že některé AAL, široce využívané v kosmetice, mohou způsobovat kožní vyrážky, ale i nevolnost, závrať, bolest hlavy, kašel aj. [26,27].

Z důvodu možných nežádoucích účinků bylo v rámci EU nařízení o kosmetických přípravcích (ES/1223/2009) určeno 26 potenciálních vonných alergenů; z nich 24 látek jsou chemicky definované těkavé sloučeniny, zbývající dvě jsou přírodní mechové extrakty. Na základě této direktivy jsou výrobci povinni deklarovat přítomnost uvedených alergenních substancí na etiketě produktu při překročení stanovené koncentrační meze: 0,01 % (100 $mg.kg^{-1}$) pro výrobky typu „rinse-off“ (smývatelné) a 0,001 % (10 $mg.kg^{-1}$) pro výrobky typu „leave-on“ (nesmývatelné). Ostatní AAL mohou být uvedeny pod souhrnným názvem „parfum“ či „aroma“ [28]. Samostatný legislativní předpis pro tyto látky přidávané do potravin však zatím neexistuje, legislativa se věnuje pouze obecně všem AAL v rámci Nařízení 1334/2008/ES a příslušných prováděcích příloh (793/2012/ES a 872/2012/ES). Příloha jmenovitě uvádí látky, které nelze přidávat do potravin jako takové, mohou se však v některých potravinách nacházet jako přirozená složka; ze sledovaných alergenů je zde limitován pouze kumarin kvůli jeho toxicitě [29].

3 POUŽITÉ METODY A INSTRUMENTÁLNÍ TECHNIKY

3.1 Senzorické metody

Z širokého spektra senzorických metod pro hodnocení vzorků v rámci prezentovaného výzkumu autorky byly použity metody vycházející z platných ČSN: pořadová zkouška (ČSN ISO 8587), párová porovnávací zkouška (ČSN EN ISO 5495); vybrané senzorické znaky (vzhled, barva, flavour, vůně, textura a celková senzorická kvalita/přijatelnost) byly hodnoceny pomocí pěti nebo sedmibodových kategorových ordinálních stupnic intenzitního (neznatelná ⇒ velmi silná) nebo hédonického (vynikající ⇒ nepřijatelný) typu, případně grafických stupnic (10 cm) (ČSN ISO 4121); v rámci profilového testu byla hodnocena intenzita vybraných deskriptorů vůně, chuti/flavouru a případný off-flavour (ČSN ISO 11035, ČSN EN ISO 13299) [30-34].

Senzorickému hodnocení flavouru různých typů potravin se věnuje velká řada publikací (viz níže citované), často v kombinaci se stanovením vonných a/nebo chuťových látek. Většinou jsou aplikovány různé typy deskriptivních metod a/nebo spotřebitelské testy. Hlavním cílem **spotřebitelských testů** je získání názoru spotřebitelů/konzumentů na daný výrobek, používají se neškolení hodnotitelé/spotřebitelé [2,9]. Na základě spotřebitelského hodnocení se provádí rozvoj produktů, optimalizace, analýza trvanlivosti apod. Potravinářská praxe si vyžádala vývoj i nových moderních spotřebitelských testů, např. „Free Choice Profile“ nebo „Flash Profile“, které jsou sice náročnější pro hodnotitele, ale podávají více informací o vzorcích [35]. Používají se poměrně často a lze nalézt publikace o sýrech [11,12,36-38], tavených sýrech a analozích [39,40] aj.

Rozlišovací zkoušky jsou používány ke stanovení rozdílu mezi vzorky [1,2]. V publikovaných pracích jsou aplikovány méně často, nejčastěji se používá párová porovnávací (ČSN EN ISO 5495), pořadová (ČSN ISO 8587) nebo trojúhelníková (ČSN EN ISO 4120) zkouška. V praxi nejrozšířenější jsou zkoušky s použitím **stupnic** a lze najít i některé aplikace v odborných publikacích, např. na sýry [41,42], tavené sýry a analogy [43,44] aj.

Deskriptivní metody jsou vysoce sofistikované metody, pomocí nichž lze provést důkladnou, hloubkovou senzorickou analýzu. Postupem času se vyvinula řada variant, např. „Flavour Profile“, „Quantitative Descriptive Analysis“, „Quantitative Flavor Profiling“ aj., které se liší způsobem výběru deskriptorů, výběru a školení posuzovatelů, zpracováním výsledků aj. [2,35]. V publikovaných pracích se používají nejčastěji, jsou zvláště vhodné pro korelaci s instrumentálními nebo konzumentskými daty a lze nalézt aplikace na široké spektrum různých typů potravin. Na sýry např. [10-12,45-49], tavené sýry a analogy [50-52], bobulové ovoce a ovocné šťávy [24,53,54] aj. Mezi deskriptivní metody patří i **senzorický profil** (ČSN EN ISO 13299), aplikovaný v rámci této práce [1,2,34]. Kompletní vyjádření profilu je velmi složité, je zapotřebí sledovat velmi mnoho parametrů, tzv. deskriptorů (u některých typů potravin až 100-150), proto se většinou stanovují profily jen určité vlastnosti. V rámci našeho výzkumu jsme se samozřejmě zaměřili na hodnocení senzorického profilu flavouru. Deskriptory byly vybírány, s ohledem na účel výzkumu, na základě provedené rešerše, zkušeností autorky s daným typem produktu a případně expertů v daném oboru [33].

3.2 Instrumentální metody

Přímé instrumentální stanovení AAL je možné jen zřídka, protože se v potravinách vyskytují většinou ve velmi nízkých koncentracích (ng–mg.kg^{-1}); kromě izolace z matrice vzorku bývá nezbytné i jejich zakoncentrování [55,56]. Izolaci komplikuje řada faktorů: (i) AAL tvoří široké spektrum různých chemických sloučenin odlišných fyzikálních a chemických vlastností; to usnadňuje jejich separaci, ale komplikuje kvantitativní izolaci; (ii) mnoho sloučenin je nestabilních a snadno podléhají vytěkání, oxidační nebo tepelné degradaci; (iii) netěkavé složky vzorku, jako jsou lipidy, proteiny a sacharidy, mají pěnivý a emulzifikační charakter; (iv) AAL jsou často intracelulární a je nutné rozrušení vzorku, přičemž použitý způsob (krájení, mletí aj.) může vést k různému spektru získaných AAL [55,57,58].

Pro **extrakci/izolaci** těkavých sloučenin ze vzorku lze použít různé metody, z nichž každá má určité výhody a nevýhody. Většina z nich využívá jejich těkavost nebo nepolární charakter. V ideálním případě má být izolační proces jednoduchý, rychlý, levný, selektivní, kvantitativní a umožňující automatizaci; podmínky mají být mírné k zábraně oxidačních, tepelných i jakýchkoli jiných změn vzorku [55,57]. Při výběru je třeba mít na paměti, že různé metody zachytí pouze určitou část z celkového obsahu těkavých sloučenin a je otázkou, která poskytne nejvíce reprezentativní směs AAL, tedy nejvíce se blížící vnímání v ústech [18,59]. Tyto úvahy vedly k vývoji nejnovějších systémů simulujících zpracování potravin v ústech (umělá ústa-„Artificial Mouth“) [49], nebo přímo analýza headspace ústní dutiny („Buccal Headspace Analysis“) [60,61].

Jedny z prvních používaných metod byly jednoduchá extrakce rozpouštědlem, destilace s vodní parou (nebo vakuová destilace) a později jejich kombinace, tzv. „Simultánní destilace/extrakce“. Tyto metody lze nalézt zvláště ve starších publikacích, v současné době jsou používány zřídka, především kvůli řadě nevýhod souvisejících s aplikací vysoké teploty a/nebo rozpouštědla [55]. Přesto lze nalézt některé novější aplikace, na sýry např. [47,62-66], bobulové ovoce [67-69] aj. Dnes se tyto klasické techniky nahrazují novými, např. vysokotlaká extrakce rozpouštědlem, extrakce superkritickou tekutinou, mikrovlnná nebo ultrazvuková extrakce, miniaturizovaná verze mikroextrakce kapalnou fází aj., které především usnadňují a urychlují extrakční proces [55,57,70], přesto jsou zatím pro izolaci AAL z potravin používány minimálně, pravděpodobně kvůli jejich horší dostupnosti a vyšší ceně.

Další možností jsou tzv. headspace techniky, tj. extrakce plynem. Z výhod lze zdůraznit především šetrnost procesu, z nevýhod např. vyšší cenu, časovou náročnost, nízkou reprodukovatelnost [56,57]. Zvláště dynamická headspace patří v současnosti k často používaným extrakčním technikám pro zisk AAL a byla aplikována i při analýze sýrů [41,45,71], tavených sýrů a analogů [72], bobulového ovoce [73-75] aj.

Klasickou extrakci kapalina-kapalina nahrazuje v posledních letech stále populárnější zachycení těkavých látek na vhodný sorbent, sem patří tzv. extrakce a mikroextrakce pevnou fází. Extrakci pevnou fází lze v zásadě použít pro zisk nebo přečištění extraktu a/nebo destilátu [55], pro izolaci AAL z potravin se zatím příliš nepoužívá.

Mikroextrakce pevnou fází („Solid Phase Microextraction“ – SPME) je miniaturizovaná verze předchozí metody; analyt je zachycen na vhodný sorbent, zakotvený v tenké vrstvě na povrchu křemenného vlákna. Na rozdíl od klasických extrakčních metod není analyt extrahován ze vzorku úplně, ale pouze do dosažení rovnováhy [58,76]. Vlákno se umísťuje

přímo do vzorku, pro stanovení těkavých AAL je však vhodnější varianta, kdy se vlákno umístí do uzavřeného prostoru nad vzorkem (headspace - HS-SPME). Tento postup je rychlejší, citlivější, vykazuje vyšší selektivitu pro těkavé látky a v neposlední řadě se prodlužuje životnost vlákna. K desorpci dochází v injektoru plynového chromatografu účinkem vysoké teploty, v případě spojení SPME s kapalinovou chromatografií jsou analyty desorbovány vhodným rozpouštědlem [58,76]. Z výhod lze zdůraznit malý objem vzorku, jednoduchost, rychlost (cca 15–60 min.), nízká cena, možnost automatizace, minimalizace tepelné, mechanické i chemické modifikace vzorku. Z nevýhod je to především nízká výtěžnost, robustnost a horší reprodukovatelnost; tyto nevýhody, a především možnost tzv. kompetitivní sorpce (tj. vytlačování již nasorbovaných sloučenin látkami s vyšší distribuční konstantou), limitují použití SPME pro kvantifikaci [58,76].

I přes zmíněné nevýhody a vzhledem k uvedeným výhodám se SPME stává dominantní technikou, byla použita i pro extrakci AAL při stanovení flavouru/off-flavouru v mnoha publikacích; na sýry např. [37,42,47, 65,77-84], na tavené sýry a analogy [85-87], na bobulové ovoce a ovocné šťávy [24,68,88-90] aj.; byly publikovány i některé přehledné práce shrnující možnosti aplikace SPME při měření AAL potravin [58,91,92]. Především pro svou jednoduchost byla zvolena i pro experimenty v rámci předkládaného výzkumu autorky. Pro extrakci těkavých AAL se v současnosti doporučují dva typy vláken: Carboxen/PDMS tloušťky 75 nebo 85 μm, a DVB/CAR/PDMS 50/30 μm [58,91]; tato jsou používána ve většině publikací. Vlákno CAR/PDMS je vhodné pro extrakci molekul s uhlíkatým řetězcem C2-C12 (molekulová hmotnost asi do 200), výrobcem doporučováno obecně pro nízkomolekulární látky. Vlákno DVB/CAR/PDMS je určeno pro látky s řetězcem C3-C20 a molekulovou hmotností cca 40–275, výrobcem je přímo doporučeno pro extrakci těkavých až středně těkavých AAL [56,91]. Vzhledem k dosaženým výtěžkům a tehdejší dostupnosti bylo pro naši práci zvoleno vlákno CAR/PDMS 85 μm.

Ke **stanovení** AAL lze v současné době použít především plynovou chromatografii (GC), kapalinovou, resp. vysokoúčinnou kapalinovou chromatografii (HPLC) a jejich kombinace s hmotnostní detekcí (MS). Z moderních metod lze zmínit elektronický nos nebo jazyk [57,93].

HPLC je méně účinná, pomalejší a nákladnější, používá se spíše pro sledování chuťových látek [57,91], např. organických kyselin, meziproduktů při degradaci aminokyselin aj. [93]. Nejvhodnější a nejčastěji používaná metoda je GC, většinou v kombinaci s plamenově ionizačním (FID) nebo hmotnostním detektorem [55,91]. Byla aplikována ve většině níže citovaných prací a byla použita i v rámci našeho výzkumu. Celá HS-SPME-GC-FID/MS metoda byla optimalizována a validována pro analýzu konkrétních níže diskutovaných potravinářských matric.

Hlavní nevýhodou ostatních technik z hlediska stanovení AAL je fakt, že měří všechny těkavé látky, přestože pravděpodobně jen část z nich je aromaticky aktivní. Tento problém lze překonat kombinací GC s tzv. olfaktometrií (GC-O), kdy jsou jednotlivé těkavé látky separovány pomocí GC a následně identifikovány čichem [17,18,94,95]. Tato metoda je v podstatě jediná možnost, jak zjistit, zda daná sloučenina vykazuje nějakou vůni a/nebo pach; zároveň lze získat popis charakteru vůně a případnou intenzitu v daném vzorku. Pro zajímavost je u dále diskutovaných sloučenin v závorkách uveden charakter jejich vůně/aroma, získaný z literatury; jak je patrné vjem je velmi individuální a v různých publikacích se může výrazně lišit.

Metoda má některé nedostatky: je málo reprodukovatelná, časově náročná, poměrně namáhavá, nelze zjistit synergický efekt [18,94]; v současné době je však velmi používaná a byla publikována řada prací, které se zabývají charakterizací těkavých vonných látek různých typů potravin, vč. sýrů [41,46,64,71,77,79,94], tavených sýrů a analogů [52,72], bobulového ovoce a ovocných šťáv [67,68,73,75] aj.

4 APLIKACE NA VYBRANÉ VZORKY POTRAVIN

4.1 Sýry a sýrové analogy

Vzhledem k předchozímu dlouholetému působení v mlékárenském průmyslu byly vědecko-výzkumné aktivity autorky započaty spoluprací s průmyslem sledováním výroby a zrání přírodních sýrů, konkrétně sýrů plísňových. Díky výrazné pikantní chuti a aroma plísňové sýry patří mezi nejoblíbenější, což bylo potvrzeno i průzkumem mezi studenty FCH VUT v Brně. Podstatou experimentů byl odběr a analýza vzorků během procesu zrání, posouzení kvality konečných výrobků a faktorů, které ji mohou během výroby ovlivňovat. Hlavním záměrem bylo dosáhnout zlepšení celkové senzorické kvality výrobku a její zachování bez ohledu na použité podmínky zpracování a skladování a podpořit tak české výrobce, resp. české výrobky na našem trhu.

4.1.1 Přírodní sýry

Současná česká legislativa, vyhláška č. 397/2016 Sb., definuje **sýr** jako „*mléčný výrobek vyrobený vysrážením mléčné bílkoviny z mléka působením syřidla nebo jiných vhodných koagulačních činidel, prokysáním a oddělením podílu syrovátky*“, zrající sýr jako „*sýr, u kterého po prokysání došlo k dalším biochemickým a fyzikálním procesům*“ [96].

Výroba sýrů je poměrně složitý technologický proces, který vychází ze základní suroviny - mléka. Podstatou je jeho přeměna v gelovitou sýřeninu buď působením bakterií mléčného kvašení (BMK), nebo pomocí syřidla. Byť rozdílným principem, dochází v obou případech k porušení stability kaseinových micel, tím k jejich vysrážení a následnému oddělení mléčného séra, tj. syrovátky. Při následném zpracování vzniklé sýřeniny se lisováním oddělí syrovátka, sýřenina se zformuje do požadovaného tvaru, prosolí a je podrobena procesu tzv. zrání, kde dochází následkem celé řady enzymových i neenzymových reakcí k tvorbě žádoucího vzhledu, barvy, textury, chuti a vůně (flavouru) [97,98].

Z hlediska výživy jsou sýry cenným zdrojem živin, neboť v sobě koncentrují základní složky sušiny mléka; obsahují tedy vysoký podíl proteinů (kaseinů), lipidů, vitaminů a minerálů (vápník) [97].

Senzorická kvalita, vzhledem k velkému počtu různých druhů sýrů, je velmi různorodá. Každý typ sýra má svoje unikátní senzorické vlastnosti, které jsou důsledkem vlastností mléka, jednotlivých kroků výroby a také podmínek v průběhu zrání. U sýrů lze senzoricky posoudit zvuk (souvisí s přítomností ok), chuť, vůni a vizuální aspekty. Při posuzování vzhledu se hodnotí povrch sýra (barva, kůra nebo ochranná vrstva, plísňový porost nebo maz aj.), poté je sýr hodnocen na řezu (barva, přítomnost a tvar ok, struktura, textura aj.). Textura zahrnuje fyzikální vlastnosti, které spotřebitel vnímá prostřednictvím kombinace hmatu, zraku a sluchu. U tvrdých sýrů se hodnotí stlačením vykrojené části mezi ukazovákem a palcem a dokončí se při hodnocení flavouru v dutině ústní [99]. Rozhodujícím faktorem pro chuť (ale i texturu) sýra je poměr mezi sušinou a tučností (tuk v sušině-tvs), tučnější sýr je vždy jemnější, lahodnější a chutnější. Tento fakt potvrzují i studie sýrů se sníženým obsahem tuku, které prokázaly výrazně zhoršený flavour těchto výrobků [85,93,100]. Sloučeniny, které přímo přispívají k chuti sýrů, jsou: kyselina mléčná (kyselá), NaCl (slaná), minerální soli draslíku, vápníku a hořčíku (slaná) a volné aminokyseliny a peptidy (sladká, hořká, umami).

Základní chuť je v různé intenzitě společná všem sýrům a zahrnuje mléčnou chuť (výrazná u čerstvých sýrů), která během zrání přechází v chuť sýrovou (prozrálé sýry). Všechny druhy sýrů mají více nebo méně výraznou kyselou chuť, která se stupňuje od jemně mléčně nakyslé až po silně kyselou; podobně, podle druhu sýra, je gradována i slaná chuť. Druhová chuť a vůně (flavour) je souhrnem vlastností přítomných AAL vzniklých zráním (viz dále) [97].

4.1.1.1 Vznik a vývoj flavouru přírodních sýrů

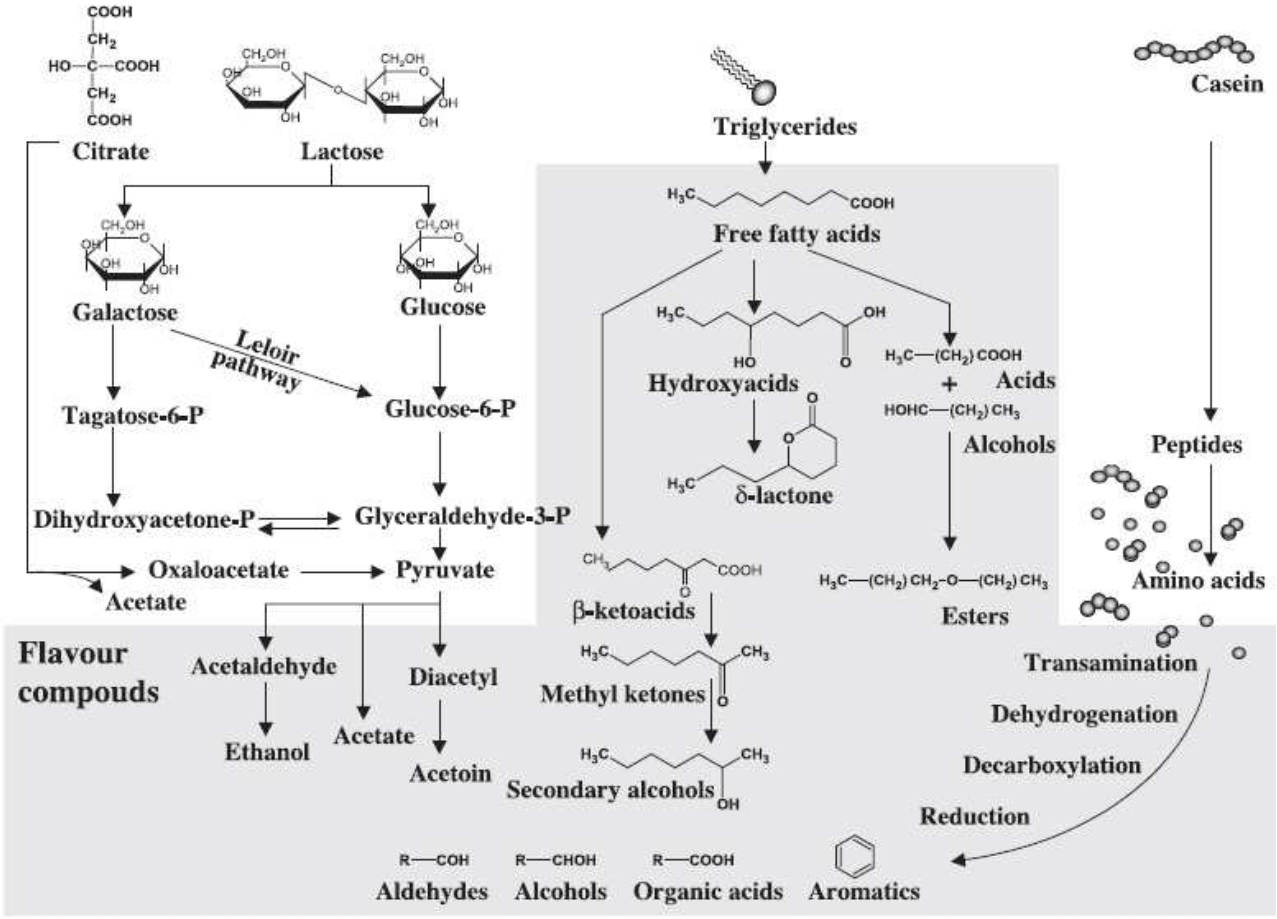
Dosud bylo v sýrech identifikováno více než 600 těkavých látek, za významné pro flavour sýrů jsou považovány zejména mastné kyseliny, estery, aldehydy, alkoholy, ketony a sirné sloučeniny [101-103]. U některých druhů sýrů jsou známy konkrétní typy „klíčových" sloučenin, jako např. volné mastné kyseliny (VMK) u sýrů typu parmezán nebo methylketony u plísňových sýrů [100]. Nicméně flavour sýrů pravděpodobně nezáleží na koncentraci jedné (několika málo) sloučenin, ale spíše na přesně vyváženém poměru mnoha sloučenin, často ty nejvíce obsažené mohou mít malý nebo vůbec žádný vliv [17,94]. Některé látky, které jsou v nízkých koncentracích součástí přirozeného aroma sýra, mohou při vyšší koncentraci způsobovat off-flavour. Např. nižší VMK mohou způsobovat žádoucí „sýrový" flavour nebo „žluklý/zatuchlý" off-flavour [16,41].

Nejdůležitější fází výroby sýrů je zrání, protože právě při něm dochází k tvorbě charakteristického **flavouru**, **vůně**, ale také **textury**. Obecně je sýr zralý, když je po stránce senzorických vlastností nejvhodnější k lidské konzumaci. Hlavní biochemické procesy, které doprovázejí zrání, lze rozdělit na primární a sekundární. Primární děje zahrnují metabolismus laktosy, laktátu a citrátu, lipolýzu a proteolýzu. Během primárních dějů vzniká kyselina mléčná, VMK a volné aminokyseliny; v rámci sekundárních dějů jsou tyto meziprodukty metabolizovány za vzniku AAL [93,101,102,104]. Zmíněné procesy probíhají jednak postupně, jednak souběžně a jsou vyvolány činností syřidlových enzymů (chymosin, pepsin, proteasy z plísní a rostlin), nativních enzymů mléka (plasmin, cathepsin D aj.) a mikroorganismů, specifických pro každý druh sýra. Jejich rozsah se liší v závislosti na typu sýra [100,105]. Přehledné schéma vzniku AAL v sýrech je uvedeno na *Obr. 1*.

Mikroorganismy, které se podílejí na zrání sýrů, jsou především BMK použitých čistých mlékařských (tzv. startovacích) kultur, tzv. non-starterové BMK, jejichž zdrojem je mléko (hl. syrové). Kombinace startovacích (mezofilní *Lactococcus* a *Leuconostoc* spp., a termofilní *Lactobacillus* spp. a *Streptococcus* spp.) a sekundárních startovacích kultur (*Penicillium* spp., *Propionibacterium* spp. aj.) je specifická pro každý druh sýra [106-109].

Konečné produkty metabolismu **laktosy**, považované za významné AAL, jsou např. butan-2,3-dion (biacetyl)(„máslové", „oříškové"), 3-hydroxybutan-2-on (acetoin)(„krémové", máslové"), kyseliny octová a propionová („octové", „štiplavé"), acetaldehyd, ethanol („jemné etherové"), nižší mastné kyseliny (C2–C6) apod. [66,93].

Proteolýza je u většiny druhů sýrů nejdůležitější metabolický proces, hraje důležitou roli ve vývoji **textury**, **flavouru** a, při nesprávném průběhu, také **off-flavouru** (kyselost, hořkost). Začíná přídavkem syřidla, jehož proteolytické enzymy štěpí kasein až na úroveň polypeptidů. Tyto jsou následně pomocí proteas a peptidas (čistých kultur, případně mikroflóry mléka) štěpeny na oligopeptidy, dipeptidy a volné aminokyseliny, jež jsou základem pro vznik AAL [105-107]. Peptidy jsou charakterizovány sladkou, hořkou nebo oříškovou chutí. Pokud dojde během zrání k nahromadění některých peptidů, u sýrů se objeví hořká chuť [106].



Obr. 1: Biochemické procesy vedoucí ke vzniku aromaticky aktivních látek v sýrech. Sloučeniny označené šedě jsou považovány za aromaticky aktivní (převzato z [93]).

Reakce aminokyselin mohou vést k celé řadě sloučenin, jako např. amoniaku, aminů, aldehydů, alkoholů, fenolů, kyselin, esterů a sloučeniny síry, z nichž všechny mohou přispívat k výslednému flavouru sýrů [94,104,106]. Při nevhodných podmínkách zrání však mohou vznikat i nežádoucí produkty – močovina, kyselina máselná, vodík, putrescin, kadaverin, sirovodík apod. Zvláště aminokyseliny obsahující síru, rozvětvené a aromatické jsou prekursory důležitých AAL [93,94,106]. Od aromatických aminokyselin jsou odvozeny např. benzaldehyd, fenylacetaldehyd, fenylethanol, fenyl-acetát a fenyl-propanoát. Z produktů metabolismu aminokyselin s rozvětveným řetězcem jsou to např. aldehydy 2- a 3-methylbutanal a 2-methylpropanal („sladové“, ale v nízkých koncentracích příjemné „ovocné“), kyseliny 2-methylpropanová, 2- a 3-methylbutanová („sýrové“, „žluklé“, „shnilé“, „zpocené“) a alkoholy 2- a 3-methylbutanol („alkoholové“, „ovocné“) [66,93,94].

Hlavním procesem rozkladu mléčných tuků je **lipolýza**, primárními produkty jsou VMK, z nichž některé, zejména s kratším nebo středně dlouhým řetězcem, přímo přispívají k flavouru sýrů: máselná („žluklé“, ale v nižších koncentracích „sýrové“), hexanová („štiplavé“, „po modrém sýru“, „kozí“), oktanová („voskové“, „mýdlové“, „kozí“, „plesnivé“, „žluklé“, „ovocné“), dekanová („žluklé“) [77,94]; překročení jejich určité koncentrace však vede ke vzniku nežádoucího off-flavouru, označovaného jako „žluklý“, „kozí“, „kovový“,

„mýdlový“ [93,110]. Jiné jsou prekursory pro vznik dalších AAL, jako jsou (methyl)ketony, aldehydy, laktony, alkoholy a estery [94,100]. Rozsah lipolýzy se výrazně liší v závislosti na typu sýra; významnými producenty lipas jsou plísně (*Penicillium* spp.), rozsáhlá je zejména u sýrů s modrou plísní v těstě [100,105].

Problematice výroby a zrání sýrů se věnuje řada autorů. V odborných databázích lze najít poměrně velké množství publikací, které se zabývají senzorickou kvalitou, flavourem a vznikem a vývojem AAL v různých typech sýrů. Většina z nich je však zaměřena na sýry ovčí, kozí, různé místní speciality často ze syrového mléka; z klasických sýrů je to pak hlavně Čedar, což je z celosvětového hlediska nejvíce vyráběný a konzumovaný sýr [17]. Co se týče plísňových sýrů, publikováno je méně, z novějších lze uvést např. práce Price a kol. [37], Gkatzionis a kol. [38,65], Wolf a kol. [82], Cakmakci a kol. [42,83], Cao a kol. [84] na sýry s modrou plísní v těstě a Galli a kol. [48], Chen a kol. [81] na sýry s camembertového typu.

4.1.1.2 Sýry s modrou plísní v těstě

Tato problematika probíhala ve spolupráci s mlékárnou Madeta a.s. České Budějovice, závod Český Krumlov. Podstatou bylo sledování změn vybraných charakteristik v průběhu zrání.

Tyto sýry patří do skupiny měkkých sýrů a vyznačují se v nákroji typickým porostem modré/modrozelené plísně *Penicillium roqueforti*, která se vysokou proteolytickou a lipolytickou aktivitou výrazně podílí na zrání a jejich typickém flavouru [38,65]. Pod různými názvy jsou rozšířeny po celém světě, typickým představitelem je Roquefort, vyráběný ve Francii z ovčího mléka nebo italská Gorgonzola aj. Jednotlivé druhy se liší původem a druhem mléka, jeho zpracováním a dobou zrání [97]. V naší zemi se vyrábí jediný zástupce těchto sýrů – Niva. Madeta a.s. ji v současnosti uvádí na trh pod názvem „Jihočeská Niva“, nabízí i variantu s vyšším obsahem tuku „Zlatá Niva“, oba tyto výrobky jsou nositelem Chráněného zeměpisného označení EU.

V naší studii byly použity vzorky sýrů Niva (suš. 50 %, tvs 55 %) a Zlatá niva (tvs 60 %.), vyrobené v mlékárně Madeta a.s. obvyklým technologickým postupem [98]. Průběh zrání byl sledován se vzorky dovezenými přímo z výrobny v různém stupni zrání. Sýry zrají cca 2 měsíce, vzorky byly odebírány v intervalech cca 10 dní.

První získané výsledky identifikovaných sloučenin ve vzorcích a jejich změny v průběhu zrání byl prezentovány na konferenci „IDF Symposium on Cheese: Ripening, Characterization & Technology“ v Praze[1] a „Súčasný stav a perspektívy analytické chemie v praxi“ v Bratislavě [2]. Souhrnné výsledky stanovení AAL a jejich změny v průběhu zrání

[1] VÍTOVÁ, E., ZEMANOVÁ, J., BEZDĚKOVÁ, Š., FIŠERA, M., BŘEZINA, P. Monitoring volatile compounds with SPME-gas chromatography during mould cheese ripening. In *IDF Symposium on Cheese: Ripening, Characterization & Technology*. Praha. 2004. p. 83.

[2] VÍTOVÁ, E., LOUPANCOVÁ, B., ZEMANOVÁ, J., ŠTOUDKOVÁ, H., BABÁK, L. Změny těkavých aromatických látek v průběhu zrání sýra Niva. In *Súčasný stav a perspektívy analytické chemie v praxi*. Bratislava (SR). 2005.

sýrů byly zpracovány do publikace[3] uvedené v **Příloze 1.** V publikaci je uvedena optimalizace použité metody HS-SPME-GC-FID/MS a vybrané validační parametry: RSD ploch píků se pohybovaly v rozmezí 2-11 %; meze detekce v rozmezí 0,003-0,2 $\mu g.g^{-1}$ pro různé sloučeniny; linearita byla testována v koncentračním rozsahu 0,003-200 $\mu g.g^{-1}$, korelační koeficienty všech sloučenin > 0,98. Metoda je tedy citlivá, reprodukovatelná a vhodná pro stanovení stopových koncentrací AAL v sýrech.

V publikaci jsou dále diskutovány identifikované sloučeniny a možnosti jejich vzniku během zrání. Výsledky byly porovnávány s tehdy dostupnými publikacemi o sýrech s modrou plísní v těstě, např. [49,71,111,112]. I když proteolýza je u plísňových sýrů poměrně rozsáhlá, je pravděpodobně pro typický flavour méně významná, než změny mléčného tuku [106,111]. Methylketony s lichým počtem uhlíků C3-C15 („ovocné“, „květinové“, ale také „zatuchlé/plesnivé“), především heptan-2-on („modrého sýra“) a nonan-2-on, sekundární alkoholy (např. pentan-2-ol, heptan-2-ol a nonan-2-ol) a VMK jsou hlavní složky tvořící charakteristický flavour plísňových sýrů. Typický „zatuchlý“ pach je způsobený nahromaděním ketonů [71,111,112]. Alkoholy mají menší vliv, nicméně nepřímo přispívají díky své schopnosti tvořit estery [94,101,102].

Při zrání se mohou uplatňovat i kvasinky, zkoumány byly např. *Kluyveromyces lactis* [37], *Torulopsis sphaerica* nebo *Yarrowia lipolytica* [38]. Přítomností kvasinek se může laktosa částečně rozkládat na ethanol, který s VMK tvoří estery, sýry tak mají bohatší flavour a proto je v současnosti výroba s kvasinkami v praxi často používána [37,38]. Ve větší míře je však alkoholové kvašení v sýrech nežádoucí, neboť může způsobit off-flavour [102].

Ve vzorcích sýrů Niva bylo identifikováno celkem 54 sloučenin, methylketony a alkoholy jako klíčové složky byly sledovány v průběhu zrání. Methylketony vznikají β-dekarboxylací mastných kyselin, primární alkoholy jsou tvořeny redukcí odpovídajících aldehydů a sekundární alkoholy redukcí methylketonů [94]. Během zrání docházelo k jejich významným změnám ($P < 0,05$); podle očekávání byl pozorován celkový nárůst.

Výsledky měření AAL a jejich korelace se senzorickými vlastnostmi byly prezentovány na konferenci „Mléko a sýry 2004“ v Praze[4]. Mladý, nezralý sýr obsahuje malé množství těkavých látek, je poměrně tvrdý a bez výraznějšího flavouru. S růstem plísně však vzrůstá její enzymová aktivita, v důsledku rozkladu proteinů se mění textura a dochází k produkci AAL. Zralý sýr se vyznačuje tzv. mramorovitým nárůstem plísně, měkkou až mazlavou texturou; flavour je výrazně slaný díky vysokému obsahu soli (4-5,5 % hm.), charakteristický pikantní po ušlechtilé plísni [49,71,100].

Výsledky hodnocení potvrzovaly předpokládaný vývoj senzorických vlastností; intenzita deskriptorů flavouru charakteristických pro plísňové sýry (sýrový, plesnivý, pikantní, štiplavý) se postupem zrání zvyšovala, stejně tak intenzita slané chuti. Naopak tvarohovitá a kyselá chuť postupně ztrácela na intenzitě v důsledku oxidace laktátu až na CO_2 a H_2O [101,102]; nízká intenzita hořké chuti svědčí o správném průběhu proteolýzy. Pro dosažení charakteristických vlastností by měl sýr zrát min. 2 měsíce [49,71], nicméně vzorky byly už

[3] VÍTOVÁ, E., LOUPANCOVÁ, B., ZEMANOVÁ, J., ŠTOUDKOVÁ, H., BŘEZINA, P., BABÁK, L. Solid-phase microextraction for analysis of mould cheese aroma. *Czech Journal of Food Scencies* (Special Isue). 2006, 24(6), pp. 268-274.

[4] VÍTOVÁ, E., ZEMANOVÁ, J., BEZDĚKOVÁ, Š., BŘEZINA, P., FIŠERA, M. Identifikace aromaticky aktivních sloučenin sýra Niva. In *Mléko a sýry 2004*. Praha. 2004. p. 184.

po cca 1 měsíci zrání hodnoceny jako velmi dobré. Důvodem může být i to, že naši spotřebitelé jsou z tržní sítě zvyklí na nedozrálý sýr. Zlatá niva má díky vyššímu obsahu tvs jemnější flavour a jemnější texturu; většina deskriptorů flavouru byla hodnocena jako mírně intenzivnější, nicméně nebyla nalezena výraznější preference oproti Nivě klasické. Ze statistického porovnání senzorických a analytických dat lze usuzovat, že k celkové kvalitě/přijatelnosti sýrů nejvíce přispíval podle očekávání sýrový, pikantní, štiplavý a slaný flavour.

Získané poznatky přispívají k objasnění procesů probíhajících během zrání a umožní standardizaci výroby sýrů, optimalizaci doby zrání a lepší kontrolu celého procesu.

4.1.1.3 Sýry s bílou plísní na povrchu (camembertového typu)

Tato problematika probíhala ve spolupráci s mlékárnou Pribina, spol. s r.o., Přibyslav. Podstatou bylo opět sledování změn vybraných charakteristik v průběhu zrání.

V současné době jsou tyto sýry pod různými názvy rozšířeny po celém světě, typickými představiteli jsou Camembert, Olivet, De Brie aj. [97]. U nás jsou vyráběny pod obchodními názvy Hermelín, Sázavský sýr, Kamadet, Premium, Plesnivec apod. Sýry s bílou plísní patří mezi sýry sladké, měkké, aerobně zrající. Základní technologie odpovídá operacím pro měkké sýry [97]; v procesu zrání se vedle běžné mikroflóry účastní i ušlechtilá plíseň *Penicillium camamberti*, která svou lipolytickou a proteolytickou činností výrazně ovlivňuje vzhled, texturu, ale zejména flavour těchto sýrů [101,102].

V této studii byly analyzovány sýry s bílou plísní vyráběné ve společnosti Pribina, spol. s r. o.: Hermelín (suš. 50 %, tvs 50 %), Premium (suš. 55 %, tvs 60 %) a Hermelín ke krájení (blokový) (hm. 1 kg)(suš. 55 %, tvs 60 %). Sýry byly vyrobeny obvyklým technologickým postupem [98]. Průběh zrání byl sledován se vzorky dovezenými přímo z výroby, experiment trval cca 2 měsíce (až po přezrálé sýry), vzorky byly odebírány v intervalech cca 10 dní. Cílem práce bylo posoudit průběh zrání z hlediska změn AAL a srovnat aromatický profil tří uvedených různých typů sýrů. Senzorické hodnocení prováděla skupina expertů přímo ve výrobě a nebylo součástí této studie.

Ač se jedná o sýry stejného typu, liší se svým složením, technologií výroby i dobou zrání. Klasický Hermelín je sýr středně tučný, blokový Hermelín a Premium jsou sýry tučné. Při výrobě blokového Hermelínu byla použita mezofilní startovací kultura, u ostatních dvou kombinace mezofilní a termofilní kultury, přičemž u Hermelínu převažuje mezofilní, u sýra Premium naopak termofilní kultura. Textura má být v konzumní zralosti mírně gumovitá, jemná, máslovitá; u blokového Hermelínu, zřejmě vzhledem k jeho velikosti a faktu že zrání postupuje od povrchu sýra [101,102], je na řezu výrazné neprozrálé tvarohovité jádro.

Pro zlepšení flavouru se v současnosti, podobně jako u sýrů s modrou plísní, používá přídavek vybraných kmenů kvasinek a/nebo koryneformních bakterií, které přispívají především tvorbou sirných sloučenin. Při výrobě tučných sýrů Premium a blokový Hermelín byla použita kultura *Geotrichum candidum*, která navíc výrazně napomáhá růstu plísně úpravou pH povrchu sýra a snížením vlhkosti [94]. Flavour se výrazně mění v průběhu zrání; zatímco mladé sýry se vyznačují „mléčně tvarohovým“ aroma, během zrání převládá „plísňové“, „žampionové“ aroma a po delší době zrání se může projevit až „čpavkové“ nebo „sírové“ aroma. Chuť přechází od čistě kyselé a slané až po hořkost [19]. Klasický a blokový Hermelín mají výrazný flavour, u Hermelínu se často na konci konzumní trvanlivosti

vyskytuje zmíněné silně „čpavkové“ aroma a občas hořká chuť, blokový Hermelín je kyselejší a často trpký a hořký. Premium má, vzhledem k vyššímu obsahu tuku, aroma slabé a jemné, převládá máslový flavour (pocit plnosti, tučnosti), chuť je spíše sladká a slaná.

První získané výsledky identifikovaných sloučenin ve vzorcích sýrů byly prezentovány na konferenci „11th International Symposium on Separation Science“ v Pardubicích [5] a „2nd International Symposium on Recent advances in food analysis“ v Praze [6]. V sýrech bylo identifikováno celkem 32 těkavých sloučenin. Za klíčové AAL sýrů s bílou plísní jsou považovány sloučeniny síry, methylketony (především pentan-2-on, heptan-2-on, oktan-2-on a nonan-2-on) tvořené β-oxidací mastných kyselin, těkavé VMK (octová, máselná) a alkoholy. Tyto látky jsou tvořeny v průběhu zrání především proteolytickou a lipolytickou činností plísně *Penicillium camamberti* [21,22,113]

Těkavé sloučeniny síry pocházejí z degradace methioninu; nejčastěji je nacházen methional („palčivé/štiplavé“, „vařené/pečené brambory“, „vařený květák“), jeho degradační produkt methanthiol („vařené zelí“), jež je dále prekursorem dimethylsulfidu („sirné“, „vařené zelí“), dimethyldisulfidu (česnek) a dimethyltrisulfidu („vařené zelí“, „brokolice“, „květák“, „česnek“) [93,94,114]. Jejich obsah se zvyšuje u přezrálých sýrů. Z metabolismu laktátu a citrátu byl jako klíčová složka Camembertu identifikován biacetyl („máslové“, „oříškové“). Rozkladem kyseliny linolové a linolenové vznikají okt-1-en-3-ol („žampiónové“, „zemité“, „plísňové“) a okt-1-en-3-on („žampiónové“), působí synergicky a jsou považovány za původce charakteristického aroma těchto sýrů [94]. U přezrálých sýrů, díky hlubokému rozkladu bílkovin, bývá detekován amoniak („čpavkové“) ve vysokých koncentracích [101,102]. Někteří autoři uvádějí jako klíčovou složku Camembertu také laktony, pocházející z lipolýzy. Hlavními laktony v sýrech jsou γ- a δ- laktony („broskvové“, „meruňkové“, „kokosové“, „máslové“) [20,25]. Přestože jejich aroma není „sýrové“, mohou přispívat k flavouru sýrů [100]; především δ-laktony udělují „smetanové“, „máslové“ aroma [20,25]. V plísňových sýrech byly identifikovány δ-dekalakton, δ-dodekalakton a γ-dodekalakton, první dva jako klíčová složka Camembertu [94].

Dílčí výsledky srovnání identifikovaných sloučenin v různých typech sýrů byly prezentovány na konferenci „Bezpečnosť a kontrola potravín“ v Nitře [7]. Kompletní výsledky byly zpracovány do publikace[8] uvedené v **Příloze 2**. V publikaci jsou diskutovány identifikované sloučeniny ve třech typech analyzovaných sýrů, možnosti jejich vzniku a změny během zrání. Výsledky byly porovnávány s tehdy dostupnými publikacemi o sýrech s bílou plísní, např. [21,22,113]. Během zrání docházelo ke změnám v obsahu jednotlivých

[5] VÍTOVÁ, E., LOUPANCOVÁ, B., HRADILOVÁ, J., BEZDĚKOVÁ, Š., ZEMANOVÁ, J. Aroma Compounds of White Surface Mould Cheeses. In *11th International Symposium on Separation Science.* Pardubice. 2005. p. 272.

[6] VÍTOVÁ, E., LOUPANCOVÁ, B., HRADILOVÁ, J., ZEMANOVÁ, J., BEZDĚKOVÁ, Š. Analysis of white surface mould cheeses aroma by SPME-GC method. In *2nd International Symposium on Recent advances in food analysis*. Praha. 2005. p. 269.

[7] VÍTOVÁ, E., LOUPANCOVÁ, B., ŠTOUDKOVÁ, H., ZEMANOVÁ, J. Srovnání aromatického profilu sýrů s bílou plísní. In *Bezpečnosť a kontrola potravín*. Nitra (SR). 2006, p. 287-290.

[8] VÍTOVÁ, E., LOUPANCOVÁ, B., ŠTOUDKOVÁ, H., ZEMANOVÁ, J. Application of SPME-GC method for analysis of the aroma of white surface mould cheeses. *Journal of Food and Nutrition Research*. 2007, 46(2), pp. 84-90.

sloučenin, nelze však určit jednoznačný trend. Některé sloučeniny během zrání vznikají, u některých dochází k jejich degradaci. Průběh zrání se odlišoval i u jednotlivých typů sýrů, ale z hlediska celkového obsahu identifikovaných sloučenin byl průběh obdobný; v počáteční fázi zrání došlo k nárůstu s maximem po cca 15 dnech zrání, poté k pomalému poklesu. To by odpovídalo aplikovaným podmínkám zrání, sýry camembertového typu zrají cca 6 – 12 dnů při 12 – 15 °C až do rozvoje plísně; v této fázi tedy dochází k výrazné tvorbě těkavých látek. Poté se sýry balí a uchovávají při teplotě 5°C, kdy pokračuje „pomalé zrání“. V této fázi už pravděpodobně u sledovaných sloučenin dochází k pomalé degradaci [101,102]; řada z nich měla ve zralých sýrech nižší koncentraci než v sýrech nezralých.

Ze srovnání analyzovaných tří typů sýrů je patrné, že obsah a složení identifikovaných těkavých látek se u jednotlivých sýrů podle očekávání liší; ve všech byly nalezeny téměř totožné sloučeniny, lišil se však jejich obsah. Díky výše zmíněným odlišnostem při výrobě, a zvláště u sýrů s vyšším obsahem tuku a přídavkem kultury *Geotrichum candidum* (Premium a blokový Hermelín), lze očekávat vyšší obsah a celkově bohatší spektrum AAL. Na druhou stranu sýr Premium by měl mít spíše slabé, jemné aroma (dle senzorického hodnocení). Celkový obsah identifikovaných sloučenin klesal v pořadí: blokový Hermelín (62,2 ± 3,1 $\mu g.g^{-1}$), Hermelín (51,8 ± 2,6 $\mu g.g^{-1}$) a Premium (32,8 ± 1,4 $\mu g.g^{-1}$). V blokovém Hermelínu byl nalezen nejvyšší obsah ketonů, alkoholů a mastných kyselin, tj. sloučenin typických pro aroma těchto typů sýrů. Výsledky zhruba odpovídají zmíněným předpokladům, nicméně vzhledem k dostupným standardům nebyly kvantifikovány všechny potřebné sloučeniny, tento problém by si zasloužil hlubší studium a je možné se k němu v budoucnu vrátit. I tak získané poznatky přispěly k optimalizaci senzorické jakosti sýrů v praxi.

4.1.2 Tavené sýry

Ústav chemie potravin a biotechnologií FCH VUT v Brně dlouhodobě spolupracuje s Univerzitou Tomáše Bati ve Zlíně. V rámci této spolupráce je řešena řada projektů, často ve spolupráci s průmyslem, zaměřených na výrobu modelových přírodních a tavených sýrů, a sýrových analogů; jde o komplexní chemickou a senzorickou charakterizaci produktů s cílem jejich eventuálního praktického využití. Náš výzkumný tým se na spolupráci podílí posuzováním senzorické kvality (flavouru) vzorků.

Problematika tavených sýrů je řešena v rámci probíhající disertační práce [9]. Práce je součástí rozsáhlé studie, která se zabývá studiem senzorické kvality přírodních sýrů eidamského typu a tavených sýrů, hlavním cílem je popsat změny flavouru a obsahu AAL v průběhu jejich výroby a skladování.

Zatímco přírodní sýry byly známy už od starověku, výroba tavených sýrů začala až začátkem 20. století. Nevznikly náhodným objevem jako klasické sýry, ale byly plánovitě vyvíjeny s cílem získat trvanlivý sýr schopný skladování a transportu. První tavený sýr vznikl na počátku 20. století, přesněji v roce 1911, ve švýcarské firmě Gerber a spol. Tavené sýry byly nezávisle vyvinuty i v USA, cca v r. 1916 začala s výrobou firma Kraft [115-118].

Tavené sýry lze zařadit do současného trendu „convenience food“; k jejich přednostem patří: různé varianty chuti, vůně, vzhledu a textury a z toho vyplývající široké možnosti

[9] MAHDALOVÁ, M. Vliv tepelného záhřevu na změny obsahu senzoricky aktivních látek v sýrech [disertační práce, školitel specialista E. Vítová].

použití, relativně nízká cena, poměrně dlouhá trvanlivost. V současné době se tavené sýry stále více využívají nejen k přímému konzumu, ale také jako ingredience do některých potravinových výrobků, např. do salátů, sendvičů, sýrových omáček, hamburgerů, pomazánek a polotovarů apod. [115,116].

Ve srovnání s přírodními sýry mají tavené sýry nižší výživovou hodnotu. Je to způsobeno použitím zvýšené teploty při tavení a přídavkem tavicích solí, což má za následek zhoršenou vstřebatelnost vápníku, snížení obsahu vitaminů a biologické hodnoty proteinů. Řada tavených sýrů má poměrně vysoký obsah tuku a jsou tak významným zdrojem energie, cholesterolu a v neposlední řadě také sodíku [118,119].

Podle vyhlášky č. 397/2016 Sb., je **tavený sýr** definován jako „*sýr, který byl tepelně upraven za přídavku tavicích solí*“ [96]. Základní surovinou pro výrobu jsou přírodní sýry, jejich obsah v tavených sýrech musí být min. 50 % hm.; v ČR se nejčastěji jedná o sýry eidamského typu, minoritně sýry švýcarského typu. S výhodou je možné použít sýry nevhodné pro přímý prodej (mechanické vady, praskliny aj.), nesmí se však jednat o vady chemické nebo mikrobiální, neboť mohou být příčinou senzorických vad, nebo ohrozit bezpečnost konečného výrobku [120]. Kromě toho se přidávají další složky **mléčné** povahy: např. máslo, tvaroh, smetana, sušené mléko, podmáslí, syrovátka aj.). Do některých typů tavených sýrů se používají další **nemléčné** přísady ovlivňující chuť, vůni a barvu, např. koření, masné výrobky, žampiony, zelenina, ořechy apod., v zahraničí dokonce i kakao, med, vanilka, kávový extrakt aj. [115-118].

Klíčové jsou **tavicí soli**; přírodní sýry není možné běžně zahřívat, aniž by došlo k rozdělení směsi na tři fáze: vysráženou bílkovinu na dně, vodní fázi a volný tuk na povrchu. Tomuto rozdělení zabraňují tavicí soli, které zajišťují výměnu Ca^{2+} iontů v tavenině za Na^{+} (nebo K^{+}) ionty, emulgují tuk, rozpouštějí proteiny a zamezují tak jejich srážení, upravují pH na optimální 5,6–5,9 [119]. Kombinací všech těchto účinků dochází k tvorbě žádoucí textury a struktury taveného sýra. Nejčastěji se jedná o sodné nebo draselné soli kyseliny citronové, fosforečné a polyfosforečné. Obvykle se používají ve směsích v množství 2-3 % hm. finálního výrobku [118,119].

Technologie výroby tavených sýrů spočívá v tavení směsi výše zmíněných surovin. Použitím vysoké teploty (cca 80–100 °C), za stálého míchání a částečného vakua, dochází k žádoucí přeměně směsi na homogenní hladkou lesklou hmotu požadovaných texturních, strukturních aj. senzorických vlastností. Důležitou roli hraje teplota, doba působení, rychlost míchání, množství a druh tavicích solí [117,118]. Je třeba říci, že dosud nejsou uspokojivě vysvětleny reakce probíhající během tavení [119]. Proces tavení je především fyzikálně chemický proces, který vyvolává změny v koloidním a disperzním stavu sýrové hmoty (tzv. krémování), následkem čehož dochází ke tvorbě žádoucí textury výrobku. Vlivem vysoké teploty dochází ke tvorbě flavouru (viz dále) a v neposlední řadě ke zničení nežádoucích mikroorganismů, čímž se zajistí bezpečnost a trvanlivost výrobku. Tavení může probíhat buď kontinuálním (110-145 °C; 5-20 sekund), nebo diskontinuálním (90-100 °C; 4-15 min.) způsobem [115-118]. V ČR se většinou používá diskontinuální způsob výroby [119].

4.1.2.1 Vznik a vývoj flavouru tavených sýrů

Zatímco problematice výroby a zrání přírodních sýrů se věnuje řada autorů a bylo publikováno mnoho prací, podstatně méně je věnována pozornost sýrům taveným; existuje

poměrně málo publikací zabývajících se změnami, které v tavených sýrech probíhají během procesu tavení, balení nebo skladování, většina z nich se navíc zaměřuje na sledování textury. Flavouru tavených sýrů zatím v dostupné literatuře není věnována velká pozornost. Asi nejstarší práce je od autorů Gupta a kol. [121], kteří se věnovali studiu vlivu tavicích solí na flavour a texturu tavených sýrů. Podobně autoři Cunha a kol. [40] sledovali vliv tavicích solí na celkovou senzorickou kvalitu/přijatelnost. Ve skupině Muir a kol. [122] srovnávali flavour a texturu tavených sýrů klasických a se sníženým obsahem tuku; podobně Drake a kol. [51] se pokusili vytvořit lexikon termínů, na jejichž základě bude možné kompletně popsat flavour tavených sýrů a rozlišit jejich různé typy. Pouze autorský tým Sunesen a kol. [72] se ve své práci zabývali identifikací a srovnáním obsahu AAL různých typů tavených sýrů a jejich změnami během skladování. Zjištěné poznatky lze shrnout takto: flavour tavených sýrů je ovlivněn mnoha faktory, od vlastností mléka až po podmínky při výrobě a skladování. Z velké části je determinován flavourem přírodních sýrů použitých k jejich výrobě; část AAL pravděpodobně pochází z nich [120]. Nejdůležitějším ukazatelem se zdá být stáří, tj. stupeň prozrání sýra a jeho pH [51], plný a výrazný flavour získáme použitím velmi zralých sýrů, nevýhodou je možnost vzniku příliš ostrého flavouru; naopak u mladé suroviny narážíme na tzv. prázdnost chuti [120].

Stejně jako v případě přírodních sýrů důležitý je obsah mléčného tuku. Tavené sýry se sníženým obsahem tuku vykazují zhoršení, naopak přídavek mléčného tuku přispívá ke zlepšení flavouru [118,122]. Za tímto účelem se většinou do tavených sýrů přidává máslo, které se tak stává dalším zdrojem AAL. Obsahem těkavých látek v másle a mléčném tuku se zabývalo již mnoho autorů, jejich výsledky jsou shrnuty v práci Mallia a kol. [123], podle nich bylo v másle identifikováno už více než 280 těkavých sloučenin.

Také nevhodné složení a především nadměrné množství (> 2 % hm.) tavicích solí může vést k negativním změnám. Fosfáty mohou být příčinou nežádoucí hořké chuti, případně off-flavouru popisovaného jako „mýdlový“ či „chemický“ [119,121].

Velmi důležitým faktorem je tepelný záhřev, při němž některé AAL vznikají, jiné jsou rozloženy nebo přeměněny [51,118]. Mezi nejvýznamnější reakce, které mohou vést ke vzniku nebo změně flavouru a vzniku off-flavouru během výroby a skladování patří Maillardova reakce a oxidace lipidů [72,124]. Maillardova reakce, kromě změny barvy a nutriční hodnoty, vede ke vzniku širokého spektra nízkomolekulárních sloučenin, které tvoří důležitou součást flavouru, ale také off-flavouru [124]. Probíhá mezi redukujícími cukry a aminosloučeninami; z redukujících cukrů se v tavených sýrech vyskytuje laktosa (a její metabolit galaktosa), která pochází přímo z mléka, nebo je přidávána ve formě sušeného mléka nebo syrovátky [125]. U tavených sýrů probíhá Maillardova reakce jednak při zvýšené teplotě během tavení a významně se projevuje během skladování, zvláště za zvýšené teploty, kdy dochází ke tvorbě typického off-flavouru (aldehydy vznikající Streckerovou degradací aminokyselin) [51].

Oxidační reakce v tavených sýrech obvykle vedou ke změně barvy a vzniku oxidovaného off-flavouru, který bývá popisován jako „kovový“, „olejovitý“, „lojovitý“, „po rybím tuku“, způsobeného tvorbou sekundárních produktů oxidace lipidů (aldehydy, ketony, alkoholy, mastné kyseliny a uhlovodíky). Jako indikátor oxidace bývá často používán hexanal („pažitka“, „nezralé ovoce“) [72,124].

V průběhu skladování tavených sýrů tedy dochází k řadě změn, které zhoršují vzhled, barvu a především flavour a texturu. Intenzita reakcí se zvyšuje s vyšší teplotou a dobou

skladování, za přístupu světla a s rostoucí koncentrací kyslíku; následky oxidace lze významně snížit použitím kvalitních obalů, nepropustných pro světlo a kyslík, případně balením v ochranné atmosféře. Obecně by tavené sýry měly být skladovány při teplotě max. 35 °C a ne déle než 6 týdnů [117,119,125].

4.1.2.2 Změny flavouru během výroby modelových tavených sýrů

V rámci naší studie byly analyzovány modelové vzorky přírodních sýrů eidamského typu (suš. 55 %, tvs 45 %) a tavených sýrů z nich vyrobených (suš. 40 %, tvs 50 %); vzorky byly vyrobeny standardním technologickým postupem [117] na UTB ve Zlíně. Pro výrobu tavených sýrů byly použity: vyrobený eidamský sýr (stáří 2 měs.), máslo, voda a tavicí soli (Fosfa a.s., Břeclav); tavicí proces 90 °C 10 min. Po celou dobu výroby byly v pravidelných intervalech odebírány vzorky, které byly podrobeny analýzám. Cílem je sledovat změny vybraných parametrů v průběhu technologického procesu výroby. Podstatou prvního experimentu bylo posoudit vliv různé teploty a doby pasteračního a následně tavicího záhřevu na AAL ve vzorcích.

Ve vzorcích bylo celkem identifikováno 42 těkavých sloučenin, jejich změny v průběhu výroby sýrů byly v souladu s naším očekáváním. V **mléce** byl nalezen nejmenší počet i obsah AAL. Ve vzorcích **nezralých sýrů** byl počet i obsah stanovených sloučenin oproti mléku výrazně vyšší, což je způsobeno pravděpodobně vyšší sušinou sýrů; měřené vzorky byly na počátku zrání, obsahovaly tedy pravděpodobně pouze sloučeniny, které se nacházely v použité surovině (mléko), a dále látky, které vznikly v průběhu výroby díky fermentaci laktosy [101,102]. Metabolismus laktátu a citrátu hraje u eidamských sýrů významnou roli, neboť touto cestou vzniká biacetyl („máslové“, „oříškové“), který byl identifikován jako klíčová složka aroma těchto sýrů. Během zrání jeho koncentrace klesá, může být přeměněn na acetoin („krémové“, máslové“), dále na butan-2,3-diol a butan-2-on („acetonové“) a ten nakonec na butan-2-ol [94]. Tyto sloučeniny, kromě butan-2,3-diolu, byly v nezralých sýrech identifikovány.

Sýry eidamského typu zrají anaerobně v celé hmotě, nejdůležitější během zrání je proteolýza, i když nepříliš rozsáhlá [62]. Lipolýza je u eidamských sýrů považována spíše za nežádoucí, vysoký obsah vzniklých VMK by mohl způsobovat např. nežádoucí žluklý off-flavour. Nicméně nízké koncentrace VMK, jsou-li ve správné rovnováze s produkty proteolýzy, přispívají k výsledné chuti eidamských sýrů [93,100]. Jejich hlavní význam však tkví v tvorbě laktonů; k nejběžnějším patří δ-dekalakton a δ-dodekalakton („smetanové“, „máslové“), nezbytné pro charakteristický flavour eidamských sýrů, kde byly nalezeny ve vysokých koncentracích [62,63]. Podle očekávání u **zralých sýrů** došlo k dalšímu výraznému nárůstu počtu i obsahu sloučenin jako důsledek jejich tvorby během zrání; zvláště v případě alkoholů, esterů a kyselin. Nebyly identifikovány žádné laktony kvůli nedostatku standardů pro jejich stanovení. Jak již bylo zmíněno, flavour tavených sýrů je ovlivněn mnoha faktory [72,121]. V našem případě došlo u **tavených sýrů** k poklesu počtu i obsahu stanovených sloučenin pravděpodobně účinkem použité tavicí teploty, kdy převážily degradační procesy nad tvorbou sloučenin nových [51,118].

Pomocí PCA byly potvrzeny významné ($P < 0,05$) rozdíly mezi vzorky, tj. mléko vs. nezralý vs. zralý sýr vs. tavený sýr. Pouze mezi vzorky pasterovaného a nepasterovaného mléka nebyl nalezen významný rozdíl; použité varianty pasteračního záhřevu tedy byly

dostatečně šetrné a nebyl potvrzen jejich vliv na vznik nebo degradaci identifikovaných sloučenin.

První výsledky AAL identifikovaných v sýrech eidamského typu, použitých jako hlavní surovina pro výrobu tavených sýrů, byly prezentovány na konferenci „Chemistry and Life 2015“ v Brně[10]. Výsledky byly porovnány s komerčně dostupnými eidamskými sýry získanými přímo od výrobce Mlékárna Miltra, Městečko Trnávka, zpracovanými do publikace v recenzovaném časopise[11]. Na základě srovnání s dostupnými publikacemi, např. Thomsen a kol. [46], Shiota a kol. [47], Alewijn a kol. [62,63], Inagaki a kol. [64], Van Leuven a kol. [66], Cavanagh a kol. [80], hlavní pozornost bude v dalších experimentech zaměřena na detekci laktonů, které by měly tvořit podstatnou složku aroma těchto sýrů.

Zralé eidamské sýry a vyrobené tavené sýry byly zároveň podrobeny senzorickému hodnocení. U eidamských sýrů vzhledem k jejich výrobě, především v důsledku (i) přídavku prací vody při dohřívání mají nižší kyselost; (ii) nízkého obsahu soli (1,5–3,0 % hm.); (iii) poměrně krátké doby zrání (cca 2-5 měs. podle velikosti) proteolýza není příliš rozsáhlá; textura je proto měkčí a flavour celkově méně výrazný. Chuť a vůně je obecně jemně sýrová, mléčně nakyslá, mírně slaná, nasládlá, lehce hořkomandlová, oříšková [62,99]. Vyrobené eidamské sýry byly hodnoceny po 2 měsících zrání; v této fázi už by měl sýr dosáhnout plně rozvinuté chuti a vůně, což bylo senzorickým hodnocením potvrzeno. Sýry jsou tedy vhodnou surovinou pro výrobu tavených sýrů.

Tavený sýr by měl mít flavour typický sýrový, u tučných sýrů až máslový, do určité míry charakteristický pro druh sýra, ze kterého byl vyroben [51]. Vyrobené sýry byly vyhodnoceny jako dobré, nicméně v následujících experimentech budou porovnávány s komerčně dostupnými tavenými sýry pro lepší posouzení chutnosti a odlišností od klasických tavených sýrů.

4.1.3 Sterilované tavené sýry

Tato problematika byla také řešena v rámci disertační práce[12]. Hlavním cílem bylo navrhnout výrobu tavených sýrů s prodlouženou trvanlivostí (sterilovaných), tak aby si uchovaly požadovanou senzorickou kvalitu (flavour) po celou dobu uvedené trvanlivosti.

Sterilované tavené sýry jsou zvláštní skupinou tavených sýrů, která byla vyvinuta ke speciálním účelům – pro stravování příslušníků Armády ČR a členů Integrovaného záchranného systému a případně i civilního obyvatelstva v krizových situacích (povodně ap.). Jsou součástí tzv. bojových dávek potravin (BDP), což jsou (zjednodušeně řečeno) balíčky obsahující potraviny pro danou osobu (vojáka) na 24 hodin. Jednotlivé potravinové komponenty (např. hotové pokrmy, sušenky, čokoláda aj.) mají trvanlivost stanovenou Standardizační dohodou Severoatlantické aliance STANAG 2937 na nejméně 24 měsíců při

[10] MAHDALOVÁ, M., VÍTOVÁ, E., RYGLOVÁ, H., SŮKALOVÁ, K., BUŇKA, F. The development of volatile flavour compounds during ripening of Gouda cheese. In *Chemistry & Life 2015*. Brno. 2015. p. 97.

[11] VÍTOVÁ, E., DIVIŠOVÁ, R., BABÁK, L., ZEMANOVÁ, J., SKLENÁŘOVÁ, K. The changes of flavour and aroma active compounds content during production of Edam cheese. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*. 2011, 59(1), pp. 255-261.

[12] LOUPANCOVÁ, B. Studium faktorů ovlivňujících tvorbu těkavých aromaticky aktivních látek v přírodních materiálech. [disertační práce, školitel specialista E. Vítová]. Brno: FCH VUT v Brně, 2011.

okolní teplotě, což v podmínkách ČR představuje cca pokojovou teplotu. Tavené sýry se ukázaly být vhodným zdrojem vápníku splňujícím všechny tyto požadavky. Oprávněnost jejich zařazení do BDP dokládá i skutečnost, že jsou součástí BDP řady armád zemí NATO. Tento nový typ výrobku by však mohl obohatit i nabídku trvanlivých potravin na našem trhu.

Vzhledem k tomu, že tavené sýry patří mezi tzv. neúdržné potraviny, je třeba pro prodloužení trvanlivosti použít vhodných konzervačních metod. Prakticky jedinou možností, jak dosáhnout u taveného sýra trvanlivosti 24 měsíců, je jeho termosterilace. Potřebné zahřátí sice významně prodlouží trvanlivost výrobků, na druhou stranu však urychluje i nežádoucí nemikrobní a neenzymové procesy, které v nezahřátých potravinách probíhají jen velice zvolna, následkem čehož dochází k nežádoucím změnám v obsahu živin, fyzikálně chemických a senzorických vlastností výrobku.

4.1.3.1 Flavour sterilovaných tavených sýrů a jeho změny během skladování

O AAL přítomných ve sterilovaných tavených sýrech nebyly v literatuře nalezeny prakticky žádné informace. Pouze Bertrand a kol. [86,87] zkoumali vliv různých sterilačních teplot na flavour a vznik off-flavouru modelových tavených sýrů.

Pro naši unikátní studii byly použity tavené sýry (suš. 38 %, tvs 45 %), speciálně pro účely diskutovaného výzkumu vyrobené ve společnosti Madeta a.s. Sýry byly vyrobeny klasickým diskontinuálním technologickým postupem [117]; pro výrobu byly použity: směs sýrů eidamského typu, máslo, voda, sušená syrovátka (0,5 % hm.) a tavicí soli (Fosfa a.s., Břeclav); tavicí proces 92 °C 5 min. Část sýrů byla okamžitě po výrobě vychlazena na 6 °C („klasické/nesterilované tavené sýry“), druhá část byla podrobena sterilaci (117 °C 20 min.)(„sterilované tavené sýry“), tyto byly následně rozděleny a uchovávány při chladírenské (6 °C), skladové (23 °C) a zátěžové (40 °C) teplotě. Vzorky byly odebírány a analyzovány v průběhu 24 měsíců skladování.

První získané výsledky identifikovaných sloučenin ve vzorcích byly prezentovány na konferenci „Vitamins 2006“ v Pardubicích[13], další výsledky shrnující vliv sterilačního záhřevu a následně podmínek skladování na obsah AAL a senzorickou kvalitu vzorků na konferenci „Chemistry and Life 2011“ v Brně [14]. Ve vzorcích bylo identifikováno celkem 45 těkavých sloučenin.

Nesterilované tavené sýry byly považovány za jakýsi standard kvality pro následná porovnání. Jak již bylo zmíněno, jedná se o neúdržné potraviny, nicméně sýry si udržely dobrou senzorickou kvalitu dokonce 1 rok, až po této době došlo k významnému zhoršení (byly silně zduřelé a na první pohled nevhodné ke konzumaci).

S**terilační záhřev** měl vliv na obsah prakticky všech identifikovaných těkavých látek, u většiny došlo ke zvýšení jejich obsahu; celkový obsah těkavých látek se vlivem sterilace významně ($P < 0,05$) zvýšil. Řada reakcí, probíhajících během tavení, působí i při sterilačním

[13] LOUPANCOVÁ, B., VÍTOVÁ, E., ŠTOUDKOVÁ, H., LAZÁRKOVÁ, Z., ZEMANOVÁ, J. Determination of Volatile Compounds in Processed cheese by Gas Chromatography. In *Vitamins 2006*. Pardubice, 2006, p. 151.

[14] VÍTOVÁ, E., LOUPANCOVÁ, B., DIVIŠOVÁ, R., SKLENÁŘOVÁ, K., FIŠERA, M. Relationship between volatile compounds content and sensory attributes of sterilized processed cheese. *Chemické Listy*. 2011. 105(S18), p. 1032.

záhřevu, díky sterilaci by však měla být většina enzymů inaktivována, v úvahu tedy přicházejí převážně změny chemické [86,87,126,127]. Lipidy podléhají především oxidačním změnám, podle dostupnosti kyslíku; za předpokladu dobrého naplnění, dobrých bariérových vlastností obalu a hermetičnosti uzavření by koncentrace kyslíku v sýrech měla být minimální. Podle očekávání následkem použitého záhřevu došlo ke ztmavnutí sýrů, vzniku tužší textury pravděpodobně uvolňováním vody a zesíťováním proteinů [124-127]. Intenzita Maillardovy reakce se zvyšuje s rostoucí teplotou záhřevu a s přídavkem surovin, které obsahují vyšší množství redukujících sacharidů (sušená syrovátka), tyto produkty tedy nejsou pro výrobu sterilovaných tavených sýrů příliš vhodné [119]. Může dojít až ke vzniku nežádoucího off-flavouru popisovaného jako „vařivý“, „připálený“, „sírový“ nebo „karamelový“ [86,87]. Bertrand a kol. [86,87] identifikovali jako hlavní příčiny off-flavouru maltol a furaneol. V našem případě sice došlo ke zhoršení flavouru, ale sýry byly stále hodnoceny velmi dobře.

Při následném skladování docházelo k dalším změnám, nelze však určit jednoznačný trend. Těkavé látky stále vznikají, ale zároveň mohou také podléhat široké škále degradačních reakcí (výše zmíněná Maillardova reakce a oxidace lipidů) [127]. Se vzrůstající **teplotou skladování** u většiny identifikovaných sloučenin koncentrace vzrostla, čímž došlo i k významnému ($P < 0,05$) nárůstu jejich celkového obsahu; to je v souladu se zjištěním autorů Schär a Bosseta [125]. Vliv **doby skladování** byl opět individuální, nejpočetnější skupinou byly sloučeniny, u nichž došlo cca po 16 měsících ke snížení koncentrace (v této fázi došlo k prudkému poklesu celkového obsahu těkavých látek), v dalších fázích však došlo k opětovnému nárůstu.

Zároveň došlo během skladování k výraznému zhoršení senzorických ukazatelů, nejhůře byly hodnoceny sýry skladované v termostatu (40 °C); vysoká skladovací teplota pravděpodobně urychluje výše zmíněné chemické změny [126]. U těchto sýrů došlo k výraznému zhoršení barvy (vznik tmavšího odstínu), textury (zvýšená tuhost), dokonce už po 6 měsících byly hodnoceny negativně ve většině ukazatelů, po 12 měsících pak došlo k dalšímu zhoršení, byla detekována netypická vůně a hořká chuť, což může poukazovat na přítomnost hořkých peptidů vznikajících degradací bílkovin [125-127]. Později byly tyto sýry hodnoceny jako krupičkovité, s jemnými hrudkami, nepříjemné na jazyku, špatně se rozplývající; to může souviset s krystalizací některých složek, zejména tavicích solí, uvolněného tuku [127] nebo laktosy přidané ve formě sušené syrovátky [117]. Původně smetanově nažloutlá barva se změnila na naoranžovělou až hnědočervenou. Celkově byly hodnoceny jako nevyhovující až nepřijatelné. Sýry uchovávané v lednici (6 °C) byly hodnoceny nejlépe; sterilované sýry si tedy udrží dobrou senzorickou kvalitu po celou dobu požadované trvanlivosti (2 roky), ale pouze za vhodných skladovacích podmínek (teplota < 6 °C). Kvalita (přijatelnost) klasických tavených sýrů je sice vyšší, zvláště v případě flavouru a textury, nicméně jejich trvanlivost je pouze několik měsíců (v závislosti na typu sýra, balení apod.) [118,125], při skladování ve stejných podmínkách si sterilované tavené sýry udrží původní kvalitu podstatně déle.

Jak vyplývá z uvedených výsledků, s aplikací sterilačního záhřevu a s vyšší teplotou skladování se zvyšuje obsah těkavých látek v sýrech, ale z hlediska senzorického jsou sýry hodnoceny hůře. Vlivem vyšší teploty tedy v sýrech pravděpodobně vzniká (případně se zvyšuje jejich koncentrace) řada AAL, které negativně ovlivňují chutnost sýrů (furfural („karamelové“, „ovocné“), aceton („acetonové“), atd. a způsobují výše zmíněný off-flavour [125].

Podstatné vybrané výsledky byly zpracovány do souhrnné publikace[15] uvedené v **Příloze 3**. V rámci zmíněné spolupráce s UTB ve Zlíně byly vzorky podrobeny komplexnímu rozboru: základní chemická analýza (pH, obsah tuku, sušiny a amoniaku, hrubý protein), doplněná o stanovení obsahu aminokyselin pomocí iontově výměnné chromatografie, proteinového profilu metodou SDS-PAGE a velikosti tukových kuliček mikroskopickou obrazovou analýzou. Mikrobiologický rozbor zahrnoval stanovení celkového počtu mezofilních, koliformních, sporotvorných mikroorganismů a plísní/kvasinek. Z výsledků získaných na FCH VUT v Brně byly do publikace zahrnuty výsledky senzorického hodnocení, které bylo pro srovnání provedeno na obou pracovištích. Veškeré výsledky byly v souladu s naším očekáváním; ani po dvou letech skladování nebyly ve vzorcích detekovány žádné mikroorganismy, což potvrzuje, že použitý sterilační záhřev je dostatečný pro zabezpečení požadované trvanlivosti. Sterilační záhřev a dlouhodobé skladování vzorků, zvláště při vyšší teplotě, však měly významný ($P < 0,05$) dopad na ostatní sledované ukazatele; snížení celkového obsahu aminokyselin, zvětšování tukových kuliček a především již zmíněné zhoršení senzorické kvality vzorků. Tyto změny souvisejí především s Maillardovou reakcí a s oxidací lipidů [86,87,127], k úbytku aminokyselin může docházet také vlivem Streckerovy degradace [125]. Dobrou senzorickou kvalitu po celou dobu požadované trvanlivosti lze udržet pouze skladováním při nízkých teplotách (< 6 °C).

4.1.4 Tavené sýrové analogy

Problematika sýrových analogů je řešena v rámci další probíhající disertační práce[16]. Hlavním cílem je navrhnout výrobu analogů tavených sýrů s obsahem rostlinných tuků, tak aby měly dobrou senzorickou kvalitu (flavour) a případně i zvýšenou nutriční hodnotu.

Se stoupající spotřebou a nárůstem ceny přírodních sýrů se objevil požadavek na výrobu levnějšího a dostupnějšího produktu, tak vznikly tzv. sýrové analogy (imitace, substituty), které se objevují na trhu počátkem roku 1970 [128,129]. Při jejich výrobě je mléčný tuk a/nebo mléčná bílkovina částečně nebo zcela nahrazena nebílkovinnou složkou zejména rostlinného původu. Kromě nižších výrobních nákladů mohou tyto výrobky nabízet i řadu pozitivních nutričních aspektů: vyšší podíl nenasycených mastných kyselin, nižší nebo žádný obsah cholesterolu, snížený obsah bílkovin/fenylalaninu, redukované množství nasycených tuků a sodíku, nižší energetická hodnota, bezlaktosové, obohacené vitaminy aj.; z těchto důvodů se analogy stávají vyhledávanými potravinami. Nalézají uplatnění v domácnostech, restauracích, školních jídelnách nebo provozovnách typu „fast-food“ jako cheeseburgery, přídavky na pizzu, do salátů, omáček, pomazánek, sendvičů aj. Výhodou může být využití v rozvojových zemích, kde jsou mléko a mléčné výrobky drahé a nedostatkové zboží [128,129].

V současné době se komerčně vyrábějí analogy širokého rozsahu přírodních (např. Cheddar, Mozzarella aj.) i tavených sýrů [130,131]. V ČR analogy tvoří asi 10 % z výroby

[15] BUBELOVÁ, Z., TREMLOVÁ, B., BUŇKOVÁ, L., POSPIECH, M., VÍTOVÁ, E., BUŇKA, F. The effect of long-term storage on the quality of sterilized processed cheese. *Journal of Food Science and Technology*, 2015, 52(8), pp. 4985 – 4993.

[16] SŮKALOVÁ, K. Faktory ovlivňující senzorickou jakost analogů tavených sýrů [disertační práce, školitel specialista E. Vítová].

tavených výrobků a jen desetiny % z výroby přírodních sýrů, ale má je ve svém sortimentu řada sýráren (TPK, spol. s r.o., Hodonín, Pribina s.r.o., Přibyslav aj.). Česká legislativa zatím pojem „analog“ nebo „imitace“ nezná. Jedinou zmínku lze nalézt ve vyhlášce MZ č. 4/2008 Sb., v platném znění, kde je řešeno nejvyšší povolené množství fosforečnanů do „tavených sýrů a jejich analogů“ [132]. Z platné potravinářské legislativy však jednoznačně vyplývá, že pokud byla některá ze základních složek mléka nahrazena jinou nemléčnou složkou, nesmí být takovýto výrobek již více označován jako mléčný výrobek, ani jako sýr [96]. V obchodech nesmí být nabízen způsobem, který by ve spotřebiteli vyvolával dojem, že jde o skutečný mléčný výrobek [131].

Sýrové analogy lze rozdělit na **mléčné**, **částečně mléčné** a **nemléčné**. Mléčné analogy jsou vyrobeny užitím pouze mléčných surovin, částečně mléčné užitím mléčných proteinů a rostlinných tuků a nemléčné užitím rostlinných tuků i proteinů. Mléčné analogy jsou drahé a téměř se nevyrábí, stejně tak nemléčné kvůli výrazně horším senzorickým vlastnostem; hlavním produktem jsou tedy analogy částečně mléčné. Dále lze tyto výrobky dělit na **imitace** nebo **substituty**; substituty mají stejnou nutriční hodnotu jako odpovídající sýr, zatímco imitace mají nutriční hodnotu nižší [128,131].

Výrobní technologie je obdobná jako u tavených sýrů. Z výše uvedeného vyplývá, že se od klasických sýrů liší především ve složení; do surovinové skladby mohou být zařazeny rostlinné oleje (sójový, palmový, řepkový aj.), jiné mléčné proteiny (kaseiny, kaseináty, syrovátkové proteiny), nemléčné (rostlinné) proteiny, přírodní (kukuřičný) či modifikované škroby. Výhodou použití těchto ingrediencí je samozřejmě nižší cena, přídavek však může výrazně ovlivnit senzorické vlastnosti výrobku, především flavour a texturu. Změny záleží na jednotlivých druzích „náhražek“ a na jejich přidaném množství. Především rostlinné proteiny (sójový, bavlníkový, pšeničný aj.) způsobují typické vady textury (snížená elasticita, nižší tvrdost, lepivé/mazlavé těsto aj.), slabý flavour a případně off-flavour [128,131]. Kromě toho se používá množství přídatných látek ovlivňujících barvu (annatto, paprika, syntetická barviva), vaznost vody (arabská, xanthanová, guarová guma, karagenany, alginát sodný, pektiny aj.), příchutě a zvýrazňovače chuti (NaCl, kvasnicové extrakty, extrakty z kouře, enzymově modifikované sýry, glutamát sodný, koření aj.), konzervanty (nisin, kyselina askorbová, propionáty, sorbáty aj.), v zahraničí dokonce i sladicí činidla, zvláště do produktů určených pro děti (sacharosa, dextrosa, kukuřičný sirup, hydrolyzované škroby) [131].

4.1.4.1 Flavour tavených sýrových analogů

Přestože se tyto výrobky na trhu už běžně vyskytují, dosud je známo a publikováno minimum odborných informací o jejich složení a vlastnostech. Většina prací se zaměřuje na sledování textury a jejích změn v důsledku přídavku jiných surovin (škrobů aj.). Přestože největší nevýhodou sýrových analogů je jejich flavour, není mu zatím v dostupné literatuře věnována velká pozornost. Lze uvést práce Cunha a kol. [39], Drake a kol. [51], Yalman a kol. [52], Noronha a kol. [85], Muir a kol. [133], Rouse a kol. [134], které většinou srovnávají flavour tavených sýrů vs. odpovídajících analogů a potvrzují fakt, že flavour analogů je málo výrazný, popisovaný jako „fádní“, „prázdný“ a většinou nedosáhne klasického sýra. Použité nemléčné suroviny mohou navíc způsobit různý off-flavour; např. kasein „stájový“, nevhodné rostlinné oleje „olejovitý/oxidovaný“ aj. [51]. Aby se analogy co

nejvíce blížily odpovídajícím sýrům, jsou v praxi používány různé příchutě a zvýrazňovače chuti, v současné době lze vyrobit analog s prakticky libovolným flavourem [39,128].

V rámci navazující originální studie prezentované v předložené práci byly analyzovány tavené sýrové analogy (suš. 40 %, tvs 50 %) s obsahem různých druhů tuků/olejů (kokosový, slunečnicový, palmový a mléčný). Vzorky byly vyrobeny na UTB ve Zlíně, pro výrobu byly použity: eidamská cihla (tvs 30 %), máslo a uvedené tuky, voda a tavicí soli (Fosfa a.s., Břeclav); tavicí proces 90 °C 10 min. Tuky/oleje byly vybrány jako nejčastěji v praxi používané a vzhledem k jejich poměrně snadné dostupnosti [128]. Máslo se používá pro výrobu klasických tavených sýrů, tento vzorek byl tedy považován za standard pro porovnání. Senzorické kvalitě a obsahu těkavých sloučenin v rostlinných olejích není věnována velká pozornost. Dostupné publikované práce se většinou věnují stanovení obsahu mastných kyselin a/nebo sledování oxidačních procesů [135,136]. Vezmeme-li v úvahu, že většina olejů je během výroby podrobena procesu deodorace, lze předpokládat, že obsah těkavých látek zde bude velmi malý a flavour kvalitního oleje není prakticky vnímatelný, jak potvrzují ve své práci např. Villarino a kol. [137]. Nicméně některé oleje slabý flavour vykazují, ten je ale velmi pravděpodobně tvořen netěkavými sloučeninami, např. mírně slaná chuť kokosového oleje [137], nebo nasládlá chuť a „fialkové“ aroma palmového tuku [138].

Dílčí výsledky identifikovaných AAL v analozích byly prezentovány na konferenci „Chemistry and Life 2011“ v Brně[17]. Kompletní výsledky identifikovaných AAL v jednotlivých druzích analogů a jejich srovnání byly zpracovány do publikace [18] uvedené v **Příloze 4**. V publikaci je uveden přehled identifikovaných sloučenin v analozích, zároveň i v tucích použitých pro jejich výrobu, jsou diskutovány rozdíly mezi jednotlivými vzorky a možný příspěvek použitých tuků k celkovému profilu analogů. Ve vzorcích bylo celkem identifikováno 31 těkavých sloučenin; z nich kvantitativně nejvýznamnější byly alkoholy a kyseliny. Mezi vzorky byly nalezeny významné ($P < 0,05$) rozdíly v počtu i obsahu identifikovaných sloučenin; nejvyšší celkový obsah byl nalezen v analogu s kokosovým ($547,3 \pm 9,8\ \mu g.g^{-1}$), nejnižší v analogu s palmovým tukem ($372,0 \pm 16,2\ \mu g.g^{-1}$). Flavour tavených sýrů i analogů je z velké části determinován flavourem přírodních sýrů použitých k jejich výrobě [131]; srovnáním aromatického profilu analogů, tuků a eidamského sýra lze říci, že část AAL přechází do analogů z použitých tuků; podobně jako u tavených sýrů podstatná část pochází z použitého sýra a část se pravděpodobně tvoří účinkem vysoké teploty během tavení, na druhou stranu u některých sloučenin pravděpodobně došlo naopak k jejich rozkladu nebo vytěkání.

Všechny vyrobené vzorky byly zároveň senzoricky hodnoceny, cílem bylo zhodnocení vlivu přídavku různých druhů tuků na senzorickou kvalitu (flavour). Výsledky senzorického hodnocení byly zpracovány do publikace v recenzovaném časopise[19]. Výsledky porovnání obsahu AAL a senzorické kvality byly prezentovány na „64. Sjezdu Asociace českých

[17] SKLENÁŘOVÁ, K., VÍTOVÁ, E., DIVIŠOVÁ, R., LOUPANCOVÁ, B. Aroma active compounds in several types of processed cheese analogues. *Chemické listy*. 2011. 105(S18), p. 1044.

[18] VÍTOVÁ, E., LOUPANCOVÁ, B., SKLENÁŘOVÁ, K., DIVIŠOVÁ, R., BUŇKA, F. Identification of volatile aroma compounds in processed cheese analogues based on different types of fat. *Chemical Papers*, 2012, 66(10), pp. 907 - 913.

[19] VÍTOVÁ, E., DIVIŠOVÁ, R., SŮKALOVÁ, K., OMELKOVÁ, J., VESPALCOVÁ, M. Srovnání senzorické kvality různých druhů tavených sýrů. *Potravinárstvo*. 2013, 7(SI), pp. 134-137.

a slovenských chemických společností“ v Olomouci[20]. V rámci pořadového testu byl jako nejlepší/nejpřijatelnější hodnocen podle očekávání sýr z másla, z analogů vzorek s kokosovým tukem; jako významně ($P < 0,05$) nejhorší analog se slunečnicovým olejem. Při hodnocení vzhledu, barvy a lesku nebyly mezi vzorky shledány statisticky významné rozdíly; vzorky byly vesměs hodnoceny jako výborné. Textura sýrů z másla a analogů s kokosovým a palmovým tukem byla velmi dobrá, analogů ze slunečnicového a mléčného tuku dobrá. Největší rozdíly byly nalezeny při hodnocení flavouru: sýry z másla a analogy s kokosovým a mléčným tukem byly hodnoceny jako velmi dobré; vzorky se slunečnicovým a palmovým tukem jako dobré nebo méně dobré, byl zde detekován off-flavour označovaný jako „žluklý“, „olejovitý“, „nahořklý“, „po plastu“, „po rybách“ či „dřevu“. Tato zjištění podporují i výsledky profilového testu, největší intenzitu off-flavouru vykazoval analog se slunečnicovým olejem. Tento typický off-flavour je způsoben tvorbou produktů lipolýzy (VMK) a/nebo oxidace lipidů, z nichž některé mají poměrně výraznou vůni; literatura uvádí např. hexanal („zelená“), heptanal („olejovitá“), (E)-2-nonenal („lojovitá“) aj. [72,124,125]. Z těchto sloučenin byly v analozích nalezeny hexanal a heptanal, i když ve velmi nízkých koncentracích (0,01 – 0,02 $\mu g.g^{-1}$ ve vzorcích se slunečnicovým olejem). U slunečnicového oleje lze tento výsledek očekávat; na rozdíl od palmového tuku, který má, stejně jako kokosový, vzhledem ke složení (převaha nasycených MK), vynikající oxidační stabilitu [137,138]. Oxidace byla pravděpodobně ovlivněna záhřevem při tavicím procesu [125].

Lze tedy konstatovat, že druh použitého tuku při výrobě analogů neovlivňuje významně vzhled, barvu a lesk, mírně ovlivňuje texturu, ale významně ovlivňuje flavour a tedy i přijatelnost spotřebiteli. Přestože žádný s posuzovatelů nerozpoznal kokosovou chuť, analogy s kokosovým tukem byly hodnoceny obdobně jako sýr s máslem, mohly by tedy být zajímavým zpestřením nabídky na trhu.

4.2 Tradiční i méně známé bobulové ovoce

V této oblasti ÚCHPBT FCH VUT v Brně dlouhodobě spolupracuje se Zahradnickou fakultou Mendelovy univerzity v Brně, Výzkumným ústavem šlechtitelským a ovocnářským v Holovousích a několika soukromými pěstiteli. V rámci této spolupráce je řešena řada probíhajících projektů, zaměřených na pěstování a šlechtění vybraných druhů tradičního (angrešt, rybíz), známého (černý bez) i méně známého (rakytník, aronie, dřín, muchovník aj.) drobného bobulového ovoce. Tyto plodiny jsou významným zdrojem různých bioaktivních látek, především charakteru antioxidantů, prospěšných lidskému zdraví; mnohé z nich zatím nejsou v dostatečné míře využívány. V rámci předložené studie jde o komplexní chemickou a senzorickou charakterizaci vypěstovaných produktů s cílem jejich praktického využití. Různé odrůdy byly pěstovány na pokusných pozemcích Mendelovy univerzity v Brně v Žabčicích u Brna, poté ve větším rozsahu ve výzkumném ústavu v Holovousích; byly hledány nejlepší odrůdy, podmínky pěstování apod. Hlavním záměrem je podpořit rozvoj jejich pěstování ve větším rozsahu a zavedení na český trh jako čerstvých plodů pro přímý konzum, případně v podobě moderních potravinářských výrobků. Výzkumný tým dr. Vítové se na spolupráci podílí zejména posuzováním senzorické kvality (flavouru) vzorků a obsahem některých biologicky aktivních látek.

[20] SKLENÁŘOVÁ, K., VÍTOVÁ, E., BUŇKA, F., DIVIŠOVÁ, R. Srovnání analytické a senzorické chutnosti tavených sýrových analogů. *Chemické listy*. 2012. 106(6), p. 571.

Postihnout senzorickou kvalitu ovoce je pro velký počet druhů a odrůd obtížné. U ovoce se hodnotí (i) **vzhled** – tvar, textura, barva (barevný tón, sytost a jas barvy, typičnost odpovídající druhu, odrůdě nebo stupni zralosti) a velikost (požaduje se vyrovnanost). Důležitá je celistvost, zdravost, čerstvost, dužnatost, povrchová čistota a suchost; (ii) **textura** a (iii) **flavour**. Významným znakem je tzv. harmoničnost chuti, kdy se hodnotí vzájemný poměr jednotlivých chuťových složek, zejména poměr mezi sladkostí a kyselostí [139].

4.2.1.1 Vznik a vývoj flavouru ovoce

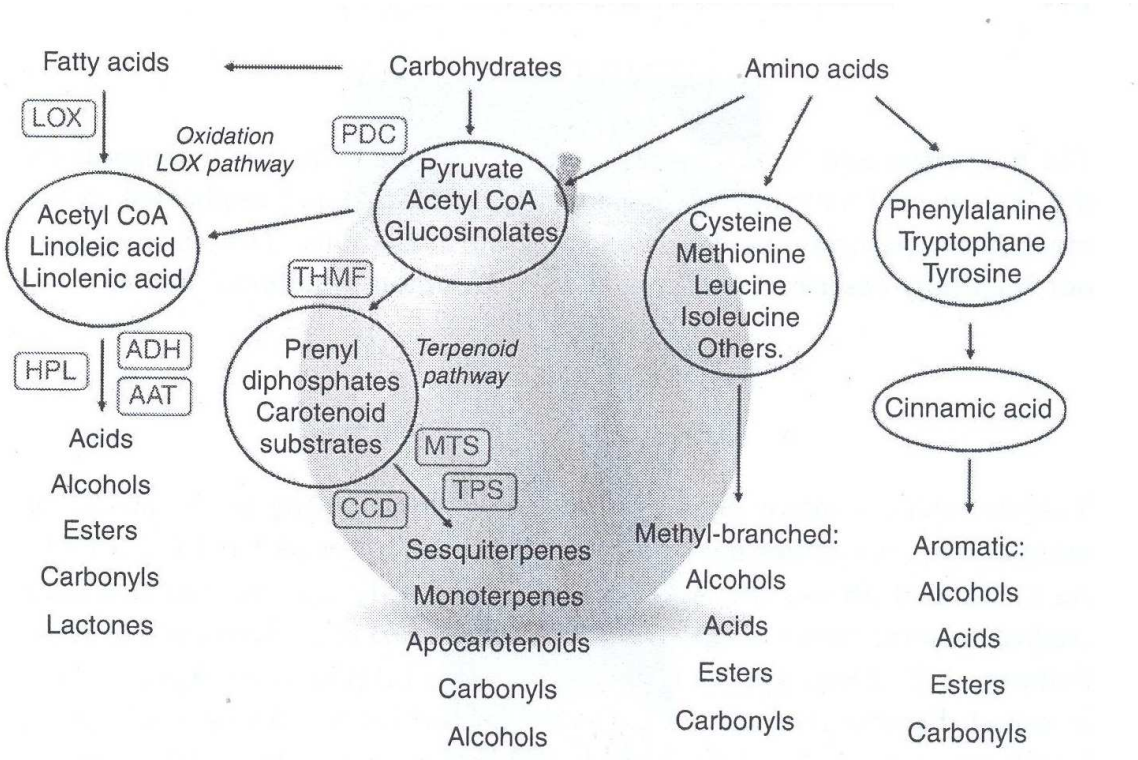
Obsah všech chemických látek (vč. AAL) v ovoci kolísá v závislosti na mnoha faktorech: odrůda, stupeň zralosti a obecně před- a posklizňové podmínky [139,140]. Flavour je tvořen obsahem AAL v kombinaci s netěkavými složkami; chuť souvisí s obsahem sacharidů a kyselin, vůně s obsahem AAL [73,74]. Zralost je považována za klíčový faktor ovlivňující flavour ovoce, během zrání obsah kyselin (kyselá chuť) a nerozpustných pektinových látek (pevnost plodu) klesá, naopak obsah sacharidů (sladká chuť), AAL a výživných látek stoupá. Ovoce by mělo být sklizeno ve stadiu optimální zralosti [141].

Počet AAL se pohybuje podle druhu ovoce od 50 do 1000; nacházejí se zde ve vyšších koncentracích (často > 30 $mg.kg^{-1}$) [17], celkové množství se pohybuje v rozmezí cca 7-200 $mg.kg^{-1}$ [142]. Mezi nejvýznamnější těkavé látky ovoce patří estery, alkoholy, kyseliny, terpeny a karbonylové sloučeniny, přičemž největší skupinu tvoří estery. V esterech bývá nejčastěji vázána kyselina octová, méně často mravenčí, propionová, máselná, 2-methylpropanová aj.; z alkoholů ethanol. Obecně mají estery „sladké“, „ovocné“ nebo „květinové“ aroma [20,25]. Z alkoholů se uplatňují hlavně nižší (do 18 C), významnými AAL jsou některé nenasycené alifatické alkoholy, např. (Z)-3-hexenol, (E)-3-hexenol, (E)-2-hexenol (tzv. „zelené“, „trávové“) [20,25].

Tyto látky jsou v ovoci produkovány většinou jako sekundární metabolity **během zrání**, v menší míře potom během sklizně, posklizňového zpracování a skladování; řada vonných látek je v ovoci chemicky vázána a podstatná část aroma se tvoří až po rozrušení buněk v důsledku zrání, kdy se uvolní příslušné enzymy [25]. Tvorba je komplexní proces zahrnující mnohé biochemické dráhy, z nichž většina není dosud plně objasněna, nicméně většina AAL v rostlinách pochází z metabolismu **aminokyselin**, membránových **lipidů** a **uhlovodíků** [141-145]. Jednoduché schéma metabolických cest tvorby AAL v ovoci je uvedeno na *Obr. 2*.

Přestože lipidy jsou v ovoci zastoupeny v zanedbatelném množství [139], většina nezralého ovoce produkuje různé mastné kyseliny, které jsou v průběhu zrání metabolizovány v rámci α- a β-oxidace nebo účinkem enzymu lipoxygenasy na alkoholy, aldehydy, ketony, kyseliny, estery a laktony [141]. Ač vycházejí ze stejných prekursorů, každý typ rostliny má svůj vlastní soubor enzymů, který, v součinnosti s podmínkami prostředí, vede k různým rozkladným drahám vedoucím k různým těkavým produktům [25].

Také aminokyseliny, hlavně rozvětvené a aromatické, jsou významné prekursory AAL ovoce, především alkoholů, karbonylových sloučenin, kyselin a esterů [141]. Z rozvětvených aminokyselin se tvoří rozvětvené alkoholy, např. 2- a 3-methylbutanol („alkoholové“, „ovocné“) a estery [25,142-144]. Z aromatických aminokyselin se odvozují další AAL, z nichž nejvýznamnější je benzaldehyd („sladké“, „hořké mandle“) a fenylacetaldehyd („medové“); z aromatických esterů pak fenyl-acetát („medové“) a fenyl-propanoát („květinové“) [66,93].



Obr. 2: Biochemické procesy vedoucí ke vzniku aromaticky aktivních látek v ovoci. (převzato z [145]). LOX-lipoxygenasa; HPL-peroxidasa mastných kyselin; ADH-alkoholdehydrogenasa; AAT-alkohol acyltransferasa; PDC-pyruvátdekarboxylasa; THMF-3-ketoacyl-CoA thiolasa; CCD-karoten dioxygenasa; MTS-monoterpen synthasa; TPS-terpen synthasa.

Terpenové uhlovodíky a především jejich kyslíkaté deriváty tvoří významnou složku aroma téměř všech druhů ovoce. Terpeny („ovocná“) limonen, α- a β-pinen, kamfen a 3-karen a terpenové alkoholy („sladké“, „květinové“) linalool, geraniol, nerol, α-terpineol a citronellol jsou v přírodě nejrozšířenější [20,25]. Jejich hlavním prekursorem je kyselina mevalonová, některé z nich pravděpodobně vznikají i jinými cestami, např. degradací karotenoidů [141-144].

Stanovením AAL v různých typech ovoce a ovocných šťáv, většinou v kombinaci se senzorickým hodnocením, se zabývá řada autorů, nejvíce prací je zaměřeno na jablka, pomeranče a víno. Cílem autorů je dokonale porozumět změnám AAL v průběhu zrání a následného zpracování ovoce, stejně tak je málo známo o příspěvku jednotlivých sloučenin ke flavouru. Zvláště žádoucí pro praxi by bylo umět objektivně popsat např. „čerstvost“, „zralost“ případně „přezrálost“ ovoce [142]. Co se týče bobulového ovoce, a zvláště druhů uvedených v rámci této práce, většina publikovaných prací se věnuje studiu jejich složení především z hlediska obsahu bioaktivních sloučenin a antioxidačního potenciálu. Problematikou jejich senzorické kvality, flavouru a obsahu AAL se zabývá velmi málo studií (viz dále). Další výzkum v této oblasti je tedy velmi žádoucí.

4.2.2 Srstka obecná (*Ribes grossularia* L.)

Srstka obecná (*Ribes grossularia* L.) je běžný zahradní keř lidově známý jako angrešt; patří do čeledi meruzalkovité (*Grossulariaceae*). Plody jsou kulaté, podlouhlé nebo hruškovité bobule s hladkou nebo chlupatou slupkou, velikosti cca do 2 cm. Velikost, tvar, barva, pevnost a flavour plodů záleží především na odrůdě a stupni zralosti, chuť je charakteristicky natrpklá (díky obsahu třísloviny), sladká a/nebo kyselá; podle barvy slupky se odrůdy angreštu dělí na běloplodé, červenoplodé, zelenoplodé a žlutoplodé [53].

Angrešt získal význam dlouholetým šlechtěním; cílem bylo vypěstovat odrůdy s většími plody, odolné proti chorobám a škůdcům, a v neposlední řadě bez trnů. V ČR angrešt patří mezi tradičně pěstované, ovocnářsky využívané ovoce, statistiky však uvádějí jeho velmi nízkou spotřebu, cca 0,3 kg na osobu za rok. I když se v poslední době zvyšuje zájem, čerstvé plody se na našem trhu objevují jen sporadicky. Plody angreštu jsou vhodné pro přímý konzum i konzervárenské zpracování. Používají se např. při přípravě kompotů, džemů nebo marmelád, džusů, vín, likérů, octa aj. Patří mezi ovoce s vysokým obsahem vlákniny (pektin), vitaminů (C, E, skupiny B aj.), minerálních látek (Si, K aj.) a dalších výživově cenných složek (flavonoidy, fenolové kyseliny, anthokyany a třísloviny). Mnohé studie dokazují, že zvláště tmavé odrůdy jsou bohatým zdrojem antioxidantů [139,140].

4.2.2.1 Senzorická kvalita (flavour) různých odrůd angreštu

V rámci studie prezentované v předložené práci bylo analyzováno 17 vybraných odrůd angreštu vypěstovaných v ČR. Vzorky byly odebírány v průběhu dvou let (2014–2015). Dílčí výsledky měření AAL byly prezentovány na „64. Sjezdu Asociace českých a slovenských chemických společností“ v Olomouci [21]. Kompletní výsledky včetně senzorického hodnocení byly zpracovány do publikace[22] uvedené v **Příloze 5**. Ve vzorcích bylo identifikováno celkem 52 těkavých látek, z nich kvantitativně nejvýznamnější byly alkoholy a kyseliny. Pomocí metody PCA bylo vybráno 12 sloučenin jako nejvíce variabilních, tyto byly použity pro vyjádření rozdílů mezi vzorky. Mezi odrůdami byly nalezeny významné ($P < 0,05$) rozdíly v obsahu i složení těkavých látek, stejně tak se odrůdy lišily i v senzorických parametrech. Naproti tomu rozdíly mezi roky produkce byly většinou malé, statisticky nevýznamné, přestože z dostupných meteorologických dat vyplývá, že počasí v obou letech bylo mírně odlišné.

Výsledky byly porovnány a diskutovány s dostupnými informacemi, složení AAL angreštu se však věnuje minimum publikací. Pouze Hempfling a kol. [23] a Nikfardjam a kol. [146] nedávno publikovali výsledky stanovení těkavých sloučenin v několika vybraných odrůdách angreštu a jejich příspěvek k aroma plodů. V angreštu identifikovali celkem 122 těkavých sloučenin. Podle nich je typické aroma angreštu charakterizováno kombinací „zelené“ a „ovocné“, přičemž zráním se zvýrazňuje aroma ovocné. Za nositele považují (Z)-3-hexenal („zelené“, „trávové“) a ethyl- a/nebo methyl-estery organických kyselin s krátkým řetězcem,

[21] VÍTOVÁ, E., DIVIŠOVÁ, R., SKLENÁŘOVÁ, K. Aromatický profil plodů angreštu (*Ribes grossularia* L.). *Chemické Listy*. 2012. 106(6), p. 569.

[22] VÍTOVÁ, E., SŮKALOVÁ, K., MAHDALOVÁ, M., BUTOROVÁ, L., MATĚJÍČEK, A., KAPLAN, J. Influence of volatile compounds on flavour of selected cultivars of gooseberry. *Chemical Papers*, 2017 (přijato do tisku)

především methyl-butanoát („ovocné“, “zelené”), ethyl-butanoát („ananas“) a ethyl-acetát („ovocné“) [23,146]. Pomocí vypočtených hodnot OAV bylo v prezentované publikaci (**Příloha 5**) určeno celkem 7 nových sloučenin jako možné složky aroma analyzovaných plodů.

V publikaci[22] je dále podrobně diskutována senzorická kvalita jednotlivých odrůd; vzhledem k tomu, že některé odrůdy jsou nově vyšlechtěné, jedná se o nové a z hlediska pěstitelů i spotřebitelů velmi důležité poznatky. Harb a Streif [53] ve své práci identifikovali pevnost plodu, vyváženost sladké a kyselé chuti a možnou přítomnost off-flavouru jako hlavní ukazatele senzorické kvality. Některé odrůdy mají sladší, některé naopak kyselejší chuť, trpká chuť je pro angrešt typická, ale ve vyšší intenzitě může být vnímána jako nepříjemná. Pro přímý konzum lépe chutnají plody s jemnou slupkou, ale pevnou texturou; konzumenti požadují charakteristické křupnutí při skousnutí. Slupka se zjemňuje během zrání, zároveň ale dochází k měknutí plodů, které dále měknou i během skladování. V našem případě příliš měkká textura výrazně negativně ovlivňovala přijatelnost plodů. Je tedy nezbytné plody sklízet v optimální zralosti a co nejrychleji zkonzumovat, případně zpracovat [53]. K celkové senzorické kvalitě našich vzorků nejvíce přispíval vzhled, barva a celková chuť a vůně.

Na základě našich prvních výsledků lze označit červenoplodou odrůdu Karát a žlutoplodý Darek jako nejlepší/nejpřijatelnější ze senzorického hlediska, bude však potřeba provést další experimenty v průběhu několika let, aby bylo možné posuzovat stabilitu (a/nebo variabilitu) senzorické kvality těchto odrůd.

4.2.3 Bez černý (*Sambucus nigra* L.)

Kromě tradičních druhů bobulového ovoce (angrešt, rybíz) se stále více zvyšuje zájem o méně tradiční druhy dosud nevyužívané v průmyslovém měřítku. Většinou jsou to dřeviny, které se nehodí k pěstování ve velkém, např. kvůli obtížné sklizni, nízkým výnosům, nerovnoměrnému zrání, krátké trvanlivosti apod. K tomu může často přispívat nezvyklá chuť jejich plodů. Některé z nich však obsahem nutričních látek značně převyšují běžně pěstované ovocné druhy [139,140].

Bez černý (*Sambucus nigra* L.) patří do čeledi zimolezovitých (*Caprifoliaceae*). Plody jsou drobné, kulaté malvice velikosti cca 6 mm. Zralé mají tmavě fialovou až černou barvu, jsou vyplněny šťavnatou tmavě červenou dužninou slabě kyselé chuti bez výrazného aroma.

Pro své léčivé účinky je již po staletí používán v léčitelství, pro své vonné a chuťové vlastnosti v kuchyni. Plody černého bezu jsou ceněny zejména kvůli vysokému obsahu vitaminů (A, B1, B2, B3 a především C), organických kyselin (citrónová, jablečná, šikimová a fumarová), sacharidů, minerálních látek (draslík, vápník, fosfor) a flavonoidů [139]. Je zpracováván v potravinářském průmyslu na výrobu šťáv, džusů, sirupů aj. Pro svůj vysoký obsah anthokyanových barviv se užívá k barvení potravinářských výrobků, jako jsou cukrovinky, rosoly, džemy, víno nebo mléčné výrobky [73,74].

Poptávka po černém bezu se neustále zvyšuje kvůli jeho využití v potravinářském, kosmetickém a farmaceutickém průmyslu. Začíná se s jeho šlechtěním, kultivací a pěstováním; tím lze získat odrůdy, které mají vyšší výtěžky, vyšší obsah žádaných komponent, nižší obsah toxického sambunigrinu, lepší senzorické vlastnosti aj. [139].

4.2.3.1 Senzorická kvalita (flavour) různých odrůd bezu černého

V rámci této studie bylo analyzováno celkem 16 šlechtěných odrůd a pro srovnání i planý bez. Cílem bylo porovnat jednotlivé vzorky a doporučit kultivary vhodné pro pěstování v ČR a eventuální praktické využití. První získané výsledky identifikovaných AAL ve vzorcích bezu byly prezentovány na konferenci „Chemistry and Life 2011" v Brně [23]. Kompletní výsledky byly zpracovány do publikace [24] uvedené v **Příloze 6**.

Aroma bezových plodů i květů je již poměrně dobře popsáno. Bez má obecně vysoký obsah AAL, různými autory již bylo v bezových produktech identifikováno více než 100 těkavých AAL, většina z nich jsou degradační produkty mastných kyselin (alifatické alkoholy, aldehydy, estery, ketony, furany, terpenoidy) a deriváty kyseliny šikimové [73-75]. S využitím GC-O Jensen a kol. [67] rozdělili AAL v bezu podle charakteru jejich vůně do 6 skupin: „bezová", „květinová", „ovocná", „trávová", „venkovská" a „ostatní". Tohoto rozdělení se víceméně drží a k podobným závěrům docházejí i další autoři, např. Kaack a kol. [73,74]. Pro celkové aroma bezu jsou podle nich nejdůležitější sloučeniny s charakteristickou vůní bezu, doplněné látkami v „ovocné" a „květinové" skupině. Těkavé látky v „trávové" skupině jsou důležité pro „čerstvost" produktů [67,73,74]. V našich vzorcích bylo celkem identifikováno 102 těkavých sloučenin. Na základě srovnání s jinými autory [67,73-75], a také vzhledem k jejich vysokému obsahu ve vzorcích bylo vybráno 36 sloučenin jako nejvýznamnějších, tyto byly ve vzorcích dále sledovány a použity pro vyjádření rozdílů mezi odrůdami. Ve shodě s ostatními autory byly mezi odrůdami nalezeny významné rozdíly ($P < 0,05$) v obsahu různých skupin i jednotlivých sloučenin; zajímavé je, že poměrně vysoký obsah byl nalezen ve vzorcích planého bezu, přestože jeho aroma je spíše jemné a málo intenzivní [73,74]. Navíc jedním z cílů šlechtění bezu je dosáhnout lepší flavour, a tedy lze očekávat i vyšší obsah AAL. Na základě našich výsledků se odrůdy Korsör, Pregarten a Samdal jeví jako slibné pro eventuální praktické využití.

Pro posouzení chuťových a aromatických vlastností jednotlivých odrůd byly vzorky také senzoricky hodnoceny (výsledky nebyly zahrnuty do publikace), za tímto účelem z nich byla vylisována šťáva. Jako standard chutnosti byla použita 100% pasterovaná (78 °C, 20-30 s) bezová šťáva (Vitaminátor s.r.o.), získaná od soukromého pěstitele z eko- nebo integrované produkce; tato šťáva byla senzoricky posouzena skupinou expertů (odborníci z praxe) jako výborná. Podle očekávání vzorky samotné bezové šťávy nebyly hodnoceny příznivě. Bez má zvláštní, nakysle sladkou, charakteristickou chuť, vysoký obsah třísloviny mu dodává chuť více či méně trpkou/svíravou [73,74]. Jednotlivé odrůdy se překvapivě poměrně výrazně lišily i z hlediska senzorické jakosti. Vzorky planého bezu byly podle očekávání hodnoceny hůře (nevyhovující), stejně jako některé šlechtěné odrůdy (Albida, Aurea, Haschberg, Sambu a Samyl), u nichž nebyla vůbec nebo velmi málo znatelná charakteristická bezová chuť a byly spíše hořké a trpké, v některých případech i více než vzorky planého bezu. Co se týče odrůd

[23] VÍTOVÁ, E., VESPALCOVÁ, M., LOUPANCOVÁ, B., DIVIŠOVÁ, R., SKLENÁŘOVÁ, K., KORHOŇOVÁ, M. The comparison of content of aroma active compounds in samples of elderberries (*Sambucus nigra* L.). *Chemické Listy*. 2011. 105(S18), s. 1031.

[24] VÍTOVÁ, E., DIVIŠOVÁ, R., SŮKALOVÁ, K., MATĚJÍČEK, A. Determination and quantification of volatile compounds in fruits of selected elderberry cultivars grown in Czech Republic. *Journal of Food and Nutrition Research*. 2013, 52(1), pp. 1-11.

slibných z hlediska obsahu AAL (Korsör, Pregarten a Samdal – viz výše) byly ohodnoceny jako dobré nebo méně dobré. Jako nejlepší byl vždy hodnocen standardní vzorek.

4.2.3.2 Příprava funkčního ovocného nápoje na bázi bezové šťávy

Vzhledem ke vzrůstající popularitě tzv. funkčních nápojů, v rámci řešení problematiky bezu a jeho zpracování bylo naším hlavním záměrem navrhnout optimální složení bezové šťávy (koncentrátu) pro komerční využití. Samotná bezová šťáva je však v čistém stavu kyselá, trpká až hořká (viz výše), pro případné využití v praxi bylo nezbytné ji smíchat se šťávou jiného druhu ovoce. Výsledná směs by měla být ze senzorického hlediska co nejpřijatelnější s tím, že si pokud možno zachová charakteristický bezový flavour a příjemnou tmavě červenou barvu typickou pro černý bez. Jako standard byla použita komerčně získaná koncentrovaná bezová šťáva z Rakouska, kde se běžně prodává jako doplněk stravy.

První dílčí výsledky byly prezentovány na konferenci „Bezpečnosť a kontrola potravín" v Nitře[25]. Kompletní výsledky pak byly zpracovány do publikace v recenzovaném časopise [26] uvedené v **Příloze 7.** Publikace uvádí postup optimalizace složení šťávy především na základě senzorického hodnocení. Nejprve byly připraveny vzorky smícháním bezové šťávy s vybranými druhy šťáv (mrkvová, jablečná, hroznová, pomerančová, rybízová-černý rybíz). Vzorky bezové a hroznové šťávy byly získány od soukromého pěstitele, ostatní z běžné tržní sítě. Jako nejlepší/nejpřijatelnější byl vyhodnocen vzorek rybíz-bez a hrozny-bez. Vzhledem k příjemné sladké chuti, a v neposlední řadě i přijatelné ceně a snadné dostupnosti, byla nakonec vybrána pro praktické využití šťáva hroznová; následně byly připraveny další vzorky smícháním hroznové a bezové šťávy v různém poměru (50-90 % obj. bezové). Jako senzoricky nejlepší (nejpřijatelnější) byl hodnocen vzorek obsahující 60 % obj. bezové šťávy; přesto byl zvolen poměr 70 % obj. bezové šťávy, který byl senzoricky hodnocen také jako velmi dobrý. Je to především proto, aby konečný nápoj obsahoval co nejvyšší koncentrace biologicky účinných látek, které se v bezu nacházejí. Mírně natrpklá chuť tohoto vzorku pak byla vylepšena přídavkem glukosy, jako optimální byl hodnotiteli určen přídavek 8 % hm. Přídavkem glukosy lze korigovat i eventuální sezónní změny obsahu cukru ve víně, které mohou být značné.

Pro doplnění je v publikaci zahrnuto i stanovení a srovnání obsahu AAL; eventuální příspěvek identifikovaných sloučenin k flavouru ovocných šťáv byl posouzen s využitím konceptu OAV. Ve vzorcích bylo identifikováno celkem 57 těkavých sloučenin. Nejvyšší obsah byl nalezen v bezové šťávě (1694,4 ± 16,6 $\mu g.ml^{-1}$), naopak hroznová šťáva měla překvapivě nízký obsah těchto sloučenin (828,9 ± 4,9 $\mu g.ml^{-1}$), k jejímu flavouru přispívá především sladkost. Zajímavé je, že vzorky mrkev-bez a pomeranč-bez, které byly senzoricky hodnoceny nejhůře, měly poměrně vysoký obsah těkavých látek. Lze tedy soudit, že ne všechny AAL přispívají k celkovému flavouru pozitivně. Praktickým výsledkem této práce je

[25] VÍTOVÁ, E.; VESPALCOVÁ, M.; OMELKOVÁ, J.; DIVIŠOVÁ, R.; SKLENÁŘOVÁ, K. Obsah aromaticky aktivních látek ve šťávě z bezu černého (*Sambucus nigra* L.). In *Bezpečnosť a kontrola potravín*. Nitra (SR). 2011, p. 257-260.

[26] VÍTOVÁ, E., SŮKALOVÁ, K., MAHDALOVÁ, M., BUTOROVÁ, L. BABÁK, L., MATĚJÍČEK, A. Comparison of flavour and volatile flavour compounds of mixed elderberry juices. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, 2015, 63(1), pp. 147-152.

tedy komerčně využitelný směsný ovocný nápoj na bázi bezové šťávy, příjemné mírně sladké chuti s vysokým obsahem antioxidantů a dalších zdraví prospěšných látek obsažených v bezu.

4.2.4 Rakytník řešetlákový (*Hippophae rhamnoides* L.)

Rakytník řešetlákový (*Hippophae rhamnoides* L.) náleží do čeledi hlošinovitých (*Eleagnaceae*). Jeho plody jsou nepravé peckovice, které mají v době zralosti žlutou, oranžovou až červenou barvu (danou vysokým obsahem karotenoidů), oválný až vejčitý tvar a velikost cca 5 mm]. Obsahem biologicky aktivních látek patří rakytník mezi nejcennější potravinářské a léčivé rostliny. Plody jsou bohaté na vitaminy (C, E, B_1, B_2 aj.), karotenoidy, flavonoidy, organické kyseliny, polynenasycené mastné kyseliny aj. [88,147,148].

Plody rakytníku byly využívány už v tradiční tibetské, mongolské a čínské medicíně. I dnes má rakytník široké spektrum uplatnění v medicíně, farmacii a kosmetice (anti-age krémy, masky, pleťové vody aj.) [149]; zvyšuje se zájem i o rakytník jako o potravinu. Plody se mohou konzumovat čerstvé nebo se z nich připravují různé produkty, jako např. šťávy, džemy, kompoty, povidla, sirupy, alkoholické i nealkoholické nápoje, cukrovinky aj. [148,149]. V dnešní době se rakytník používá hlavně jako výživový doplněk (olej, kapsle s olejem, tablety), v poslední době se začíná více uplatňovat i v potravinářství např. jako součást některých funkčních nebo biopotravin [54].

4.2.4.1 Senzorická kvalita (flavour) rakytníku řešetlákového

Problematikou senzorické kvality rakytníku se dosud zabývalo poměrně málo studií. Plody rakytníku mají jemné unikátní aroma nepodobné jakémukoli jinému běžnému ovoci. Tang a kol. [54] ve své práci označili flavour rakytníkové šťávy jako jahodový, broskvový, mango nebo jablko, přičemž nejčastěji se objevoval termín jahodový, nicméně tento výraz byl spojován se slovy "zmražený" a/nebo "fermentovaný". Tiitinen a kol. [89] popisují flavour rakytníku jako exotického, bobulového nebo citrusového ovoce, Li a Beveridge [148] jako ananas nebo maracuja. Chuť plodů rakytníku je typicky kyselá, hořká a trpká; může se lišit mezi odrůdami, stejně jako jejich velikost, tvar nebo barva. Kyselost je dána přítomností organických kyselin (jablečná), přítomnost fenolických sloučenin vyvolává trpkou chuť; sladkost, stejně jako poměr cukr/kyseliny, je ve srovnání s ostatními druhy bobulového ovoce velmi nízká [54,88,89]. Také problematice stanovení AAL v plodech rakytníku a případných produktech (šťáva, víno) se zatím věnovalo jen málo autorů; lze uvést práce [69,89,90,150-152].

První dílčí výsledky naší studie zahrnovaly těkavé sloučeniny identifikované ve vzorcích rakytníku a popis jeho senzorických vlastností a byly prezentovány na konferenci „Bezpečnosť a kontrola potravín“ v Nitře [27]. Vzhledem k tomu, že se jedná o v Česku poměrně méně známý a málo využívaný druh drobného ovoce, hodnocení bylo nejprve zaměřeno na celkový popis, intenzitu a příjemnost chuti a vůně. Zajímalo nás, jak posuzovatelům vzorky chutnají a zda by je konzumovali v syrovém stavu. Chuť i vůně

[27] VÍTOVÁ, E., DIVIŠOVÁ, R., SKLENÁŘOVÁ, K., OMELKOVÁ, J., ZEMANOVÁ, J. Obsah aromaticky aktivních látek v plodech rakytníku řešetlákového (*Hippophae rhamnoides* L.). In *Bezpečnosť a kontrola potravín*. Nitra (SR). 2012, pp. 262-265.

rakytníku byla hodnocena jako celkem příjemná, vůně střední intenzity. Jako hlavní chuť, která v rakytníku převažuje, byla označena kyselá. Sladká chuť byla neznatelná, trpká a hořká se podílejí na celkové chuti slabě. Dosti silně k celkovému flavouru rakytníku přispívají další chutě, které posuzovatelé popisovali jako jahodovou, ananasovou a/nebo ovocnou, což je v souladu s výsledky jiných autorů [54,89]. Především kvůli jeho výrazně kyselé chuti posuzovatelé doporučili případné výrobky z rakytníku přisladit, podobně jako Tang a kol. [54] ve své práci. Tiitinen a kol. [89] navrhují smíchat rakytníkovou šťávu s jiným ovocem, např. jablko nebo pomeranč.

Výsledky AAL identifikovaných ve 13 různých odrůdách rakytníku a jejich porovnání byly prezentovány na konferenci „CECE 2014, 11th International Interdisciplinary Meeting on Bioanalysis“ v Brně [28]. Kompletní výsledky byly zpracovány do publikace[29] uvedené v **Příloze 8**. Celkem bylo analyzováno 13 odrůd sbíraných během dvou let (2012-2013). Tyto odrůdy jsou zamýšleny a zkoumány pro možné pěstování v ČR. Ve vzorcích bylo celkem identifikováno 69 těkavých sloučenin, výsledky byly porovnány s dostupnými publikacemi [69,89,90,150]. Podobně jako v práci autorů Tiitinen a kol. [89] byly nalezeny významné rozdíly ($P < 0,05$) mezi odrůdami. Pomocí PCA bylo vybráno 18 reprezentativních sloučenin, tyto byly ve vzorcích dále sledovány a použity pro vyjádření rozdílů mezi vzorky. Při porovnání jednotlivých odrůd sklízených v různých letech však bylo patrné, že obsah identifikovaných sloučenin sice mírně kolísá, na rozdíl od práce Tiitinen a kol. [89] však rozdíly mezi roky sběru byly shledány nevýznamné. Z hlediska případného praktického využití je dosažení standardního složení a vlastností plodů velmi žádoucí.

Pro posouzení chuťových a aromatických vlastností jednotlivých odrůd byly vzorky také senzoricky hodnoceny (výsledky nebyly publikovány). Výsledky odpovídají výše zmíněným poznatkům a byly také v souladu s již publikovanými [54,89]. Kromě poměrně výrazně kyselé a trpké chuti byli posuzovatelé schopni rozpoznat i podstatně jemnější, charakteristickou chuť plodů rakytníku, kterou popisovali jako jahodová, ananasová, citrusová, jablečná nebo po tropickém ovoci. Jak uvádějí Tang a kol. [54] vzhledem ke specifické chuti nejsou plody rakytníku spotřebiteli dobře přijímány a nejsou tedy příliš vhodné k přímému konzumu; náš další výzkum tedy směřuje k jejich zpracování na vhodný produkt, podobně jako v případě bezové šťávy.

4.2.5 Temnoplodec černoplodý (*Aronia melanocarpa* L.)

Temnoplodec černoplodý (*Aronia melanocarpa* L.), běžněji nazývaný aronie, patří do čeledi růžovité (*Rosaceae*). Plody jsou černě zbarvené malvice velikosti 6−13 mm a hmotnosti 0,5−2 g, dužnina je tmavě fialová se svíravou, natrpklou chutí. Aronie je bohatým zdrojem polyfenolických sloučenin (polyfenolické kyseliny, prokyanidiny, anthokyany a flavonoly), minerálních látek (draslík, zinek), vitaminů (skupina B, C, E a K) a tříslovin.

[28] VÍTOVÁ, E., SŮKALOVÁ, K., MAHDALOVÁ, M., BUTOROVÁ, L. Identification and quantification of aroma compounds of Sea buckthorn berries. In *CECE 2014, 11th International Interdisciplinary Meeting on Bioanalysis*. Brno: Ústav analytické chemie AV ČR, 2014. pp. 450 - 453.

[29] VÍTOVÁ, E., SŮKALOVÁ, K., MAHDALOVÁ, M., BUTOROVÁ, L., MELIKANTOVÁ, M. Comparison of selected aroma compounds in cultivars of sea buckthorn (*Hippophae rhamnoides* L.). *Chemical Papers*, 2015, 69(6), pp. 881-888.

Plody využívali v tradiční medicíně i jako potravinu již původní severoameričtí indiáni; mnohé studie prokazují antioxidační, protirakovinné, protimutagenní, kardioprotektivní, hepatoprotektivní, antidiabetické a protizánětlivé účinky [153,154]. V dnešní době jsou z plodů aronie vyráběny různé potravinářské, farmaceutické (tablety, sirupy, doplňky stravy) i kosmetické produkty. Aronie má kyselou, trpkou chuť, proto je vhodnější ke zpracování na produkty, např. šťávy, džusy, koncentráty, džemy, čaje, likéry, ovocná vína aj. Díky vysokému obsahu anthokyanů se používá jako přírodní potravinářské barvivo, v poslední době si získává oblibu jako funkční potravina nebo biopotravina [154].

4.2.6 Muchovník olšolistý (*Amelanchier alnifolia*)

Muchovník olšolistý (*Amelanchier alnifolia*) patří do čeledi růžovité (*Rosaceae*). Vzhledem k taxonomické příbuznosti vykazuje mnohé obdobné vlastnosti jako aronie. Plody jsou šťavnaté červeně až tmavě fialově zbarvené malvice o velikosti 5–15 mm s příjemnou nasládlou chutí. Je dobrým zdrojem polyfenolických sloučenin (prokyanidiny, anthokyany), minerálních látek (draslík, železo, hořčík) a vitaminů (A, skupina B, C a E) [155,156]. I plody muchovníku byly využívány původními domorodci v tradiční medicíně. Studie prokazují antioxidační, protirakovinné, antidiabetické a protizánětlivé účinky. Ač u nás téměř neznámý, např. v Kanadě jsou plody běžně konzumovány v čerstvém stavu nebo zpracovávány na různé potravinářské výrobky (koláče, muffiny, sušenky, chléb, sirupy, mošty, pivo, víno, likéry, džemy, cukrovinky aj. Podobně jako v případě bezu i aronie, potenciálním nebezpečím mohou být kyanogenní glykosidy (sambunigrin, amygdalin a prunasin) [155,156].

4.2.6.1 Flavour aronie a muchovníku

Problematikou obsahu AAL v aronii, resp. muchovníku se dosud zabývalo poměrně málo studií. V případě aronie lze uvést práce Kraujalytė a kol. [68], Hirvi a Honkanen [157] nebo Dolezal a kol. [158]. Celkem už bylo v aronii identifikováno > 200 sloučenin. V případě muchovníku byla nalezena jediná práce autorů Mazza a Hodgins [159], která se navíc zaměřuje pouze na benzaldehyd jako hlavní AAL muchovníku.

V rámci prezentované studie bylo analyzováno několik odrůd aronie a muchovníku. Jedná se o první vypěstované vzorky těchto plodů, k dispozici byly pouze dvě odrůdy aronie a pět odrůd muchovníku, sbíraných v letech 2011-2013. Výsledky identifikovaných těkavých látek byly zpracovány do publikace[30] uvedené v **Příloze 9**. Cílem bylo posoudit rozdíly/podobnosti mezi aronií a muchovníkem, mezi jednotlivými odrůdami a mezi roky sběru. Hlavním předpokládaným záměrem do budoucna je, podobě jako v případě bezu, návrh funkčního nápoje na bázi aronie pro komerční využití.

Aronie i muchovník obsahují především amygdalin, který je zodpovědný za charakteristický hořko-mandlový flavour plodů a zároveň je pravděpodobně prekursorem řady AAL [158,159]. Za dominantní složku aroma obou druhů lze považovat benzaldehyd („sladké“, „hořké mandle“) [68,159], vznikající hydrolýzou amygdalinu [20,25]. Kraujalytė

[30] BUTOROVÁ, L., VÍTOVÁ, E., POLOVKA, M. Comparison of volatiles identified in *Aronia melanocarpa* and *Amelanchier alnifolia* using solid-phase microextraction coupled to gas chromatography-mass spectrometry. *Journal of Food and Nutrition Research*. 2016, 55(1), pp. 57-68.

a kol. [68] dále uvádí aldehydy, alkoholy a terpenoidy jako významné pro aroma aronie, které popisují jako „mandlové“, „ovocné“, „kyselé“ a/nebo „zelené“. V našich vzorcích aronie bylo identifikováno 39, ve vzorcích muchovníku 31 těkavých sloučenin; jako převládající aldehydy, alkoholy, estery a kyseliny. Podle očekávání aroma profil aronie a muchovníku byl podobný, pravděpodobně díky zmíněné taxonomické příbuznosti. Pomocí vypočtených hodnot OAV bylo celkem 14 sloučenin určeno jako složky aroma analyzovaných plodů. Pomocí metody PCA bylo vybráno 24 reprezentativních sloučenin, tyto byly použity pro vyjádření rozdílů mezi vzorky. U obou plodin byly nalezeny významné rozdíly ($P < 0,05$) nejen mezi odrůdami, ale i mezi roky sběru, což ukazuje na vliv environmentálních faktorů [139]; nejbližším cílem tedy bude standardizace produkce.

4.3 Aromatizované potraviny, pochutiny a nápoje

Společně s růstem poptávky po atraktivních produktech vyznačujících se příjemnou vůní/chutí se neustále rozšiřuje sortiment aromatizovaných potravin (cukrovinky, alkoholické i nealkoholické nápoje aj.); o těchto typech produktů je však zpracováno velmi málo informací [160]. Lze uvést např. analýzu různých druhů čajů [161-163], likérů [164,165], žvýkacích gum [166] nebo cukrovinek [167,168]. Práce se však věnují stanovení obecně všech AAL, cílem předložené studie je zaměřit se na konkrétní vonné alergeny.

4.3.1 Stanovení alergenních vonných látek v aromatizovaných potravinách

Problematika alergenních AAL byla řešena v rámci další disertační práce[31]. Hlavním cílem práce bylo vyvinout jednoduchou a rychlou rutinní metodu pro stanovení vonných látek s alergenními účinky s možnou aplikací na potraviny a kosmetické přípravky. Práce zahrnuje optimalizaci a validaci použité metody pro simultánní stanovení konkrétních 24 legislativou regulovaných vonných látek: limonen („citrusové“), amylcinnamylalkohol („ovocné“), linalool („květinové“), citronellol („květinové“, „citrusové“), geraniol („růže“), benzylalkohol („mandlově ovocné“), eugenol („hřebíček“, „karafiát“), isoeugenol („koření“), anýzalkohol („květinové“), cinnamylalkohol („květinové“), farnesol („květinové“), citral („citronové“), hydroxycitronellal („květinové“), lyral („květinové“), lilial („květinové“), cinnamal („skořice“), amylcinnamal („jasmín“), hexylcinnamal („jasmín“), benzyl-benzoát („hořkosladké“), benzyl-salicylát („balzamické“, „ovocné“), benzyl-cinnamát („balzamické“), methyl-2-oktynoát („květinové“), α-isomethylionon („květinové“), kumarin („vanilka“). Přehledná tabulka je uvedena v publikaci[33] v **Příloze 10.**

Stanovení vonných alergenů je založeno na již ověřené a používané HS-SPME-GC-FID/MS metodě, bylo však nutné ověřit a upravit některé parametry pro dokonalou separaci a zisk maximálního extrakčního výtěžku cílených sloučenin během přijatelně dlouhé doby analýzy. Villa a kol. [169] vyvinuli a publikovali metodu stanovení těchto látek založenou na použití HPLC. Uvádějí její výhody, jako jednoduchost, rychlost (doba analýzy 40 min), snadno dostupnou instrumentaci. Na druhou stranu některé typy vzorků bylo třeba extrahovat rozpouštědlem (acetonitril), z tohoto hlediska je námi vyvinutá metoda šetrnější, bez použití

[31] DIVIŠOVÁ, R. Alergenní vonné látky v potravinách a předmětech běžného užívání. [disertační práce, školitel specialista E. Vítová]. Brno: FCH VUT v Brně, 2014.

rozpouštědel. První dílčí výsledky byly prezentovány na „64. Sjezdu Asociace českých a slovenských chemických společností“ v Olomouci[32]. Metoda byla následně aplikována na vybrané vzorky aromatizovaných potravin, kosmetiky a hraček. Byly vybrány výrobky, u kterých lze předpokládat, že byly aromatizovány. Celkem bylo analyzováno 82 komerčně dostupných vzorků: 34 kosmetických výrobků (zubní pasty, ústní vody, pleťové vody, krémy a masky, balzám na rty, dětské krémy a oleje, vody po holení, tělová kosmetika a krémy na ruce, deodoranty a antiperspiranty, sprchové gely a šampóny a avivážní prostředky); 42 vzorků potravin (sypané a porcované čajové směsi, želatinové bonbóny, žvýkací gumy a alkoholické případně nealkoholické nápoje); a 3 druhy dětských hraček, zakoupených v běžné tržní síti. Přítomnost a obsah sledovaných látek ve vzorcích byl porovnán s informacemi na obalu a s příslušnými legislativními požadavky za účelem vyhodnocení možných negativních účinků na zdraví uživatelů.

Podrobný postup optimalizace a validace metody a vybrané výsledky analýz kosmetických produktů byly zpracovány do publikace [33] uvedené v **Příloze 10**. Z hlediska komplexnosti této práce zde budou uvedeny a diskutovány pouze výsledky analýz vzorků potravin. Jak již bylo zmíněno, potravinářská legislativa zatím použití alergenních vonných látek nereguluje (s výjimkou kumarinu) [29]. Přídavek vonných látek do potravin je na obale výrobku většinou shrnut pod pojem „aroma“ případně konkrétněji „jahodové, citrónové aj. aroma“ [29]. I když se měřené vzorky, i stejného typu, poměrně výrazně lišily, z naměřených výsledků je patrné, že obsah některých vonných alergenů ve vzorcích je poměrně vysoký. Z důvodu absence jejich legislativního omezení v potravinách byla pro hrubé přiblížení a porovnání zvolena jako limitní koncentrace stanovená pro nesmývatelné kosmetické výrobky (10 $\mu g.g^{-1}$). Nejčastěji (> 90 % vzorků) byly ve vzorcích detekovány limonen a linalool.

Velké množství alergenních AAL bylo nalezeno ve vzorcích čajů, obsahujících (a na obalu uvedených) sušené části ovoce (jablko, šípek, citrón, pomeranč, ale také exotické plodiny goji a papája), byliny (meduňka, vousatka citrónová, zázvor, ženšen), léčivé rostliny (květy šafránu, chrpy, pivoňky, bodláku, slunečnice) a koření (bílý a červený pepř, santalové dřevo, skořice). Zastoupení vonných alergenů bylo sledováno jak v původní směsi, tak ve výluhu, který spotřebitel konzumuje. Všechny vzorky obsahovaly téměř veškeré sledované vonné alergeny v různých koncentracích, ve výluhu byl většinou obsah sledovaných látek významně nižší ($p < 0,05$) než v suché směsi. Mezi nejčastěji se vyskytujícími byly: limonen, amylcinnamylalkohol, linalool, citral, amylcinnamal, α-isomethylionon, benzyl-benzoát. Koncentrace byly velmi rozdílné, pohybovaly se od jednotek až ke stovkám $\mu g.ml^{-1}$, v některých případech i k tisícům $\mu g.ml^{-1}$. Většina z nich přesahovala stanovený limit, dokonce i v čajovém výluhu.

V případě cukrovinek a žvýkacích gum bylo zastoupení vonných látek podobné, v každém vzorku bylo nalezeno v průměru 10 vonných alergenů s koncentracemi v jednotkách až desítkách $\mu g.g^{-1}$. Z cukrovinek byly vybrány různé druhy želatinových bonbónů. Na obale

[32] DIVIŠOVÁ, R., ZEMANOVÁ, J., VÍTOVÁ, E., SKLENÁŘOVÁ, K. Stanovení alergenních vonných látek metodou mikroextrakce tuhou fází ve spojení s plynovou chromatografií. *Chemické Listy*. 2012. 106(6), s. 503.

[33] DIVISOVA, R., VITOVA, E., DIVIS, P., ZEMANOVA, J., OMELKOVA, J. Validation of SPME-GC-FID Method for Determination of Fragrance Allergens in Selected Cosmetic Products. *Acta Chromatographica*, 2015, 27(3), pp. 509-523.

těchto produktů je často kromě termínu „aroma“ uvedeno použití ovocných případně bylinných koncentrátů nebo extraktů; tyto sice většinou plní primárně funkci barviv, ale nepochybně přispívají i k flavouru a mohou být zdrojem vonných alergenů. Téměř ve všech vzorcích byly identifikovány limonen, linalool, amylcinnamylalkohol, methyl-2-oktynoát, α-isomethylionon, benzylalkohol a benzyl-benzoát. Nejvyšší koncentrace, často přesahující limit, byly nalezeny u limonenu, linaloolu a amylcinnamylalkoholu.

Z alkoholických nápojů byly analyzovány ovocné kvašené nápoje a likéry, z nealkoholických ochucené ovocné nápoje. Také zde bylo často uvedeno použití různých, většinou ovocných šťáv a/nebo koncentrátů. Ve vzorcích bylo nalezeno v průměru 14 vonných alergenů na výrobek, jejich koncentrace se pohybovaly v jednotkách až desítkách $\mu g.ml^{-1}$, výjimečně ve stovkách až tisících. Jelikož se jedná o ovocné nápoje, byly ve všech vzorcích opět nalezeny limonen, linalool a amylcinnamylalkohol.

Všechny identifikované látky se používají do syntetických aromat, většina z nich je i přirozenou součástí různých rostlin, popřípadě ovoce. Ale vzhledem k tomu, že o jejich možných účincích na lidský organismus po konzumaci je známo jen velmi málo, i při takto vysokých koncentracích se lze pouze dohadovat o jejich případném negativním působení. Nicméně není vyloučeno, že v budoucnu budou tyto účinky potvrzeny a podobné limity budou zavedeny i v potravinářské legislativě.

5 ZÁVĚR

Předložená habilitační práce představuje nejvýznamnější odborné aktivity uchazečky v oblasti využití kombinace senzorické analýzy a moderních instrumentálních metod pro kontrolu kvality a bezpečnosti potravin.

Senzorická analýza jako vědecká analytická metoda je dnes neodmyslitelnou součástí výzkumu, vývoje a především každodenní praxe v potravinářských, ale i nepotravinářských organizacích. Pomocí ní lze více či méně podrobně charakterizovat senzorickou kvalitu potravin. V potravinářském průmyslu je dnes vedle fyzikální, chemické a mikrobiologické kontroly nezastupitelnou součástí hodnocení kvality surovin, polotovarů a hotových výrobků.

Vzhledem k určitým nevýhodám bývá v poslední době kombinována s vhodnými instrumentálními technikami, které při použití v potravinářské praxi zjednodušují a urychlují získání relevantních informací, umožňující okamžitou korekci výroby. Ve vědecké práci pak umožňují důkladnější charakterizaci vlastností produktu, ale i procesů probíhajících během jejich výroby, skladování, při kulinářském zpracování aj.

Hlavním objektem výzkumu v této práci je flavour, který je považován za podstatnou část senzorické kvality potravin a je také spotřebiteli nejvíce sledován. Je třeba znát, odkud pochází originální flavour jednotlivých typů potravin a co se s ním děje během zpracování. Pokud potravina nemá očekávaný flavour, může to být známka kažení nebo kontaminace ohrožující zdraví. I z těchto spotřebitelských a komerčních důvodů je třeba mít spolehlivou metodu, jak flavour stanovit. Z chemického hlediska je flavour tvořen především obsahem těkavých látek. Pro jejich stanovení byla v rámci této práce optimalizována a validována metoda HS-SPME-GC-FID/MS pro různé typy potravin. Metoda vykazuje dobrou linearitu, reprodukovatelnost, je dostatečně citlivá a vhodná pro stanovení stopových koncentrací těchto látek v potravinách, jak bylo prokázáno aplikací na vybrané vzorky potravin. Metoda je jednoduchá a rychlá, což umožní i její případné rutinní využití v praxi.

Pokud se podaří porozumět chemickému složení flavouru a mechanismu jeho tvorby u jednotlivých typů potravin, umožní to lepší praktickou kontrolu, směřující ke standardizaci výroby, zlepšení flavouru a potlačení vzniku off-flavouru, a v konečném důsledku výrobu kvalitnějších a bezpečnějších potravin.

6 TRENDY A PERSPEKTIVA DALŠÍ PRÁCE

Autorka působí na ÚCHPBT FCH VUT v Brně cca 20 let, velká část profesního života je spojena s výukou a výzkumem v oblasti analýzy, technologie, hygieny a bezpečnosti potravin. V dalším období by ráda pokračovala v dosavadní vědecko-výzkumné linii, založené především na senzorické analýze a jejím spojení s instrumentálními metodami na jedné straně a technologickými postupy na straně druhé. Dosavadní nejvýznamnější dosažené výsledky jsou shrnuty v této práci.

V rámci spolupráce s UTB ve Zlíně bude na základě požadavku regionálního výrobce v nejbližší době realizována komplexní studie zaměřená na další přírodní sýr s vysokodohřívanou sýřeninou, Moravský bochník. Řešení této problematiky bylo již zahájeno v rámci další disertační práce[34]. Moravský bochník je sýr ementálského (švýcarského) typu českého původu; jeho výroba byla zavedena v první polovině 20. století na Moravě. Technologie výroby je podobná ementálským sýrům, oproti ementálu nemá oka a jeho flavour je méně výrazný. V současné době se vyrábí především ve tvaru hranolu, tzv. Moravský blok; dříve byl používán hlavně jako surovina pro tavení, dnes je k dostání i v tržní síti. Naším cílem bude nalézt takové podmínky výroby, aby byl dosažen nutričně cenný a zároveň senzoricky atraktivní produkt. Během výroby modelových sýrů bude sledována řada fyzikálních, chemických, mikrobiologických a senzorických parametrů, náš tým se bude opět podílet sledováním senzorické kvality (flavouru). První výsledky byly prezentovány na konferenci „Chemistry and Life 2015“ v Brně [35].

Dále pokračuje práce na tavených sýrových analozích. V dalších experimentech byla nahrazena část (1 % hm.) tradičního tuku (másla) rostlinnými oleji s vyšším obsahem biologicky aktivních látek (rybízový, meruňkový, lněný a hroznový olej) s cílem vylepšit nejen senzorickou, ale i nutriční hodnotu konečného výrobku. První výsledky byly prezentovány na konferenci „CECE 2014, 11th International Interdisciplinary Meeting on Bioanalysis“ v Brně[36], naznačují dobrou přijatelnost spotřebiteli, z hlediska praktického využití však bude určitou nevýhodou vyšší cena takovýchto produktů.

Co se týče problematiky bobulového ovoce, i zde bude další výzkum pokračovat zejména studiem dalších méně známých (resp. méně využívaných) druhů. Poslední experimenty byly zaměřeny na různé odrůdy rybízu, se stejným cílem jako v případě angreštu (viz kap. 4.2.2), získané výsledky jsou připravovány k publikování.

V rámci dlouhodobých kontaktů byla navázána též úzká spolupráce s Výzkumným ústavem potravinářským v Bratislavě (Obor chemie a analýzy potravin) zaměřená na problematiku využití určitých typů léčivých rostlin v potravinářství. Na toto téma je

[34] SÝKORA, M. Změny senzoricky aktivních látek během zrání vybraných druhů přírodních sýrů [disertační práce; školitel-specialista E. Vítová].

[35] VÍTOVÁ, E., SŮKALOVÁ, K., MAHDALOVÁ, M., BUTOROVÁ, L., MUSILOVÁ, L., PECINOVÁ, E. Comparison of sensory quality of model Swiss cheese with commercially obtained corresponding product. In *Chemistry and Life 2015*. Brno. 2015. p. 95.

[36] SŮKALOVÁ, K., VÍTOVÁ, E., BUŇKA, F. The influence of storage on the sensory quality of processed cheese analogues. In *CECE 2014, 11th International Interdisciplinary Meeting on Bioanalysis*. Brno: Ústav analytické chemie AV ČR, 2014. pp. 403 - 406.

v současnosti řešena další disertační práce[37], jejímž cílem je komplexní charakterizace nejvýznamnějších, běžně dostupných léčivých rostlin pomocí moderních analytických a senzorických metod. Hlavní pozornost je v tomto případě zaměřena na jejich antioxidační vlastnosti. Ve spolupráci s praxí budou následně vybrané komponenty aplikovány do potravinářských produktů (nápoje, pečivo); v této souvislosti je řešena i senzorická kvalita (flavour) potravinových vzorků. V rámci této problematiky se už podařilo publikovat první výsledky[38] analýz deseti vybraných, v česku nejčastěji používaných, bylin: levandule lékařská, měsíček lékařský, třezalka tečkovaná, šalvěj muškátová, meduňka lékařská, jestřabina lékařská, yzop lékařský, máta peprná, šalvěj lékařská a ostropestřec mariánský.

Velmi aktuální je v současné době problematika alergenů. Počet osob alergických na různé podněty je vysoký a stále se zvyšuje. V návaznosti na předchozí experimenty (viz kap. 4.3.1) bude další výzkumný směr věnován i sledování obsahu alergenních AAL ve vybraných typech aromatizovaných potravin, resp. pochutin. V současné době jsou studovány nečokoládové cukrovinky se speciálním důrazem na přítomnost alergenních AAL. Na trhu je dostupný široký sortiment cukrovinek a příbuzných produktů od tuzemských i zahraničních výrobců, většinou s ovocnou příchutí. Jejich kvalita tkví hlavně v senzorické atraktivitě, proto se také při jejich výrobě používá široké spektrum různých aditivních látek, např. i některých kontroverzních barviv, příp. syntetických aromat. V souvislosti se zvýšenou informovaností a zvyšujícími se nároky spotřebitelů se však dnes na trhu převážně objevují nové druhy deklarující použití pouze přírodních barviv, popř. aroma; tyto se používají většinou ve formě různých extraktů (viz např. bez, aronie aj.), i v nich se však mohou nacházet relativně vysoké koncentrace alergenních AAL. Vzhledem k tomu, že se jedná o produkty konzumované především dětmi, je obzvlášť žádoucí se touto problematikou zabývat.

Veškeré uvedené výzkumné směry budou ve vhodné formě zařazovány i do výuky. Jak již bylo uvedeno, autorka působí jako školitel-specialista 3 disertačních prací a vede řadu diplomových a bakalářských prací, jejichž tematika souvisí s výše uvedenými trendy výzkumné práce. Kromě toho jsou získané aktuální poznatky průběžně zařazovány do výuky předmětů, které autorka přednáší (senzorická analýza, technologie a hygiena potravin), i do vyučovaných praktických předmětů (především metodika v rámci praktika ze senzorické a také instrumentální a strukturní analýzy). Část poznatků bude rovněž popularizována v rámci přednášek U3V a zpřístupněna odborné veřejnosti v rámci akreditovaných kurzů Senzorické analýzy probíhajících na FCH VUT v Brně v rámci CŽV.

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8 SEZNAM SYMBOLŮ A ZKRATEK

AAL ……	aromaticky aktivní látky
BDP ……	bojové dávky potravin
BMK ……..	bakterie mléčného kvašení
CAR ……..	karboxen
DVB ……...	divinylbenzen
FID …….	plamenově ionizační detektor
GC …….	plynová chromatografie
hm. …….	hmotnost, hmotnostní
HPLC …….	vysokoúčinná kapalinová chromatografie
HS ……..	headspace
MS …….…….	hmotnostní spektrometrie
OAV …….……..	Odour Activity Value
PCA …….……...	Principal Component Analysis
PDMS ……..…	polydimethylsiloxan
RSD ……...	relativní směrodatná odchylka
SPME ……..…	Solid Phase Microextraction
VMK ……..	volné mastné kyseliny

9 SEZNAM PŘÍLOH

Publikace autorky související s tématem habilitační práce.

Příloha 1. VÍTOVÁ, E., LOUPANCOVÁ, B., ZEMANOVÁ, J., ŠTOUDKOVÁ, H., BŘEZINA, P., BABÁK, L. Solid-phase microextraction for analysis of mould cheese aroma. *Czech Journal of Food Scencies* (Special Isue). 2006, 24(6), pp. 268-274.

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Příloha 5. VÍTOVÁ, E., SŮKALOVÁ, K., MAHDALOVÁ, M., BUTOROVÁ, L., MATĚJÍČEK, A., KAPLAN, J. Influence of volatile compounds on flavour of selected cultivars of gooseberry. *Chemical Papers*, 2017 (přijato do tisku)

Příloha 6. VÍTOVÁ, E., DIVIŠOVÁ, R., SŮKALOVÁ, K., MATĚJÍČEK, A. Determination and quantification of volatile compounds in fruits of selected elderberry cultivars grown in Czech Republic. *Journal of Food and Nutrition Research*. 2013, 52(1), pp. 1-11.

Příloha 7. VÍTOVÁ, E., SŮKALOVÁ, K., MAHDALOVÁ, M., BUTOROVÁ, L. BABÁK, L., MATĚJÍČEK, A. Comparison of flavour and volatile flavour compounds of mixed elderberry juices. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, 2015, 63(1), pp. 147-152.

Příloha 8. VÍTOVÁ, E., SŮKALOVÁ, K., MAHDALOVÁ, M., BUTOROVÁ, L., MELIKANTOVÁ, M. Comparison of selected aroma compounds in cultivars of sea buckthorn (Hippophae rhamnoides L.). *Chemical Papers*, 2015, 69(6), pp. 881-888.

Příloha 9. BUTOROVÁ, L., VÍTOVÁ, E., POLOVKA, M. Comparison of volatiles identified in Aronia melanocarpa and Amelanchier alnifolia using solid-phase microextraction coupled to gas chromatography-mass spectrometry. *Journal of Food and Nutrition Research*. 2016, 55(1), pp. 57-68.

Příloha 10. DIVISOVA, R., VITOVA, E., DIVIS, P., ZEMANOVA, J., OMELKOVA, J. Validation of SPME-GC-FID Method for Determination of Fragrance Allergens in Selected Cosmetic Products. *Acta Chromatographica*, 2015, 27(3), pp. 509-523.

Solid-Phase Microextraction for Analysis of Mould Cheese Aroma

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Abstract

Vítová E., Loupancová B., Zemanová J., Štoudkova H., Březina P., Babák L. (2006): **Solid-phase microextraction for analysis of mould cheese aroma**. Czech J. Food Sci., **24**: 268–274.

Solid-phase microextraction coupled with gas chromatography was used for the analysis of volatile aroma compounds in Niva cheese. The extraction conditions were very mild, which minimises thermal, mechanical, or chemical modification of the sample; the method is rapid, simple, and cheap. In total, 54 compounds were identified in Niva cheese using this method: 3 hydrocarbons, 5 aldehydes, 11 ketones, 18 alcohols, 3 esters, 10 fatty acids, and 4 sulphur compounds. These aroma compounds were quantified and subsequently the changes in the concentrations of them were studied throughout the ripening period. Most of the volatile compounds identified were present at all stages of the cheese ripening, their amounts changing significantly, however, in most cases the final concentration in the ripe cheeses was similar to the initial concentration in the unripe cheese.

Keywords: SPME; gas chromatography; mould cheese; aroma compounds

Aroma and flavour belong among the most important food quality criteria. They are major attributes that influence the selection and consumption of food. Cheese flavour results from the breakdown of milk proteins, fat, lactose, and citrate due to enzymes from microorganisms, coagulants, and milk. Many volatile compounds are potentially involved in cheese flavour: hydrocarbons, alcohols, aldehydes, ketones, esters, fatty acids (FA), lactones, sulphur- and nitrogen-containing compounds. To analyse the cheese flavour by gas chromatography (GC), it is necessary to extract these compounds from their matrix. Many of them are present only at very low concentrations and several methods for their extraction and concentration have been developed: e.g. steam distillation, extraction with organic solvents, surfactants, and supercritical fluids, headspace techniques, dialysis, and solid-phase extraction (Carbonell *et al.* 2002; Fernández-García *et al.* 2002; Qian *et al.* 2002). However, these methods have some drawbacks: they are time-consuming, require large volumes of samples or solvents, some volatile compounds can be damaged or lost, etc.

The solid-phase microextraction (SPME) is a relatively new sample preparation technique, based on the partition of the analyte between the extraction phase on the outside of a small fused-silica fibre, and the matrix. SPME was introduced by Arthur and Pawliszyn (Pawliszyn 1997; Kataoka *et al.*

2000) for the extraction of organic compounds from environmental samples, but now it has gained a lot of interest in a broad field of analysis including the analysis of food. Very interesting possibility is the use of SPME in the food aroma analysis. Many authors describe the analysis of flavour and off-flavour of some foods, e.g. fruit, vegetables, meat, drinks and dairy products (Pérés *et al.* 2001; Frank *et al.* 2004; Mallia *et al.* 2005).

The aim of our work was to develop a simple, rapid, and cheap method for the extraction of the aroma compounds of cheese, based on SPME. Mould cheese was used to evaluate our method and the optimised method was used for monitoring the changes throughout Niva cheese ripening.

MATERIALS AND METHODS

Chemicals. The following chemicals were used as standards: pentadecane, heptadecane, dimethyl disulphide, dimethyl sulphide, dimethyl trisulphide, benzothiazol, phenylacetaldehyde, hexanal, 8-nonen-2-one, decan-2-one, heptadecan-1-ol, heptadecan-2-ol, hexadecan-2-ol, myristic acid, benzoic acid, pentadecanoic acid, palmitic acid, phenylethyl-acetate, pentyl-benzoate (Sigma-Aldrich, Germany), phenylethanol, ethanal, propanal, hexanoic acid, isobutanoic acid, isopentanoic acid, capric acid, 3-hydroxybutan-2-one, nonan-2-one, pentan-2-one, undecan-2-one, heptan-2-on (Merck, Germany), methanol, propan-1-ol, propan-2-ol, butanol, pentan-1-ol, pentan-2-ol, octan-1-ol, nonan-2-ol, 2-methylpropan-1-ol, 3-methylbutan-1-ol, acetone, propan-2-one, butan-2-one, butanoic acid, acetic acid, propionic acid, ethyl-acetate (Lachema, CR), heptane, ethanol, heptan-2-ol, dodecan-1-ol, benzaldehyde (J.T. Baker, the Netherlands), oct-1-en-3-ol, butan-2,3-dione (Fluka, Switzerland). All the chemicals were of chemically pure grade.

Samples. Niva cheese (50% dry matter, 55% fat in dry matter) was manufactured according to the traditional procedures in a dairy in Český Krumlov. The cheeses were sampled at 5, 15, 25, 35, 45, and 55 days of ripening. The cheese samples were stored at –12°C before use.

For analysis, grated cheese was placed in a vial (4 ml), sealed by a septum-type cap and held in a water bath. During this time, the sample was sometimes shaken to homogenise and to increase the transfer of the analytes to the headspace. After the equilibration time, the SPME fibre was inserted in a vial for the sampling process.

SPME. The SPME fibre Carboxen™ /polydimethylsiloxane 85 µm was purchased from Supelco (Bellefonte, PA, USA). The extraction was carried out by HS-SPME mode.

Gas chromatography and mass spectrometry (GC-MS). Gas chromatograph TRACE™ GC (ThermoQuest, I) equipped with flame ionization detection and split/splitless injection port, DB-WAX capillary column (30 m × 0.32 mm × 0.5 µm; J. & W. Scientific, Folsom, CA).

The injector – 250°C, splitless mode, the desorption time 5 min, linear purge closed for 5 min.

The oven temperature program – 40°C for 1 min, 40–200°C at 5°C/min, 200°C for 7 min. The detector 220°C. The carrier gas (N_2) 0.9 ml/min.

GC-MS analyses – gas chromatograph GC 8000 (Carlo Erba, I) coupled to a MS TRIO 1000 (Fisons Instruments, USA). The carrier gas He, the GC column and other operating parameters were the same as described.

The reproducibility, linearity and detection limits. The reproducibility of the method was determined by five replicate extractions of five standard compounds chosen under optimal conditions. The standards were added to the matrix of the ripe cheese. The results were expressed as relative standard deviations (RSD) of the peak area counts.

The method of standard addition was used for the assessment of the linearity and detection limits. The standards at different concentrations (within the range 0.003–20 µg/g) were added to the matrix (ripe cheese sample).

RESULTS AND DISCUSSION

There are two possibilities of SPME extraction: direct immersion SPME (DI-SPME), where the fibre is exposed to the liquid sample, and headspace SPME (HS-SPME), where the fibre is exposed to the headspace above the sample. The HS-SPME mode is preferred for volatile compounds because it provides greater selectivity, sensitivity, and rapidity, and an elongated fibre lifetime.

Selectivity can be altered by changing the phase type and thickness according to the characteristics of the analytes – volatile compounds require a thick phase coat. Various fibres were tested and finally Carboxen™/polydimethylsiloxane 85 µm fibre was chosen as most suitable for the extraction of

volatile aroma compounds as confirmed by many authors (Pérés *et al.* 2001; Frank *et al.* 2004).

Altering the sample conditions can optimise the extraction yield. The following parameters were optimised: equilibration time, extraction time, temperature, and sample weight, with the aim to maximise the compound recovery while keeping a reasonably short total analysis time. Also desorption parameters were optimised. SPME is an equilibrium process, hence under equilibrium conditions precision and sensitivity are expected to be optimal. However, it is not necessary to reach full equilibrium, if constant extracting conditions are maintained. The optimal SPME parameters selected for the subsequent analyses were:

– equilibration time 30 min;
– extraction time 20 min;
– extraction temperature 35°C;
– sample amount 1 g;
– desorption temperature 250°C;
– desorption time 5 min.

The reproducibility was good, RSD of the standard analyses were in the range of 2–11%. Detection limits differed for the various compounds analysed, with small volatile molecules they were higher owing to their bad affinity to the fibre, and were in the range of 0.003–0.2 μg/g. The linearity was also good, the correlation coefficients were all over 0.98.

Identification of aroma compounds in Niva cheese

The flavour of cheese originates from microbial, enzymatic, and chemical transformations. The breakdown of milk proteins, fat, lactose, and citrate during ripening gives rise to a series of volatile and non-volatile compounds which may contribute to the cheese flavour. Several degradation types occur simultaneously and the ultimate result will be a very wide range of compounds. The factual contribution of them to the flavour of cheese is largely unknown.

Proteolysis in cheese during ripening plays an important role in the development of texture. However, it also contributes to the taste of cheese by the production of peptides and free amino acids. Large peptides do not contribute directly to the cheese taste, but can be hydrolysed to shorter peptides that may be bitter. Free amino acids are the final products of proteolysis. Very extensive proteolysis occurs in blue-mould cheeses (Sorensen & Benfeldt 2001).

Lipolysis is most important for the blue cheese flavour. Lactic acid bacteria present in starter cultures are generally only weakly lipolytic, most of the FA coming from the triglycerides degradation by moulds. Compounds generated by lipid metabolism predominate among the aroma compounds identified in blue cheese (Qian *et al.* 2002).

Niva is soft blue-veined cheese manufactured from pasteurised cow milk. It has a crumbly texture, white to light beige interior with blue veining and a pleasant salty, piquant flavour. It is aged at least two months to achieve the typical appearance and flavour. It is known that free FA, odd-carbon chain methyl ketones, and secondary alcohols are the major contributors to the characteristic flavour of blue cheese, so the flavour development during the cheese ripening is dependent on milk triacylglycerols hydrolysis by *Penicillium roqueforti* lipases and subsequent oxidation of FA to methyl ketones. However, a large number of other volatile compounds have been identified in blue cheeses (Sablé & Cottenceau 1999; Ur Rehman *et al.* 2000; Qian *et al.* 2002).

The identification of the individual compounds in the sample is difficult, owing to their low concentrations in cheese and the relatively high concentrations of other compounds. The identification was carried out by GC-MS and confirmed by comparison of the retention times with those of standard substances. The mass spectra for all the compounds were compared with standard mass spectra provided by the database of the equipment. In total, 54 compounds were identified in Niva cheese: 3 hydrocarbons, 5 aldehydes, 11 ketones, 18 alcohols, 3 esters, 10 fatty acids, and 4 sulphur compounds.

Eleven ketones were identified in Niva cheese: acetone, propan-2-one, butan-2-one, pentan-2-one, butan-2,3-dione, heptan-2-one, 3-hydroxybutan-2-one, nonan-2-one, 8-nonen-2-one, decan-2-one and undecan-2-one. Ketones *a*re common constituents of most dairy products, which may be reduced to secondary alcohols (Carbonell *et al.* 2002). Methyl ketones are derived from FA by β-oxidation or from β-ketoacids and are primarily known for their contribution to the aroma of mould cheeses. They have typical odours (fruity, floral, mushroom, or musty notes) and low perception thresholds (Qian *et al.* 2002). One of the most important diketones is biacetyl (butan-2,3-dione) with sweet buttery and vanilla aroma. It is formed through lactose and citrate metabolism and its

production is mainly due to the activity of lactic acid bacteria. It can be reduced to acetoine (3-hydroxybutan-2-one) with buttery aroma and the latter can be further reduced to butane-2,3-diol, which does not have a flavour impact (Curioni & Bosset 2002). Biacetyl was identified as very important to blue cheese, in which acetoine was also detected. As mentioned previously, in mould cheese methyl ketones are the most abundant aroma compounds, the major ones being heptan-2-one and nonan-2-one (Carbonell *et al.* 2002; Qian *et al.* 2002; Frank *et al.* 2004).

Eighteen alcohols were identified in Niva cheese: ethanol, propan-2-ol, propan-1-ol, 2-methylpropan-1-ol, pentan-2-ol, butanol, 3-methylbutan-1-ol, pentan-1-ol, methanol, heptan-2-ol, oct-1-en-3-ol, octan-1-ol, nonan-2-ol, phenylethanol, dodecan-1-ol, heptadecan-1-ol, hexadecan-2-ol and heptadecan-2-ol. Primary alcohols are formed by the proper aldehydes reduction. They impart a fruity, nutty note to the cheese flavour, and in certain cheeses high levels of them can cause flavour defects. Secondary alcohols are formed by enzymatic reduction of the corresponding methyl ketones. They have similar but heavier flavour notes than methyl ketones. Ethanol comes from lactose fermentation. It has a limited role in the cheese aroma despite its high levels, but it contributes to the formation of esters (Carbonell *et al.* 2002). 3-methylbutan-1-ol was found at high concentrations in mould cheeses. The principal secondary alcohols in mould ripened cheeses are heptan-2-ol and nonan-2-ol, which correspond to the high methyl ketone contents. They have less influence on the cheese flavour than methyl ketones, however, they may indirectly contribute to it because of their ability to form esters with FA (Sablé & Cottenceau 1999).

Ten FA were identified in Niva cheese: acetic, capric, isobutanoic, propionic, isopentanoic, butanoic, hexanoic, myristic, benzoic, pentadecanoic, and palmitic acids. Fatty acids are important components of the flavour of many cheese types. They may originate from lipolysis, a lower proportion of short-chain FA originate from the degradation of lactose and amino acids, and they can also be derived from ketones, esters, and aldehydes by oxidation (Curioni & Bosset 2002). Long-chain FA (> 12 carbon atoms) play a minor role in the flavour owing to their relatively high perception thresholds. Short and moderate-chain, even-numbered FA (C4-C12) have much lower perception thresholds and characteristic notes (vinegar, sour). Moreover, free FA also serve as precursors to methyl ketones, alcohols, lactones, and esters (Sablé *et al.* 1999; Vítová *et al.* 2004). On the other hand, higher concentrations of free FA can cause off-flavours (e.g. rancid). Short-chain free FA acids are important contributors to the characteristic flavour of blue cheeses and were identified by many authors at high concentrations (Sablé & Cottenceau 1999; Qian *et al.* 2002).

Three esters were identified in Niva cheese: ethyl-acetate, phenylethyl-acetate and pentyl-benzoate. Esters are common cheese volatiles. Esterification reactions occur between short- to medium-chain FA and alcohols. Most esters in cheeses are described as having sweet, fruity, and floral notes. Some of them have very low perception thresholds and their contribution is heightened by synergistic effect. Further, they can contribute to the aroma of cheese by minimising the sharpness and the bitterness imparted by FA and amines (Curioni & Bosset 2002). Gonzales de Llano *et al.* (1990) found high proportions of methyl and ethyl esters in ripe blue cheese, Qian *et al.* (2002) consider ethyl butanoate and ethyl hexanoate to be important compounds contributing to the blue cheese aroma.

Five aldehydes were identified in Niva cheese: propanal, ethanal, hexanal, phenylacetaldehyde, and benzaldehyde. Straight-chain aldehydes may result from β-oxidation of unsaturated FA or from amino acids by Strecker degradation. Branched-chain aldehydes probably originate from amino acid degradation via enzymatic as well as non-enzymatic, e.g. Strecker degradation, processes (Curioni & Bosset 2002). Aldehydes are transitory compounds in cheese because they are rapidly reduced to primary alcohols or oxidised to the corresponding acids (Carbonell *et al.* 2002). They are characterised by green-grass or herbaceous aroma and can be very unpleasant when their concentrations exceed certain values. Qian *et al.* (2002) consider 2-methylpropanal and 3-methylbutanal to be important compounds contributing to the blue cheese aroma.

Three hydrocarbons were identified in Niva cheese in trace amounts: heptane, pentadecane, and heptadecane. Hydrocarbons are secondary products of lipid autooxidation. They do not make a major contribution to the aroma, but may serve as precursors to other aroma compounds (Ortigosa *et al.* 2001). Hydrocarbons have been frequently

reported in many cheeses, although usually at low concentrations (Carbonell *et al.* 2002).

Four sulphur compounds were identified in Niva cheese: dimethyl sulphide, dimethyl disulphide, dimethyl trisulphide, and benzothiazol. Sulphur compounds originate from methionine and cysteine degradation. These components are described as having strong garlic, onion, or very ripe cheese odours. Their perception thresholds are very low and they are probably involved in the final aroma of mould cheeses. Ortigosa *et al.* (2001) consider them to be indispensable to achieve the characteristic aroma of white mould cheese, Qian *et al.* (2002) introduce methional and dimethyl trisulfide as important compounds contributing to the blue cheese aroma.

Changes of aroma compounds during ripening of Niva cheese

SPME-GC procedure was also used to study the volatile compounds evolution during the ripening of Niva cheese, the method of standard addition was chosen for quantification, the standards were added to grated cheese in a vial.

As mentioned before, cheese ripening includes microbiological and enzymatic processes contributing to the unique flavour and textural characteristics. Knowledge of these changes could allow standardisation of the cheese manufacturing and a better control of the process. But only in a few kinds of cheese have these changes been described in depth. In the case of Niva cheese, most of the volatile compounds identified were present at all stages of the cheese ripening, however, their amounts changed significantly. The changes of the most important and most abundant compounds chosen are graphically expressed in Figures 1–4. Each value represents the mean of three replicate determinations, RSD was in all cases to 10%. Ketones, alcohols, and FA were quantitatively the most important compounds present.

The changes of ketones in Niva cheese are presented in Figure 1. As can be seen, their concentrations increased during ripening with maximum after about 40 days. Only acetoine was present at a high concentration in unripe cheese and then its amount sharply decreased. The increase in the ketones concentrations during ripening is characteristic for many kinds of cheese as confirmed by many authors, this fact being linked to lipolysis (Carbonell *et al.* 2002).

Ethanol and pentan-2-ol were the most abundant alcohols in Niva cheese. Gonzales de Llano *et al.* (1990) describe the evolution of methyl ketones and 2-alkanols in blue cheese as parallel: the contents of both increased during the first part of the ripening and then decreased after reaching their maximum after 60 days. Also in other types of cheeses, alcohols are quantitatively the main chemical family and their contents increase significantly, but at different rates during ripening (Carbonell *et al.* 2002). In our case, no significant increase was found in alcohols concentrations in Niva cheese (Figure 2), with the exception of ethanol.

To changes of short-chain fatty acids in Niva cheese are presented in Figure 3. No significant increase in their amount was found (except acetic

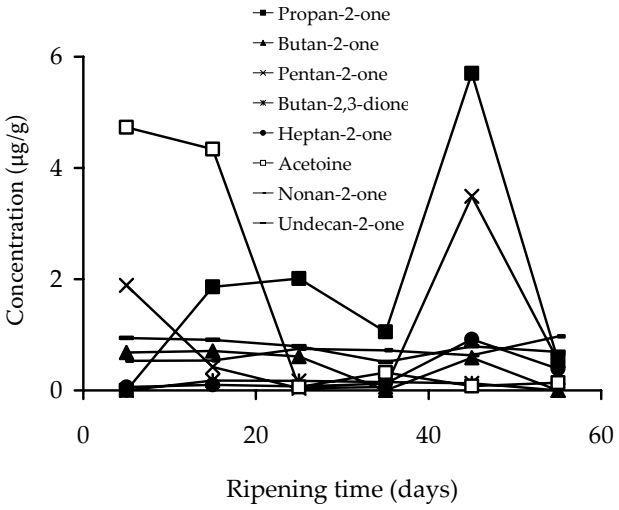

Figure 1. Changes of ketones during ripening of Niva cheese

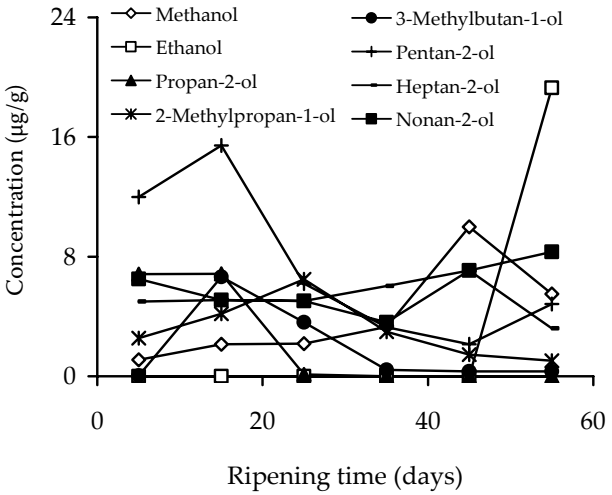

Figure 2. Changes of alcohols during ripening of Niva cheese

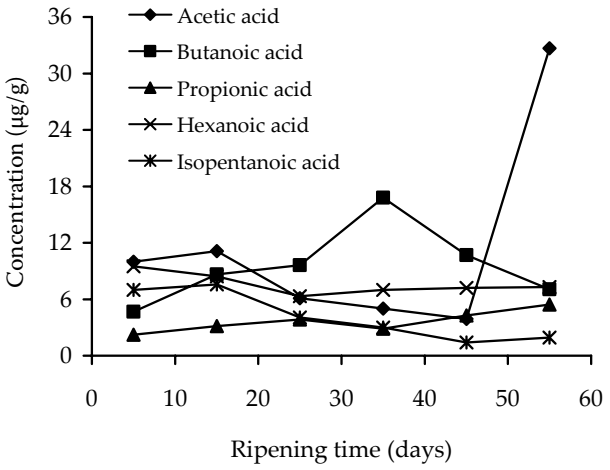

Figure 3. Changes of fatty acids during ripening of Niva cheese

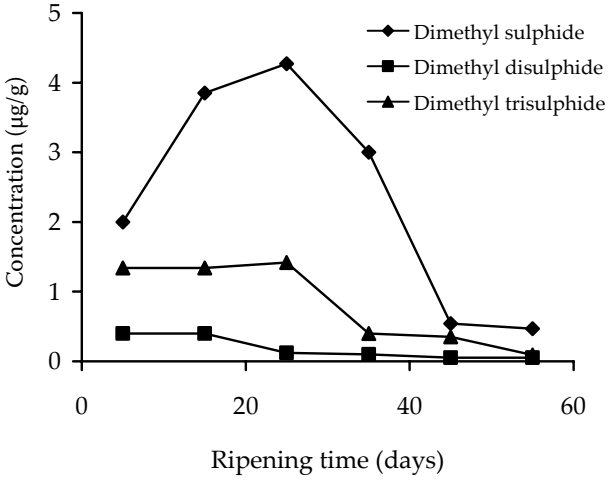

Figure 4. Changes of sulphur compounds during ripening of Niva cheese

acid), although some authors noticed an increase in the concentrations of volatile FA during the ripening of various kinds of cheese (Mulet *et al.* 1999; Vítová *et al.* 2004).

As reported by some authors, the concentrations of esters can decrease or increase during the ripening of various kinds of cheese (Mulet *et al.* 1999; Carbonell *et al.* 2002). Gonzales de Llano *et al.* (1990) describe the evolution of esters in blue cheese as similar to those of ketones and alkanols. The formation of them was slower, attaining the maximum after 180 days (end of ripening). Three esters identified in Niva cheese reached maximum concentrations (only about 0.1 µg/g) in about 40 days of ripening.

The contents of aldehydes mostly increase during the ripening of various cheeses (Carbonell *et al.* 2002). In the case of Niva cheese, the contents of the aldehydes identified reached maximum in about 40 days, the ethanal content decreased during ripening. The maximum concentrations of them were in the range of 0.4–0.8 µg/g.

The contents of sulphur compounds identified in Niva cheese decreased during ripening, dimethyl sulphide was present in the highest concentration (Figure 4).

To summarise, the analytical method based on SPME combined with GC is a simple and rapid method for the analysis of the volatile compounds in cheese. It minimises thermal, mechanical, and chemical modifications of the matrix, it is consequently suitable for the characterisation of the cheese aroma, but not only cheese as it can be applied to various foods. Some important aroma compounds in Niva cheese were identified and quantified using this method. Although important changes of them took place during ripening, in most cases, the final concentrations in ripe cheeses were similar to the initial concentrations in the unripe cheese.

List of symbols

FA – fatty acid
GC – gas chromatography
GC-MS – gas chromatography-mass spectrometry
SPME – solid-phase microextraction
DI-SPME – direct immersion SPME
HS-SPME – headspace SPME
RSD – relative standard deviation

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Application of SPME-GC method for analysis of the aroma of white surface mould cheeses

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Summary
Solid-phase microextraction coupled to gas chromatography (SPME-GC) was used for the analysis of volatile aroma compounds in cheeses which had been ripened with a white surface mould *Penicillium camemberti*. These cheeses are of a characteristic appearance, taste and aroma due to the presence of sensory-active compounds formed during the ripening. The aim of this work was to compare aroma profiles of several types of these cheeses produced in Czech Republic and to follow changes of the aroma profile during the ripening. The method was simple and fast, extraction conditions affected minimally the thermal, mechanical or chemical changes of the samples. In total, 32 compounds were identified in the samples using this method, namely, 1 hydrocarbon, 3 aldehydes, 7 ketones, 11 alcohols, 2 esters, 5 fatty acids, 2 sulphur compounds and 1 nitrogen compound. As found, their proportion varied during ripening, but there was no significant increase in their content during experiments. Aroma profiles of the tested cheeses were similar in spite of the differences in the production technologies.

Keywords
aroma; mould cheese; SPME-GC

White surface mould cheeses are covered by a coating of white mycelia of the mould *Penicillium camemberti* or closely related *Penicillium caseicolum*. The presence of moulds gives them characteristic appearance, taste and aroma. Especially in the case of traditional cheeses, they are characterized by more complex maturation, because of the diversity of the microbial flora present and the extent of the enzymatic changes that occur. A typical example of surface mould ripened cheeses is Camembert, originated from France. Traditional Camembert is made from raw milk; the other surface mould cheeses are manufactured from raw or pasteurized milk. Many types, called for example Hermelin, Kamadet, Premium or Plesnivec, are made in the Czech Republic. They are produced from pasteurized milk using starter culture consisting of thermophilic streptococci or a mixture of streptococci and lactococci, and mould cultures. In order to obtain a more aromatic product, selected strains of yeasts, corynebacteria and yeast-like mould *Geotrichum candidum* can be added to milk.

The flavour of cheese originates from microbial, enzymatic and chemical transformations. The breakdown of milk proteins, fat, lactose and citrate during ripening gives rise to a series of volatile or non-volatile compounds: hydrocarbons, alcohols, aldehydes, ketones, esters, fatty acids (FA), lactones, sulphur- and nitrogen-containing compounds [1-3]. All of them may contribute to cheese aroma, but the exact contribution is largely unknown. Moulds have a much greater enzymatic potential than bacteria, consequently, the major processes of maturation are more marked in mould-ripened cheeses than in other types [4].

Lactose decomposition is caused primarily by lactic acid bacteria enzymes [5]. In the case of white surface mould cheeses, the surface fungal flora uses created lactic acid for its growth. There is, as a result, a marked increase in the external pH and an internal migration of lactate towards the surface of the cheese. These drastic pH changes have a marked effect on the maturation process, indicated by the development on the surface of aerobic and acid-sensitive flora, consisting of corynebacteria and micrococci. This flora contributes to the development of the final organoleptic properties and cannot develop without an increase in the surface pH [4].

Lipolysis is most important for blue cheese flavour. Maturation of white surface mould cheeses

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is shorter and the degree of lipolysis is also more restricted, reaching 3–6 % and sometimes 6–10 % in the well matured traditional products. It should be stressed that these high levels of free FA do not give rise to a rancid taste as they are in a dissociated form when the pH of the curd has increased [4]. Long-chain FA (> 12 carbon atoms) play a minor role in the flavour owing to their relatively high perception thresholds. Short and moderate chain length, even numbered FA (C4–C12) have much lower perception thresholds and characteristic notes (vinegar, sour). However, free FA are not only aroma compounds by themselves, but also serve as precursors of methyl ketones, alcohols, lactones and esters [6].

Ketones are common constituents of most dairy products, but they are intermediate compounds, which may be reduced to secondary alcohols [7]. Methyl ketones are derived from FA by β-oxidation or from β-ketoacids and are primarily known for their contribution to the aroma of mould cheeses [8]. They have typical odours (fruity, floral, mushroom or musty notes) and low perception thresholds [5, 8].

Esterification of FA with primary alcohols occurs by an enzymatic or a chemical pathway [9]. The microorganisms involved in ester formation are probably mainly yeasts, but some lactic acid bacteria can be responsible [7]. Most esters in cheeses are described as having sweet, fruity and floral notes. Some of them have a very low perception threshold and their contribution is heightened by synergistic effects. Further, they can contribute to the aroma of cheese by minimizing the sharpness and the bitterness imparted by FA and amines [6, 8].

Lipases in cheese originate from milk (in the case of cheese made from raw milk), rennet and microflora. Lactic acid bacteria present in starter cultures are generally only weakly lipolytic, most of the FA come from the triglycerides degradation by moulds [6].

Proteolysis in cheese during ripening contributes to the taste of cheese by the production of peptides and free amino acids. Large peptides do not contribute directly to cheese taste, but can be hydrolysed by proteinases to shorter peptides that may be bitter [10]. Free amino acids are the final products of proteolysis. Catabolism of free amino acids is a major process for aroma development. It can result in a number of compounds, all of which may contribute to cheese flavour. Proteolysis in cheese is catalysed by enzymes from coagulant, milk, microflora and by exogenous proteinases or peptidases [10].

The study of substances creating food aroma is nowadays of great interest in quality assessment. Several methods for extraction and concentration of them have been developed: e.g. steam distillation, extraction with organic solvents, surfactants and supercritical fluids, headspace techniques, dialysis and solid-phase extraction. However, these methods have certain drawbacks [5, 9]. The solid-phase microextraction (SPME) is a relatively new sample preparation technique that can eliminate some of them. This technique has been introduced by Arthur and Pawliszyn [11, 12] for the extraction of organic compounds from environmental samples, but has now gained a lot of interest in a broad field of analysis including food. Many authors describe analysis of flavour and off-flavour of some food, e.g. fruit [13], vegetables, meat [12], drinks [14, 15] and also dairy products [16-21].

The aim of this work was to compare aroma profile of several types of white surface mould cheeses produced in Czech Republic and to follow their changes during ripening. Volatile aroma compounds of cheeses were isolated using SPME and analysed by gas chromatography (GC).

MATERIALS AND METHODS

Chemicals

The following chemicals were used as standards: pentadecane, heptadecane, dimethyl disulphide, dimethyl sulphide, dimethyl trisulphide, benzothiazol, phenylacetaldehyde, hexanal, 8-nonen-2-one, decan-2-one, heptadecan-1-ol, heptadecan-2-ol, hexadecan-2-ol, myristic acid, benzoic acid, pentadecanoic acid, palmitic acid, phenylethyl-acetate, pentyl-benzoate (Sigma-Aldrich, Deisenhofen, Germany), phenylethanol, ethanal, propanal, hexanoic acid, isobutanoic acid, isopentanoic acid, capric acid, 3-hydroxybutan-2-one, nonan-2-one, pentan-2-one, undecan-2-one, heptan-2-on (Merck, Darmstadt, Germany), methanol, propan-1-ol, propan-2-ol, butanol, pentan-1-ol, pentan-2-ol, octan-1-ol, nonan-2-ol, 2-methylpropan-1-ol, 3-methylbutan-1-ol, acetone, propan-2-one, butan-2-one, butanoic acid, acetic acid, propanoic acid, ethyl-acetate (Lachema, Brno, Czech Republic), heptane, ethanol, heptan-2-ol, dodecan-1-ol, benzaldehyde (J. T. Baker, Deventer, Netherlands), oct-1-en-3-ol, butan-2,3-dione (Fluka, Buchs, Switzerland). All the chemicals were of chemically pure grade.

Samples

Three types of white surface mould cheeses were tested in this work: Hermelin is the medium fat cheese (dry matter 47–51%, fat in dry matter

50–54%), weight 120 g, cylindrical form. Block Hermelin is fatty cheese (dry matter 50–54%, fat in dry matter 60–64%), weight 1 kg, cylindrical form. Premium is fatty cheese (dry matter 51–55%, fat in dry matter 60–64%), weight 125 g, oval form.

All of them were produced by Pribina, Přibyslav, Czech Republic. Cheeses were sampled and analysed for volatile compounds after 5, 15, 25, 35, 45 and 55 days of ripening.

SPME-GC analysis

For analysis the grated cheese (1 g) was placed in a vial (4 ml), sealed by a septum-type cap and the vial kept in a water bath. During this time, the sample was sometimes shaken to homogenize and to increase the transfer of the analytes to the headspace. After an equilibration time (30 min), the SPME fibre was inserted in a vial for the sampling process. The fibre Carboxen^{TM}/polydimethylsiloxane 85μm was purchased from Supelco (Bellefonte, Pennsylvania, USA). The extraction of volatile aroma compounds was carried out by exposure to the headspace of the sample - 20 min 35 °C.

GC conditions: Gas chromatograph TRACE^{TM} GC (ThermoQuest Italia, Milan, Italy) equipped with flame ionization detection (FID) and split/splitless injection port, DB-WAX capillary column (30 m × 0.32 mm × 0.5 μm; J&W Scientific, Folsom, California, USA). The injector - 250 °C, splitless mode, the desorption time 5 min, linear purge closed for 5 min. The detector - 220 °C. The carrier gas (N_2) 0.9 ml.min^{-1}. The oven temperature program: 40 °C 1 min, 2 °C.min^{-1} to 120 °C, 5 °C.min^{-1} to 200 °C, 5 min.

Gas chromatography and mass spectrometry (GC-MS)

Gas chromatograph GC 8000 (Carlo Erba, Milan, Italy) coupled to a MS TRIO 1000 (Fisons Instruments, Valencia, California, USA). The ionizer temperature setting was at 150 °C, using electron impact (EI) mode, with electron energy at 70 eV. The carrier gas was He with a head pressure 150 kPa, the GC column and other operating parameters were the same as described.

RESULTS AND DISCUSSION

Identification of aroma compounds in cheeses

The identification of the individual compounds in sample is rather difficult, owing to their low concentrations in cheese and the relatively high concentrations of other compounds. Identification was carried out by GC-MS and confirmed by comparison of the retention times with those of standard substances. The mass spectra for all the compounds were compared with standard mass spectra provided by the database of the equipment.

Seven ketones were identified in surface mould cheeses tested: propanone, butan-2-one, pentan-2-one, nonan-2-one, undecan-2-one, butan-2,3-dione, 3-hydroxybutan-2-one.

One of the most important diketones is biacetyl (butan-2,3-dione) with its sweet buttery and vanilla aroma. This component is formed from lactose and citrate metabolism and its production is mainly due to the activity of lactic acid bacteria. It can be reduced to acetoine (3-hydroxybutan-2-one) with buttery aroma and the latter can be further reduced to butane-2,3-diol, which does not have a flavour impact [8]. In mould cheese methyl ketones are very important aroma compounds, the major are heptan-2-one and nonan-2-one [5, 16].

In total eleven alcohols were identified in cheeses tested: methanol, ethanol, propan-1-ol, propan-2-ol, 2-methylpropanol, butanol, pentan-2-ol, heptan-2-ol, octan-1-ol, oct-1-en-3-ol, phenylethanol. Primary alcohols are formed by the reduction of the corresponding aldehydes. They impart a fruity, nutty note to the cheese flavour, but in certain cheeses, high levels of these alcohols could be responsible for flavour defects. Ethanol comes from lactose fermentation. It has a limited role in the cheese aroma despite its high levels, but it contributes to the formation of esters [7]. Secondary alcohols are formed by enzymatic reduction of the corresponding methyl ketones. They have similar but heavier flavour notes than methyl ketones. 3-Methylbutan-1-ol is present at high concentration in mould cheeses. The principal secondary alcohols in mould ripened cheeses are heptan-2-ol and nonan-2-ol. These alcohols correspond to the high methyl ketone contents of the same cheeses. They have less influence on cheese flavour than methyl ketones, however, they may contribute indirectly because of their ability to form esters with FA [6].

Five FA were identified in the cheeses tested, namely ethanoic acid, butanoic acid, 2-methylpropanoic acid, hexanoic acid and 3-methylbutanoic acid. Fatty acids are important components of the flavour of many cheese types. They may originate from lipolysis, a lower proportion of short-chain FA originate from the degradation of lactose and amino acids and they can also be derived from ketones, esters and aldehydes by oxidation [8].

Two esters were identified in the cheeses tested, namely ethyl-acetate and phenylethyl-acetate. Esters are common cheese volatiles. Esterification reactions occur between short- to medium-chain FA and primary and secondary alcohols [8, 22].

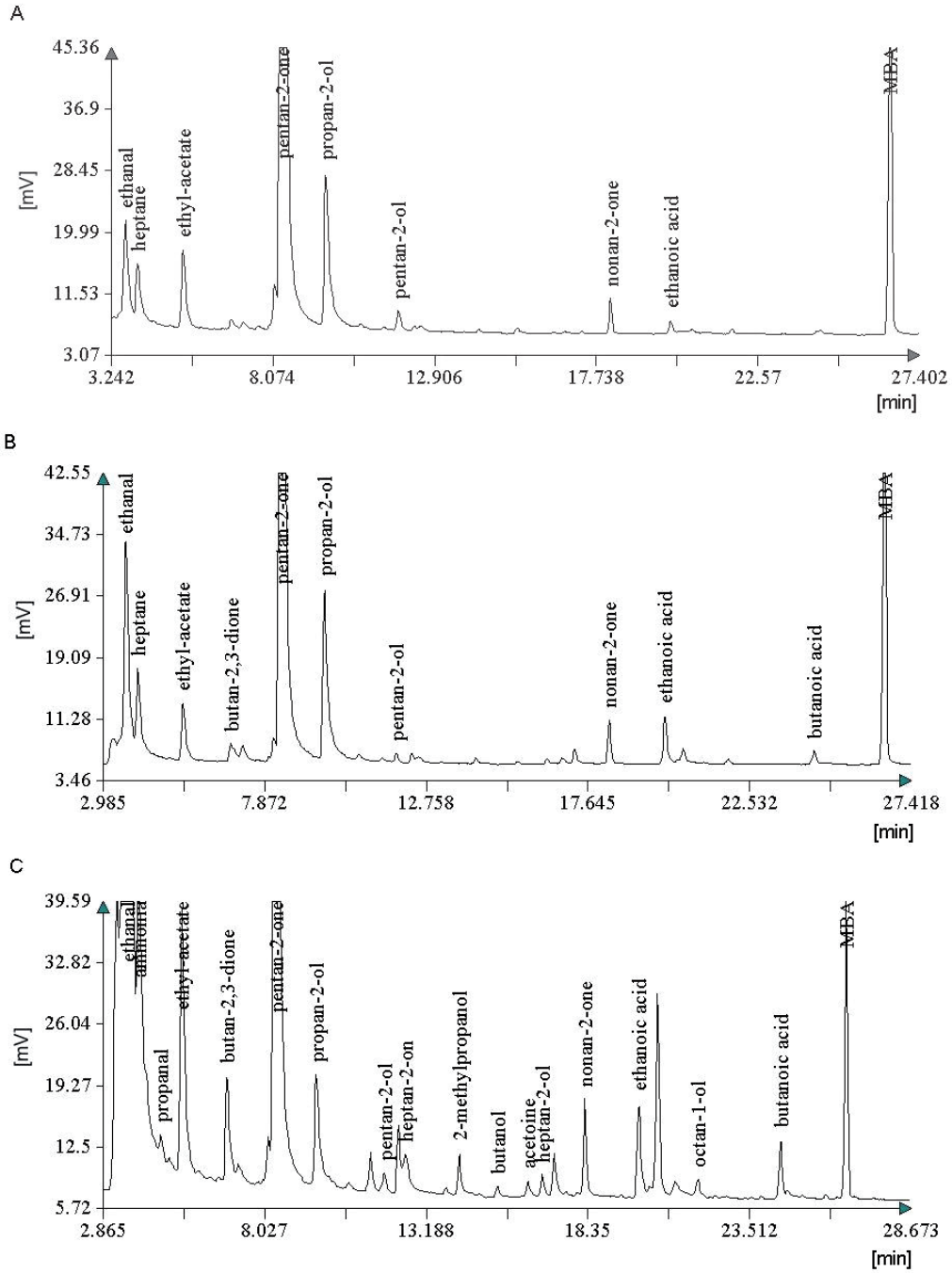

Fig. 1. Typical chromatograms of the most significant aroma compounds identified in cheeses.
A - Block Hermelin, B - Hermelin, C - Premium. MBA – methylbutanoic acid.

Three aldehydes were identified in the cheeses tested: ethanal, propanal, and phenylacetaldehyde. Straight-chain aldehydes may result from β-oxidation of unsaturated FA or from amino acids by the Strecker degradation. This reaction is simple and can occur without enzymatic catalysis during ripening. Branched-chain aldehydes probably originate from amino acid degradation via enzymatic as well as non-enzymatic, e.g. Strecker degradation, processes [8, 9]. Aldehydes are transitory compounds in cheese because they are rapidly reduced to primary alcohols or oxidised to the corresponding acids [7]. They are characterized by green-grass or herbaceous aroma and can be very unpleasant when their concentrations exceed certain value.

One hydrocarbon was identified in cheeses tested: heptane. Hydrocarbons are secondary products of lipid autooxidation. They do not make

a major contribution to aroma, but may serve as precursors for the formation of other aroma compounds [23]. Hydrocarbons have been frequently reported in many cheeses, although usually at low concentrations [7].

Two sulphur compounds were identified in cheeses tested, namely dimethyl disulphide and-dimethyl trisulphide. Sulphur compounds originate from methionine and cysteine degradation. These components are described as having strong garlic, onion or very ripe cheese odours. Their perception thresholds are very low and they are probably involved in the final aroma of mould cheeses [8].

Ammonia was the only nitrogen compound identified in the tested cheeses. Nitrogen compounds (N compounds) come from amino acids.

Typical chromatograms of most significant aroma compounds identified in cheeses are presented in Fig. 1.

Comparison of aroma profiles of ripe cheeses

SPME-GC procedure with FID detection was used for quantification of aroma compounds, the method of standard addition was chosen, the standards were added to the grated cheese in a vial. The reproducibility was good (RSD in range 2–11%). Detection limits were in the range of 0.003-0.2 $\mu g.g^{-1}$. The linearity was tested within the range of 0.003–30 $\mu g.g^{-1}$, the correlation coefficients were all over 0.99.

The aroma compounds of three different types of white surface mould cheeses produced in the Czech Republic were compared, namely Hermelin, Premium and block Hermelin. These cheeses differ in their size, shape and composition. Production technology is rather different, mainly used microbial cultures.

Comparison of these cheeses reveals differences in the content of aroma compounds. They can be ascribed primarily to the differences in the production processes, e.g. different starter cultures are used, and also to variously long ripening time. Chemical composition of cheeses, first of all the fat content, also significantly influences final aroma. The comparison of aroma profiles of ripe Hermelin, Premium and block Hermelin cheeses is graphically presented in Fig. 2.

The highest concentrations of ketones were found in block Hermelin. Propanone was quantitatively the most important ketone in all three cheese types, the other ketones identified were present only in trace amounts. The highest concentrations of alcohols were also found in block Hermelin. Ethanol was found in the significantly high amounts in all three cheese types followed by propan-2-ol. The other alcohols identified were present only in trace amounts. The highest concentrations of FA were also found in block Hermelin. Butanoic and ethanoic acids were the most significant acids in all cheese types, with the exception of Hermelin, which does not contain ethanoic acid at all. The amount of esters was similar in all cheese types, they were present only in trace amounts. The by far highest concentration of aldehydes was found in Hermelin; they were present only in trace amounts in Premium.

KARAHADIAN et al. [24] suggest that the high concentrations of secondary alcohols along with methyl ketones contribute to the flavour of the mould surface ripened cheese. The concentration of short chain free FA in Brie cheese indicates that these compounds could contribute to the flavour of mould surface ripened cheeses, although, the elevated pH (about 6.8 to 7.2) of well ripened cheese would cause a significant suppression of their flavours.

SABLÉ et al. [6] introduce the homologous series of odd-chain methyl ketones, from C3 to C15, as some of the most important compounds of white mould ripened cheese. Among volatile FA, the ethanoic, butanoic, 3-methylbutanoic and octanoic acids are the most potent odorants of Camembert cheese. Oct-1-en-3-ol, 2-phenylethanol and 2-phenylethyl acetate were quantitatively important in Camembert type cheese. These molecules together with sulphur compounds and probably lactones are reported as the key aroma substances in Camembert cheese. Sulphur containing components are considered indispensable to achieve the characteristic aroma of Camembert cheese [23]. Coryneform bacteria, especially *Brevibacterium linens*, are probably the key producers of sulphur compounds in cheeses. This explains the formation of significant concentrations of them in white mould cheese. Methanethiol appeared to be one of

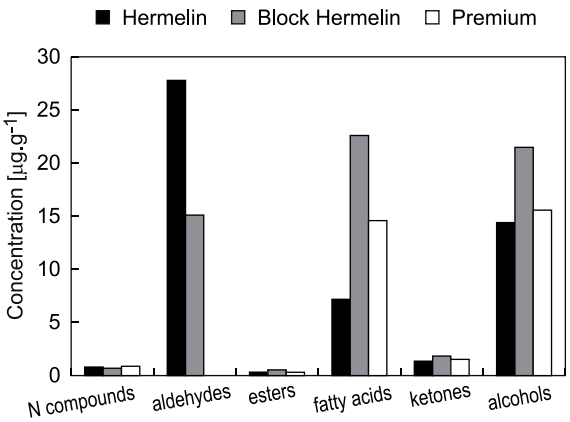

Fig. 2. Comparison of aroma profiles of three tested white surface mould cheeses.

the characteristic flavour compounds in soft white mould cheeses. Methional, dimethyl sulphide and methanethiol were also detected in significant quantities in Camembert cheese [6].

Oct-1-en-3-ol, in combination with oct-1-en-3-on, gives rise to a characteristic mushroom-like sensation of surface mould ripened cheese. Their presence might in part mask the effect of methyl ketones, also present in surface mould ripened cheeses. Sulphur compounds are key odorants of the sulphurous, garlic note in the Camembert. Butan-2,3-dione and δ-decalactone cause the buttery note. The pungent and sweaty character is mainly caused by ethanoic and butanoic acids [4].

The amount of N compounds, especially ammonia, was smaller, and similar in all three cheese types. Conversely, ammonia reaches high concentrations in the case of traditional well matured surface mould cheeses. This extensive degradation of protein is, in the main, due to the high proteolytic activity of *Penicillium* [4].

To summarize, FA, alcohols and aldehydes were quantitatively the most important compounds in cheeses tested. Block Hermelin contained the highest amounts of FA and alcohols, Hermelin contained significantly high concentrations of aldehydes and alcohols. Generally the highest amount of volatile aroma compounds was found in block Hermelin (as can be seen in figure 2), however, the difference from other cheeses is not really significant. Consequently it is possible to say, that none of cheeses investigated have stronger aroma than the others. In spite of the differences in producing technology, aroma profile (and flavour) of these cheeses is similar.

Changes of aroma compounds identified during ripening of cheeses

As mentioned before, cheese ripening includes microbiological and enzymatic processes contributing to the unique flavour and textural characteristics. Only for a few kinds of cheese have these changes been described in depth. In the case of three tested surface mould cheeses most of the identified volatile compounds were present in all stages of cheese ripening, however their relative amount changed significantly.

The concentration of ketones underwent during ripening similar changes in all cheese types tested with the maximum at about 10 days. Many authors describe the increase in the ketones concentration during ripening of various cheeses which is linked to lipolysis [7]. Alcohols are quantitatively the main chemical family in various cheese types and their concentration during ripening increases significantly although at different rates [7]. In our case no significant increase in alcohols concentration was found, their content did not change significantly. Only ethanol was present at very high concentration at the beginning of the ripening and then decreased sharply. Changes in the concentration of short-chain fatty acids were similar in all cheeses tested. Some authors note the increase in concentration of volatile FA during ripening of various kinds of cheeses [6, 16]; in our case FA reached maximum at about 20–30 days of ripening. Only ethanoic acid was present in the Premium cheese at highest concentration at about 10 ripening days and then decreased sharply. As reported by some authors, in the various kinds of cheese the concentration of esters can decrease or increase during ripening [25]. The esters in cheeses tested reached maximum at about 20–30 days of ripening. Aldehydes mostly increase during ripening of various cheeses [25]. They were not present in the Premium cheese at all or only in trace amounts, whilst in other two cheeses they appeared at the end of ripening period at relatively high concentrations. Sulphur compounds identified in cheeses tested were present only in trace amounts, although sulphur containing components are considered indispensable for achieving the characteristic aroma of Camembert cheese and were found in significant concentrations in white mould cheeses [23]. Ammonia was identified in cheeses and its concentration almost did not change during ripening.

CONCLUSIONS

Some important aroma compounds of white surface mould cheeses produced in the Czech Republic were identified and quantified using SPME coupled with GC. This method is simple and fast, minimizes thermal, mechanical, and chemical modifications of the sample. Consequently, it is suitable for the characterization of the cheese aroma.

Important changes of identified aroma compounds took place during ripening, but surprisingly no significant increase in concentration was found.

Fatty acids, alcohols and aldehydes were quantitatively the most important compounds in cheeses tested. The block Hermelin cheese contained the highest amounts of FA and alcohols, while Hermelin brand contained significantly higher concentrations of aldehydes and alcohols. Generally the highest, but not significantly higher, amount of volatile aroma compounds was found in block Hermelin. Consequently, it is possible to say, that none of the investigated brands of cheese

has stronger aroma than others. In spite of the differences in the production technology, aroma (and flavour) of these cheeses remain similar, as can also be noticed during consumption.

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The effect of long-term storage on the quality of sterilized processed cheese

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ORIGINAL ARTICLE

The effect of long-term storage on the quality of sterilized processed cheese

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Abstract The aim of this work is to evaluate the effect three different storage temperatures (6, 23 and 40 °C) on the sterilized processed cheese quality during 24-month storage. Sterilized processed cheese (SPC) is a product with extended shelf life (up to 2 years). The samples of SPC were subjected to basic chemical analyses, i.e. pH-values, dry matter, fat, crude protein and ammonia content, and microbiological analyses, i.e. total number of microorganisms, number of coliforms, colony forming units of yeasts and/or moulds and spore-forming microorganisms. Furthermore, amino acid content (ion-exchange chromatography), protein profile (SDS-PAGE) and fat globules size (image analysis of microscopic technique) were monitored and sensory analysis (scale test and pair comparative test) was implemented, too. Increasing storage temperature and length evoked decrease of total amino acid content and protein nutrition value, increase of ammonia amount, protein changes, enlargement of fat globule size and deterioration of sensory properties of SPC. All the changes grew expressive with increasing storage temperature and time.

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Keywords Sterilized processed cheese · Temperature and length of storage · Amino acids · Proteins · Fat globules · Sensory properties

Introduction

Sterilized processed cheese (SPC) represents a special group of processed cheese whose durability is prolonged by thermosterilization to minimally 24 months and which can be used in common life when refrigerator device is not available (Buňka et al. 2004). Originally, SPC was designed to be used in so-called combat rations, e.g. in armies of USA, Germany or the Czech Republic (STANAG 2937) and also to ensure boarding of the Integrated Rescue System bodies. SPC is a product that can be world-widely used. In the area of Middle Europe, SPC could be stored under ambient temperature (approx. 20–25 °C). Recently, these products are sold to Africa and/or Asia where storing under higher temperatures (approx. above 30 °C) could be expected. Unfortunately, there is a lack of information about changing of quality of SPC in literature available.

Processed cheese is produced by blending cheese in the presence of emulsifying salts and other dairy and non-dairy ingredients followed by heating and continuous mixing to form a homogenous product. Since processed cheese is not sterile, thermosterilization could be used to prolong product durability. Primarily, thermosterilization is necessary to guarantee microbiological quality and enzyme stability of the product. Nevertheless, even sterilized food is not completely stable and its long-term storage is connected with significant physico-chemical development, especially at elevated temperatures (Gliguem and Birlouez-Aragon 2005). Reactions proceeding during the storage affect all the basic components of processed cheese – proteins, fat and lactose. Maillard reaction complex (MR) ranks among the most important reactions

including both proteins (amino compounds) and carbonyl compounds (e.g. lactose). MR causes nutritional quality deterioration; primarily in consequence of essential amino acid degradation and digestibility reduction (Pizzoferrato et al. 1998). Brown pigments formation, namely darkening, and also consistency affection are regarded considerable phenomena accompanying MR in processed cheese and generally foodstuffs (Kristensen et al. 2001). The extent of non-enzymatic browning during storage advances due to rising amount of reducing saccharides, especially lactose, due to higher storage temperature and due to increasing origination of oxidizing lipids (Gaucher et al. 2008; Kristensen et al. 2001; Schär and Bosset 2002). Strecker degradation giving rise to ammonia – marker of this reaction, racemization and oxidative reaction leading to nonutilizable products creation (Adamiec et al. 2001) are considered the most common destructive reactions of amino acids. Other compounds participating in processed cheese storage reactions are lipids that undergo oxidation resulting in release of volatile carbonyl compounds causing off-flavours. Oxidation process is mostly related to higher storage temperature (Kristensen and Skibsted 1999).

Many studies dealing with the changes during storage of UHT milk have been published over the recent years. According to these papers, mainly proteolysis, protein cross-linking, colour changes and volatile components formation occur during the storage of UHT milk at both ambient and elevated temperatures (Al-Saadi and Deeth 2008; Enright et al. 1999; Valero et al. 2001). Similar reactions could be expected in SPC, especially at elevated temperatures. Besides the above mentioned chemical or physico-chemical reactions which can take place during the storage, enzymatic reaction can occur, too. Haki and Rakshit (2003) stated that some thermostable enzymes could remain active at elevated temperature such as 100–200 °C held a few seconds. Studies that have dealt with the effect of storage at higher temperature on solid dairy products are published very rarely.

The aim of this work is to evaluate the effect of three different storage temperatures (6, 23 and 40 °C) on the sterilized processed cheese quality, particularly on the amino acid content, fat globules size, protein profile and sensory properties, during 24-month storage. The results of our study could be used for the estimation of processed cheese quality changing under different conditions and contribute to improvement of product quality for consumers.

Materials and methods

All of the analyses were performed in half-year intervals, i.e. in months 0, 6, 12, 18 and 24 after production, except for amino acid content determination (months 0, 12 and 24) and SDS-PAGE analysis (month 24).

Samples of sterilized processed cheese

Storage experiment monitored SPC with 38 % *w*/*w* dry matter and 45 % *w*/*w* fat in dry matter, which was produced by Madeta, Inc., the Czech Republic. A mixture of a Dutch-type cheese with 55 % *w*/*w* dry matter and 45 % *w*/*w* fat in dry matter, butter, water, emulsifying salts (JOHA, Benckiser-Knapsack, Ladenburg, Germany) and whey powder (0.5 % *w*/*w*) was used for the processed cheese manufacturing.

Melting was accomplished at 92 °C using a Stephan TC/SK 400 batch-type industrial dairy plant equipment (Stephan Machinery, Hameln, Germany) and the product was packed into the laminated aluminium containers with seal lids. Processed cheese was sterilized in a Lubeca LW 5013 batch-type industrial autoclave (Lubeca Maschinenbau Scholz, Coesfeld, Germany) at 117 °C for 20 min. The products were cooled to 25 °C and divided into 3 parts after the sterilization. First part was stored for 2 years in a refrigerator at 6±2 °C (SR), second part at ambient temperature (23±2 °C, SA) and third part in a thermostat (40±2 °C, ST). Processed cheese manufacturing was accomplished twice for statistical purposes.

Basic chemical analyses

The samples of SPC were characterized by determining their pH, dry matter, ash, fat and crude protein content. Values of pH were measured using a pH meter with glass electrode (GRYF 209S, Havlickuv Brod, the Czech Republic). Dry matter content was determined by gravimetric method according to the ISO Standard No. 5534 (2004). Ash content was detected after burning a sample in a muffle furnace at 550 °C for 5 h. Fat content was determined according to the van Gulik acid butyrometer method (Dimitreli and Thomareis 2007) and crude protein content was assessed according to the Kjeldahl method using factor 6.38 (Dimitreli and Thomareis 2007).

Microbiological analyses

Microbiological quality of SPC was controlled by assessment of the total number of microorganisms (CFU) according to the ISO Standard No. 4833 (2003), the number of coliforms according to the ISO Standard No. 4832 (2006), the colony forming units of yeasts and/or moulds according to the ISO Standard No. 6611 (2004) and spore-forming microorganisms according to Harrigan (1998). Further, a thermostat test was accomplished at 37±1 °C for 10 days (Harrigan 1998). All media used for cultivation were obtained from HiMedia (Bombay, India).

Amino acid, ammonia, protein profile and fat globule size analyses

Total amino acid content (both free and bound) was assessed using ion-exchange liquid chromatography (IEC) as described by Buňka et al. (2009). Ammonia amount was provided by microdiffusive Conway method (Buňka et al. 2004). Protein profile was identified by SDS-PAGE (Lazárková et al. 2011). Fat globule size was assigned using image analysis of microscopic technique as described by Tremlová et al. (2006).

Sensory analysis

Sensory evaluation was accomplished using scale and pair comparative tests. A seven-point hedonic scale (1 – excellent, 4 – good, 7 – unacceptable) with the characterisation of each point was used for the assessment of appearance and colour, gloss, consistency, flavour and for overall evaluation. Moreover, three pair comparative tests were employed to confront firmness, shade and preference of the monitored cheese. Sensory panel consisted of 24 employees from the Faculty of Technology, Tomas Bata University in Zlin, trained according to the ISO Standard No. 8586 (2012).

Statistical analysis

Results of basic chemical analyses (dry matter, ash, fat and crude protein content and pH values), fat globule size analysis and determination of ammonia and amino acid content were statistically evaluated using a parametrical test comparing mean values of two independent assortments (Student *t*-test). The data from SDS-PAGE were subjected to cluster analysis using Euclidean distance measure and linking method based on average between groups. Results of sensory analysis were estimated by the Wilcoxon test and the test of binomial distribution parameter. The Unistat 5.5 software (Unistat Ltd., London, UK) was used for statistical evaluation. The level of significance was set at 95 %.

Results and discussion

Microbiological and basic chemical analyses

Microbiological analyses did not show presence of any monitored microorganism, even in thermostat test; hereby we can conclude that applied sterilizing process (117 °C, 20 min) was sufficient for inactivation of microflora and that SPC samples remained sterile even after 2-year storage. This finding agrees with published information on combined effect of temperature and time of sterilization at 110–135 °C for 5–30 min (Mafart et al. 2001).

Basic chemical analyses confirmed expectation that SPC did not show any significant differences ($P \geq 0.05$) in dry matter (35.70–37.59 % *w*/*w*), ash (3.86–4.32 % *w*/*w*), fat (17.0–18.0 % *w*/*w*) and crude protein (15.03–17.43 % *w*/*w*) content within the whole storage. Therefore, these parameters were not affected either by temperature or by length of storage. The pH-values of SPC declined ($P<0.05$) gradually during storage; total decrease was about 0.2–0.3. The highest rate and extent of pH decline during the storage was demonstrated by ST, followed by SA and SR. This pH-values reduction can be attributed to formation of acids in MR, dephosphorylation of caseins, protein-protein reactions resulting in proton release, breakdown of lactose and changes in the calcium-phosphorus equilibrium (Al-Saadi and Deeth 2008; Gaucher et al. 2008). The drop of pH during storage of UHT milk at 40 °C was observed also by Gaucher et al. (2008).

Amino acid and ammonia content analyses

Results of amino acid analysis are presented in Table 1. Storage length influenced decrease of glutamic acid, tyrosine and histidine content. Threonine, lysine, arginine and methionine showed lower concentrations during storage at 23 and 40 °C, serine and proline only at 40 °C and cysteine at 23 °C ($P<0.05$). The decline of phenylalanine and asparagic acid amount was observed only after 1-year storage. On the contrary, alanine, isoleucine and leucine did not reduce their content by 24 months ($P \geq 0.05$). Higher storage temperature affected adversely glutamic acid, tyrosine, arginine and methionine levels. Concentration of asparagic acid, serine, proline, valine and isoleucine dropped only at the highest storage temperature. In the case of threonine, leucine, phenylalanine, histidine and lysine content fall caused by temperature was detected after 2 years of storage ($P<0.05$). Glutamic acid (together with glutamine), proline, leucine and lysine ranked among the most abundant amino acids.

Concerning evaluation of total amino acid content (data not shown), definite decrease caused by both storage temperature and length can be concluded. While the amino acid decrement during the storage in refrigerator (SR) reached 2 % after 1-year and 4.5 % after 2-year storage, it grew to 7.5 % and almost to 12 % in the case of the thermostat treated samples (ST).

Further, essential amino acid indexes (EAAI) were calculated (data not shown). They exhibited slight decline owing to both storage temperature and length. During 24-month storage EAAI dropped by 4, 6 and 12 % in SR, SA and ST, respectively. This implies some, though not very considerable, protein nutrition value decrease.

Results of ammonia content determination are shown in Fig. 1. During the 24-month storage at 23 and 40 °C, the ammonia content rise occurred ($P<0.05$). Ammonia amount

Table 1 Amino acid content (g/16gN) in sterilized processed cheese depending on temperature and length of storage

Amino acid	Storage temperature (°C)	Storage length (months)		
		0	12	24
Asparagic acid	6	6,25±0,249^{a}	6,02±0,319^{b}A	5,92±0,216^{b}A
	23	6,25±0,249^{a}	6,00±0,130^{b}A	5,94±0,298^{b}A
	40	6,25±0,249^{a}	5,75±0,142^{b}B	5,65±0,354^{b}B
Threonine	6	3,19±0,144^{a}	3,18±0,204^{a}A	3,12±0,061^{a}A
	23	3,19±0,144^{a}	3,06±0,139^{b}B	2,94±0,086^{c}B
	40	3,19±0,144^{a}	3,10±0,080^{b}B	2,67±0,119^{c}C
Serine	6	4,72±0,211^{a}	4,73±0,280^{a}A	4,54±0,175^{b}A
	23	4,72±0,211^{a}	4,52±0,201^{b}B	4,46±0,198^{b}A
	40	4,72±0,211^{a}	4,53±0,173^{b}B	3,82±0,117^{c}B
Glutamic acid	6	19,88±0,979^{a}	19,05±0,638^{b}A	18,54±0,355^{c}A
	23	19,88±0,979^{a}	18,27±0,441^{b}B	17,95±0,274^{c}B
	40	19,88±0,979^{a}	17,23±0,342^{b}C	17,08±0,223^{b}C
Proline	6	9,72±0,376^{a}	9,54±0,352^{a,b}A	9,46±0,301^{b}A
	23	9,72±0,376^{a}	9,50±0,301^{b}A	9,38±0,215^{b}A
	40	9,72±0,376^{a}	9,22±0,224^{b}B	9,02±0,359^{c}B
Glycine	6	1,57±0,052^{a,b}	1,58±0,034^{a}A	1,54±0,023^{b}A
	23	1,57±0,052^{a,b}	1,59±0,033^{a}A	1,56±0,015^{b}A
	40	1,57±0,052^{a}	1,51±0,020^{b}B	1,55±0,039^{a,b}A
Alanine	6	2,37±0,074^{a}	2,43±0,141^{a}A	2,26±0,067^{b}A
	23	2,37±0,074^{a}	2,32±0,082^{a}B	2,26±0,045^{b}A
	40	2,37±0,074^{a}	2,39±0,035^{a}A	2,19±0,064^{b}B
Valine	6	5,62±0,143^{a}	5,46±0,111^{b}A	5,43±0,063^{b}A
	23	5,62±0,143^{a}	5,51±0,154^{b}A	5,40±0,154^{c}A
	40	5,62±0,143^{a}	5,39±0,118^{b}B	5,19±0,153^{c}B
Izoleucine	6	4,11±0,085^{a}	4,12±0,083^{a}A	3,96±0,099^{b}A
	23	4,11±0,085^{a}	4,10±0,126^{a}A,B	3,94±0,151^{b}A
	40	4,11±0,085^{a}	4,07±0,073^{a}B	3,78±0,124^{b}B
Leucine	6	8,18±0,108^{a}	8,20±0,342^{a}A	7,99±0,173^{b}A
	23	8,18±0,108^{a}	8,14±0,391^{a}A	7,89±0,104^{b}B
	40	8,18±0,108^{a}	8,09±0,366^{a}A	7,72±0,219^{b}C
Tyrozine	6	5,07±0,059^{a}	4,95±0,117^{b}A	4,82±0,121^{c}A
	23	5,07±0,059^{a}	4,82±0,113^{b}B	4,72±0,140^{c}B
	40	5,07±0,059^{a}	4,75±0,094^{b}C	4,51±0,168^{c}C
Phenylalanine	6	4,49±0,051^{a}	4,30±0,270^{b}A	4,28±0,108^{b}A
	23	4,49±0,051^{a}	4,22±0,124^{b}A	4,19±0,132^{b}B
	40	4,49±0,051^{a}	4,06±0,318^{b}B	3,95±0,073^{b}C
Histidine	6	2,75±0,067^{a}	2,66±0,137^{b}A	2,52±0,037^{c}A
	23	2,75±0,067^{a}	2,69±0,098^{b}A	2,43±0,032^{c}B
	40	2,75±0,067^{a}	2,43±0,100^{b}B	2,10±0,047^{c}C
Lyzine	6	6,75±0,144^{a}	6,70±0,180^{a}A	6,52±0,104^{b}A
	23	6,75±0,144^{a}	6,64±0,117^{b}A	6,45±0,066^{c}B
	40	6,75±0,144^{a}	6,00±0,097^{b}B	5,89±0,053^{c}C
Arginine	6	3,65±0,066^{a}	3,54±0,202^{b}A	3,48±0,147^{b}A
	23	3,65±0,066^{a}	3,37±0,120^{b}B	3,27±0,096^{c}B
	40	3,65±0,066^{a}	3,21±0,105^{b}C	3,08±0,114^{c}C
Cysteine	6	0,43±0,015^{a}	0,39±0,023^{b}A	0,37±0,027^{b}A
	23	0,43±0,015^{a}	0,39±0,016^{b}A	0,36±0,031^{c}A

Table 1 (continued)

Amino acid	Storage temperature (°C)	Storage length (months)		
		0	12	24
	40	0,43±0,015[a]	0,36±0,027[b]A	0,33±0,048[b]A
Methionine	6	3,16±0,037[a]	3,11±0,128[a,b]A	3,06±0,067[b]A
	23	3,16±0,037[a]	2,99±0,057[b]B	2,84±0,109[c]B
	40	3,16±0,037[a]	2,92±0,132[b]C	2,61±0,115[c]C

Amino acid content is presented as mean±SD (n=20). Means within a row (effect of storage length) with the same superscript do not differ ($P \geq 0.05$). Means within a column (effect of storage temperature) with various capital letters differ ($P < 0.05$)

increased more than double in the case of samples stored in thermostat (ST). There was no definite growing trend in ammonia concentration in cheese stored in refrigerator (SR). Storage temperature affected ammonia amount in SPC even more markedly. Ammonia concentration increase ($P < 0.05$) caused by rising temperature was observed in all samples. Growth of ammonia levels by three quarters (compared to SR) was determined after 2-year storage of ST. Increase of ammonia content is related to the above described amino acid detriment during SPC storage since ammonia is one of the amino acid degradation products (Efigênia et al. 1997). This rise could be particularly attributed to Maillard reaction complex and further reactions mentioned in the "Introduction" chapter (Adamiec et al. 2001; Pizzoferrato et al. 1998).

SDS-PAGE analysis

Dendrogram of SPC stored for 24 months is depicted in Fig. 2 (based on cluster analysis described in the part "Statistical analysis"). Seventeen proteins with molecular weight in the range 3.7–28.0 kDa were determined in SR (the electrophoregram was not shown). Thirteen proteins (3.9–28.2 kDa) were detected in SA whose protein profiles were relatively analogous to those of cheese stored in refrigerator (see similar clusters of SA and SR in Fig. 2). The samples of SPC stored in thermostat (ST) at stressed temperature mostly degraded. This statement is supported with finding a low number of proteins (5 proteins with molecular weight 9.6–28.6 kDa). The diversity of protein profile of ST is confirmed with totally different cluster compared to other clusters of SPC (see Fig. 2). These results are in good agreement with those of Al-Saadi and Deeth (2008) who observed that the electrophoretic patterns of UHT milk samples stored at 5 and 20 °C were different from those stored at 37 and 45 °C. Proteolytic reactions of proteins were more extensive with rising storage temperature which also corresponds with findings of Al-Saadi and Deeth (2008) who stated that changes in

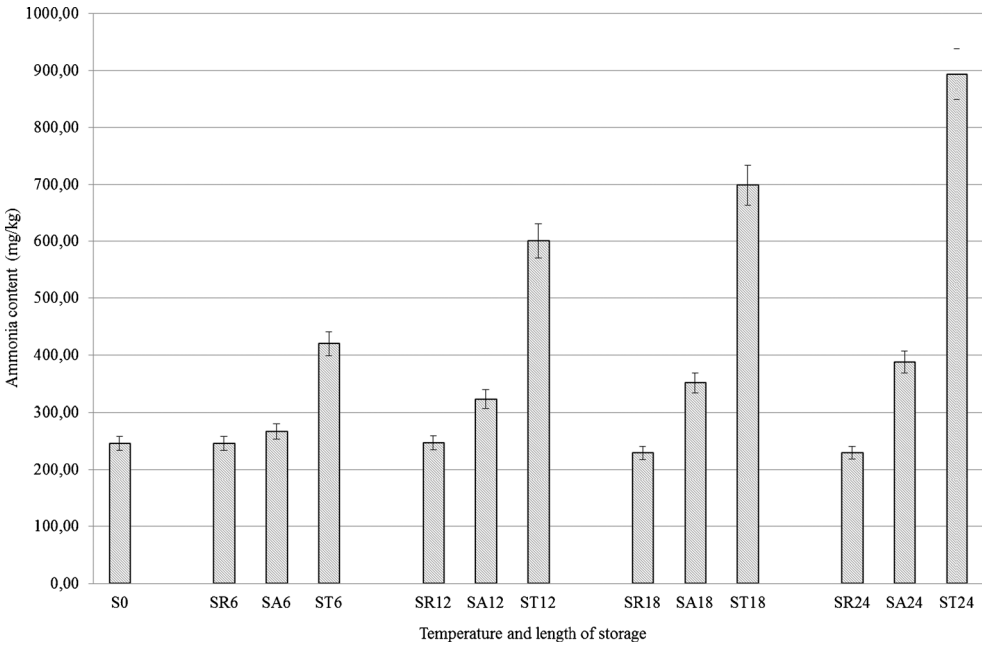

Fig. 1 Ammonia content (mg/kg) in sterilized processed cheese depending on temperature and length of storage. The values are the means with the standard errors of the means, shown by *vertical bars*. Samples of sterilized processed cheese were stored at 6 °C (SR), 23 °C (SA) and 40 °C (ST) and analysed immediately after production (0) and further in half-year intervals (6, 12, 18 and 24)

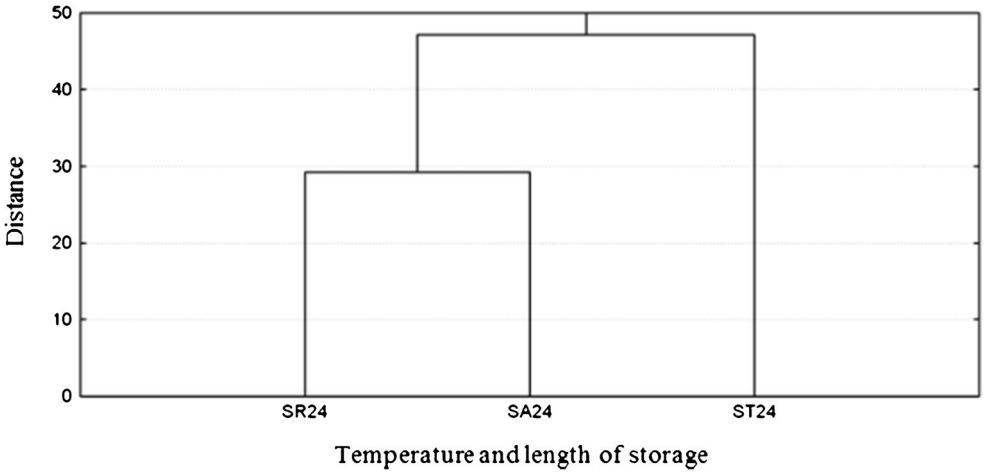

Fig. 2 Dendrogram of protein profile results of sterilized processed cheese based on cluster analysis of electrophoregram originated using SDS-PAGE analysis. Samples of sterilized processed cheese were stored at 6 °C (SR), 23 °C (SA) and 40 °C (ST) and analysed after 24 months. The distance (y-axis) is dimensionless

electrophoretic patterns of the UHT milk increased with storage temperature and time.

Fat globule size analysis

Results of image analysis expressed as rel. % of fat globule size distribution are shown in Fig. 3; it is evident that fat globules smaller than 100 μm^2 and bigger than 5,000 μm^2 occurred rarely in SPC (0.2–4.7 rel.%). The most abundant fat globule size in SPC was in the range 500–1,000 μm^2 (44.4–57.1 rel.%). Rising amount of fat globule size in the interval 1,000–5,000 μm^2 (9.1, 11.5, 16.4 and 23.0 rel.% in S0, SR6, SS6 and ST6, respectively) was observed with increasing storage temperature during the first 6 months of storage ($P<0.05$). After 1 year of storage both SR and SA showed similar fat globule size ($P\geq0.05$), whereas ST displayed bigger fat globules ($P<0.05$). Starting with 18th storage month no marked changes in fat globule size were detected ($P\geq0.05$).

Fat globule interior consists of triacylglycerols while milk fat globule membrane (MFGM) is composed of a complex mixture of proteins, glycoproteins, enzymes, phospholipids, cholesterol and other minor components. MFGM acts as a natural emulsifying agent preventing the coalescence of fat globules. However, it was stated that MFGM could be ruptured during thermal and mechanical treatment hereby resulting in aggregation and fusing of fat globules, thus

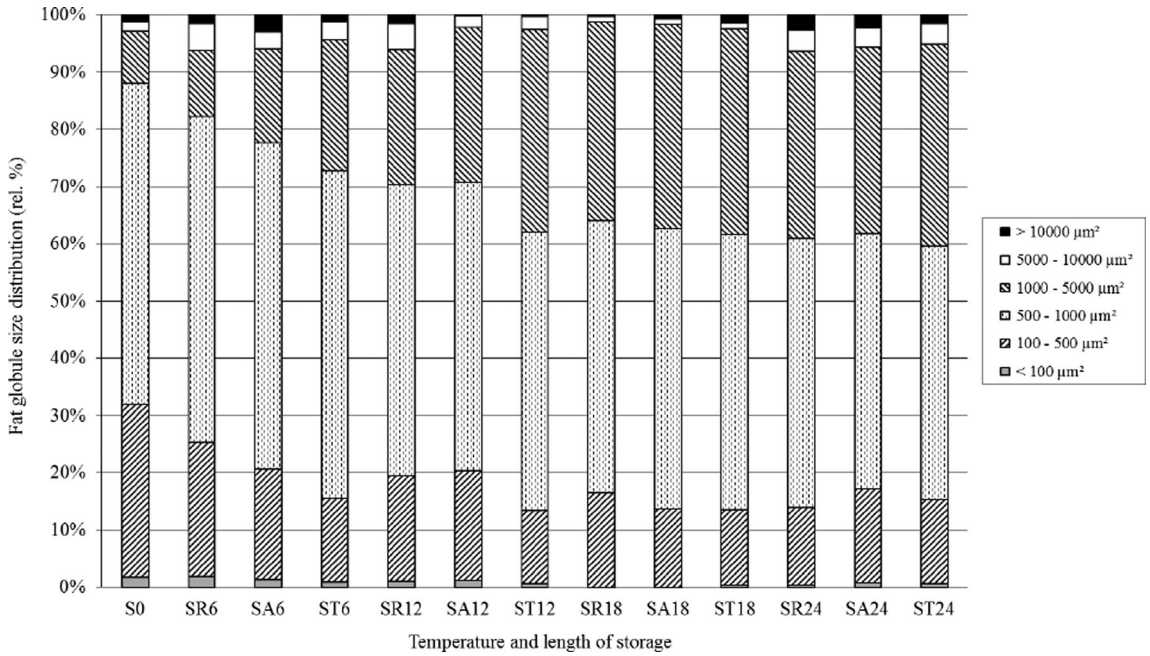

Fig. 3 Fat globule size distribution (rel. %) of sterilized processed cheese depending on temperature and length of storage. Samples of sterilized processed cheese were stored at 6 °C (SR), 23 °C (SA) and 40 °C (ST) and analysed immediately after production (0) and further in half-year intervals (6, 12, 18 and 24)

causing enlargement of fat globule size (Impoco et al. 2012). Similar clustering of fat globules caused by MFGM composition changes was described during storage of UHT milk at 30 °C for 1 year (Yamuchi et al. 1982). These findings are in good agreement with observed increase of fat globule size in SPC stored in thermostat in our study.

Sensory analysis

Results of sensory analysis of SPC with the use of a seven-point hedonic scale are presented in Table 2. All of the descriptors, namely appearance and colour, gloss, consistency, flavour and overall evaluation of SR did not deteriorate significantly ($P \geq 0.05$) until the 2nd year of storage. However, even after 2 years, these samples were evaluated as "good" or better. In the samples SA quality impairment was noticed already after 6 months of storage ($P < 0.05$), whereas after 12th month of storage no quality deterioration of most descriptors was detected ($P \geq 0.05$); samples were assessed as "good" or "less good". Most extensive changes were registered in ST during storage. Even after 6 months, these samples showed "unsatisfactory" level of most sensory descriptors. Further quality decline occurred after 12 months of storage; samples were evaluated as "unacceptable". Hence, sensory analysis of ST samples was terminated after 12-month storage.

Storage temperature significantly affected sensory quality of SPC. With rising temperature, most descriptors showed quality impairment ($P < 0.05$) which is illustrated by pair comparative test results. It was found out that samples stored at lower temperature were preferred and samples stored at higher temperature were evaluated as darker in all cases ($P < 0.05$). Presumably, darkening of the samples induced downgrade in appearance and colour descriptors. Colour changes of SPC can be supposedly attributed to reactions of nitrogen compounds, especially MR, whose intensive development is, inter alia, caused by use of whey powder in processed cheese production (Kristensen et al. 2001; Pizzoferrato et al. 1998; Schär and Bosset 2002). Browning of UHT milk stored at 37, 45 and 40 °C was reported by Al-Saadi and Deeth (2008) and Gaucher et al. (2008), respectively. Furthermore, flavour of processed cheese was significantly influenced. Storage length exerted slight impact only on samples stored at 23 and 40 °C. Contrary to the above, storage temperature influenced flavour deterioration much more markedly. Decreased sensory acceptability of UHT milk stored at ambient temperature was observed by Enright et al. (1999) and Valero et al. (2001). According to Gaucheron et al. (1999) there is a connection between MR and colour and flavour changes. Evaluation of processed cheese consistency (firmness) brought rather ambiguous results. All samples kept at higher temperature were described as tougher after 6 months ($P < 0.05$). After 12 months, samples stored at 40 °C were still tougher ($P < 0.05$) than SR and SA samples. However, firmness of cheeses stored at 6 and 23 °C did not differ significantly when compared ($P \geq 0.05$). Initial increase in firmness of cheese stored at higher temperature could be likely evoked by subsequent protein cross-linking developed due to hydrolysis of emulsifying salts and successive release of calcium ions (Schär and Bosset 2002). Incidence of two phenomena can be probably expected in longer time horizon. First, new protein bonds are formed (e.g. due to the above mentioned cross-

Table 2 Results of sensory analysis of sterilized processed cheese depending on temperature and length of storage

Descriptor	Storage temperature (°C)	Storage length (months)				
		0	6	12	18	24
Appearance and colour	6	3^{a}	$3^{b}A$	$3^{b}A$	$4^{c}A$	$4^{c}A$
	23	3^{a}	$4^{b}B$	$5^{c}B$	$5^{c}B$	$5^{c}B$
	40	3^{a}	$6^{b}C$	$6^{b}C$	ND	ND
Gloss	6	3^{a}	$3^{a}A$	$4^{a,b}A$	$4^{b}A$	$4^{b}A$
	23	3^{a}	$4^{b}B$	$5^{c}B$	$5^{c}B$	5^{c} B
	40	3^{a}	$5^{b}C$	$6^{c}C$	ND	ND
Consistency	6	3^{a}	$3^{a}A$	$4^{b}A$	$4^{b}A$	$4^{b}A$
	23	3^{a}	$4^{b}B$	$4^{b}A$	$4^{b}A$	$4^{b}A$
	40	3^{a}	$5^{b}C$	$6^{c}B$	ND	ND
Flavour	6	3^{a}	$3^{b}A$	$3^{b}A$	$4^{c}A$	$4^{c}A$
	23	3^{a}	$4^{b}B$	$5^{c}B$	$5^{c}B$	$5^{c}B$
	40	3^{a}	$6^{b}C$	$7^{c}C$	ND	ND
Overall evaluation	6	3^{a}	$3^{b}A$	$3^{b}A$	$4^{c}A$	$4^{c}A$
	23	3^{a}	$4^{b}B$	$5^{c}B$	$5^{c}B$	$5^{c}B$
	40	3^{a}	$6^{b}C$	$7^{c}C$	ND	ND

Results of sensory analysis are presented as medians. Medians within a row (effect of storage length) with the same superscript do not differ ($P \geq 0.05$). Medians within a column (effect of storage temperature) with various capital letters differ ($P < 0.05$). *ND* not determined

linking) leading to firmness enhancement. On the other hand, protein hydrolysis, e.g. owing to activity of residual thermostable proteases, can occur. Providing that this process prevails over new bond formation, either at higher temperature or sooner than at lower temperature, firmness decline can be observed. Likewise, protein degradation hypothesis is supported by deterioration of SPC flavour during storage and corresponds with the findings of Buňka et al. (2008). Declined sensory quality of SPC stored at elevated temperature corresponds with observed increase of ammonia content and protein changes.

Conclusion

The effect of storage temperature and length on processed cheese quality was monitored in this study. Storage conditions exerted impact on protein changes in SPC. Amino acid content declined only moderately due to both storage temperature and length. Nevertheless, storage length showed slightly greater influence than temperature, which was considerable only in the case of thermostat storage. Hence, storage participated in protein nutrition value decrease, especially in cheese stored at the highest temperature for 24 months. Nutrition value of SPC remained satisfactory during storage though. Protein changes were also proved by protein profile analysis using SDS-PAGE. Besides protein profile, fat globule size was also affected by storage temperature and length. Destructive protein reactions reflected adversely in organoleptic quality of SPC, particularly colour and flavour. Storage at 40 °C emerged to be entirely improper, since all the monitored descriptors deteriorated significantly. Refrigerator storage appears to be the most suitable method for long-term storage of SPC; nevertheless, acceptable products were obtained also during storage at ambient temperature even if slight quality decline can be expected compared to storage at 6 °C.

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ORIGINAL PAPER

Identification of volatile aroma compounds in processed cheese analogues based on different types of fat‡

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The simple and rapid solid-phase micro-extraction method using gas chromatography was used for the identification and quantification of volatile aroma compounds in various types of processed cheese analogues produced from different types of fat (butter, butter oil, coconut oil, palm oil, and sunflower oil). In total, 31 organic compounds belonging to five chemical groups were identified, with the alcohols and fatty acids quantitatively predominant. The contents of the aroma compounds (the so-called aroma profiles) of the analogues and corresponding fats used as raw materials were compared. Significant differences ($p < 0.05$) were found between samples. The highest total content of aroma compounds was found in coconut oil analogue ((547.30 ± 9.82) mg kg^{-1}), the lowest in palm oil analogue ((372.01 ± 16.16) mg kg^{-1}). The concentrations of aroma compounds in fats were substantially lower ($p < 0.05$) than in analogues. Hence, the largest number of aroma analogues came from Edam cheese used for production as a protein source.

Keywords: cheese analogues, fat, aroma compounds, solid-phase micro-extraction, GC-FID, GC-MS

Introduction

Cheese analogues are cheese-like products which partly or wholly substitute for or resemble cheese, and in which milk fat, milk protein, or both are partially or wholly replaced by non-milk-based components, principally of vegetable origin (Guinee et al., 2004). They were introduced onto the US market in the early 1970s. These products have numerous applications: frozen pizza toppings, slices in beef-burgers, and an ingredient in salads, sandwiches, cheese sauces, cheese dips, and ready-prepared meals (Bachmann, 2001; Guinee et al., 2004). The designation and labelling of analogues should clearly distinguish them from cheese. However, outside the USA, there is little specific legislation covering cheese analogues. Few, if any, standards relating to permitted ingredients or manufacturing procedures exist for cheese analogues (Guinee et al., 2004).

Cheese analogues are manufactured by blending various edible oils/fats (e.g. soya, peanut, palm, coconut, corn, rapeseed, cotton seed oils, and their hydrogenated equivalents) and proteins (e.g. casein, soy protein) with other ingredients and water (Guinee et al., 2004; Kapoor & Metzger, 2008). Their main advantages are low cost, consistent quality, durability, and possible nutritional advantages over the original cheese. For example, analogues containing reduced levels of sodium or saturated fats are tested; however, these reductions can alter the appearance, texture, flavour, melting properties, and other attributes (Bachmann, 2001). The most important negative property of an analogue is its flavour, which is bland and not comparable to the flavour of real cheese, so the use of some flavour system is also very important (Kilcawley et al., 1998). These flavoured analogues are very similar and difficult to distinguish from natural cheese (Bachmann, 2001).

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The effects of various ingredients, processing conditions, and storage temperature on the quality of analogues have been extensively reported; however, most reports have focused on texture and cooking characteristics (e.g. Tamime et al., 1999; Lobato-Calleros et al., 2008; Mounsey & O'Riordan, 2008; Noronha et al., 2008c), and only a few deal with the flavour of analogues (e.g. Muir et al., 1999; Noronha et al., 2008a, 2008b; Cunha et al., 2010). All these authors evaluated flavour in terms of palatability from the perspective of consumers and their demand for a pleasant flavour. Another approach to characterising flavour can be by monitoring the volatile aroma compounds. No data have yet been published on the content of aroma compounds in cheese analogues; we can assume, however, that their composition is derived from aroma compounds present in the raw materials used in their production, i.e. the source of (milk) proteins (natural Edam cheese in our case) and various types of vegetable fats. We can also use the aroma profile of traditional processed cheese for comparison, because the composition, raw materials, and production process are very similar to the processed analogues. Moreover, very little research has been conducted into the flavour of processed cheeses. Only Sunesen et al. (2002) in their work compared the content of aroma compounds in eleven types of processed cheeses, where ketones and aldehydes predominated.

The flavour volatiles and their formation in Gouda (i.e. Edam) cheese have been clearly described by many authors (McSweeney & Sousa, 2000; Sunesen et al., 2002; Liu et al., 2004; McSweeney, 2004; Alewijn et al., 2005, 2007; Van Leuven et al., 2008; Vítová et al., 2011). The aroma compounds in Edam cheese are derived from three main precursors: lipids, lactose, and proteins, all formed in the maturing of cheese (McSweeney & Sousa, 2000; Sunesen et al., 2002; McSweeney, 2004). Free fatty acids, 2-methyl ketones, δ-lactones and γ-lactones, saturated and unsaturated aldehydes, and ethyl esters are fat-derived flavour volatiles (Van Leuven et al., 2008). Methionine, the aromatic, and the branched-chain amino acids are the precursors for sulphur components, aromatic and branched-chain aldehydes and alcohols, and for branched-chain volatile acids and alcohols. Lactose, lactate, and citrate contribute to the formation of ethanol, acetate, butan-2,3-dione, and its reduction products 3-hydroxybutan-2-one and butane-2,3-diol (Van Leuven et al., 2008).

The composition of volatile compounds in butter and butter oil has been studied by many authors and a number of articles on butter aroma are available (e.g. Adahchour et al., 1999, 2005; Peterson & Reineccius, 2003; Mallia et al., 2008). The characteristic impact odour compounds of butter primarily originate from the cream used to make it. Mallia et al. (2008), in accordance with other authors, consider lactones, ketones, and aldehydes as the key aroma compounds of sweet cream butter. Several others have been found as odour-active by Peterson & Reineccius (2003); lactones have been definitively associated with butter aroma and are considered to be key character impact compounds.

However, very little information is available on the aroma composition of vegetable fats. Villarino et al. (2007) evaluated the flavour of coconut oil in respect of palatability. In lipids, the majority of volatile compounds result from lipid oxidation reactions, so several authors studied auto-oxidation and related oxidised volatile compounds in vegetable oils (Keszler et al., 1998; van Ruth & Roozen, 2000; van Ruth et al., 2000; Doleschall et al., 2001, 2003).

The aim of this work was to identify and quantify volatile aroma compounds in processed cheese analogues with different type of fat. Aroma compounds as a substantial component of flavour were assessed using gas chromatography-mass spectrometry (GC-MS) with solid-phase micro-extraction (SPME).

Experimental

Processed cheese analogues with different type of fat (butter, butter oil, coconut oil, palm oil, sunflower oil) were analysed in this work. Raw material used in the production of model cheese analogues: Edam cheese (30 % fat in dry matter), commercial emulsifying salt (sodium salts of phosphates and polyphosphates; Fosfa, Czech Republic), water, butter (82 % fat), and various types of fats (100 % fat). The declared values of analogues were 40 mass % of dry matter, 50 mass % of fat in dry matter. The precise amount of raw materials for individual types of processed cheese analogues is shown in Table 1. Raw materials were cut into pieces, transferred into a blender (model: Vorwerk Thermomix, Vorwerk, Germany), premixed with emulsifying salt at high agitation followed by the addition of water; the mixture was heated up to 90 ℃ under constant steady agitation; total melting time was 10 min. (from beginning to end of heating). The hot melt was poured into plastic cups with sealable aluminium lids (100 g), cooled and stored at (6 ± 2) ℃ until analysis. Three batches of each analogue type were melted and each sample analysed three times. For assessment of volatile aroma compounds, 1 g of sample was placed into a vial for SPME extraction.

SPME extractions were carried out using Carboxen^{TM}/Poly(dimethylsiloxane) fibre (CAR/PDMS) 85 μm (Supelco, USA) under the following conditions: extraction temperature of 35 ℃, equilibrium time of 30 min, extraction time of 20 min, desorption temperature of 250 ℃, desorption time of 5 min. The following conditions were used for gas chromatographic analysis using a gas chromatograph TRACE^{TM} GC (ThermoQuest, Italy) with a capillary column DB®-WAX (30 m × 0.32 mm × 0.5μm): injector temperature of

Table 1. Raw materials for production of model processed cheese analogues with different types of fat

Raw material	Type of processed cheese analogue/g				
	Butter	Butter oil	Coconut oil	Palm oil	Sunflower oil
Edam cheese	400	400	400	400	400
Fat	120	101	101	100	98
Emulsifying salt	19	19	19	19	19
Water	250	260	260	260	270
Total amount	789	780	780	779	787

250 ℃, split-less desorption of 5 min, carrier gas N_2, flow-rate of 0.9 mL min^{-1}, flame ionisation detector (FID) set at 220 ℃, H_2 inlet of 35 mL min^{-1}, air inlet of 350 mL min^{-1}, make up N_2 of 30 mL min^{-1}. The oven ramp temperature was 40 ℃ for 1 min, then it was increased up to 200 ℃ at a rate of 5 ℃ min^{-1} and maintained at 200 ℃ for 7 min. GC-MS analyses were performed on a GC 8000 (Carlo Erba, Italy) with MS TRIO 1000 (Fisons Instruments, USA). Helium was used as a carrier gas. GC column and conditions were as described above. The validation and validation parameters of the SPME-GC-MS method were published previously. The reproducibility was verified by repeated extraction (number of experiment, $n = 5$) of the standard mixtures (relative standard deviation < 10 %), detection limits were in a range of 0.01–0.50 mg kg^{-1}. Linearity was tested within the range of 0.01–200 mg kg^{-1}; correlation coefficients were all above 0.99 (Vítová et al., 2006, 2007).

The following chemicals were used as standards: pentanal, heptanal, hexanal, propan-2-one, nonan-2-one, decan-2-one, undecan-2-one, dodecan-2-one, 2-phenylacetaldehyde, benzaldehyde, 2-methylbutan-1-ol (all from Sigma–Aldrich, Germany). Acetic acid, propanoic acid, butanoic acid, octanoic acid, decanoic acid, 2-hydroxypropanoic acid, 2-methylpropanoic acid, 3-methylbutanoic acid, propanal, octanal, nonanal, acetaldehyde, methyl acetate, ethyl acetate, ethyl butanoate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, butyl acetate, propyl acetate, ethanol, propan-1-ol, propan-2-ol, butan-1-ol, butan-2-ol, pentan-1-ol, pentan-2-ol, hexan-1-ol, heptan-2-ol, octan-1-ol, octan-2-ol, nonan-2-ol, decan-1-ol, 2-methylpropan-1-ol, 3-methylbutan-1-ol, phenylmethanol, 2-phenylethanol, pentan-2-one, heptan-2-one, tridecan-2-one, 4-methylpentan-2-one, 3-hydroxybutan-2-one, butan-2,3-dione (all from Merck, Germany). Methanol, butan-2-one, 2-methylpropan-2-ol (all from Lachema, Czech Republic). Oct-1-en-3-ol, 3-methylbutan-1-al (both from Fluka, Switzerland). All the chemicals were of chemically pure grade.

All results were statistically processed using parametric one-way analysis of variance (ANOVA) and subsequently by Duncan test; the results are expressed as mean ± standard deviation ($n = 9$). MS Excel 2003 (Microsoft, USA) and statistical software Unistat, version. 5.5 (Unistat, United Kingdom) were used.

Results and discussion

SPME/GC-MS analysis of aroma compounds

SPME was used for the extraction of volatile aroma compounds from cheese or fat samples. The extracted aroma compounds were identified by GC-MS, confirmed using retention times of standards, and quantified using standards by GC-FID. The SPME method is simple, rapid, and very gentle towards the sample matrix; it is especially suitable for the extraction of volatile and semi-volatile organic compounds (Kataoka et al., 2000; Wardencki et al., 2004). Many authors have used it for the assessment of volatile compounds in various cheese types (Pérès et al., 2001; Ziino et al., 2005; Andic et al., 2011; Delgado et al., 2011; Majcher et al., 2011).

In total, 31 different organic compounds belonging to five chemical groups were identified in the fat and cheese analogue samples: 10 alcohols, 8 aldehydes, 6 ketones, 3 fatty acids, and 4 esters. Quantitatively, alcohols and fatty acids were the most important groups. The aroma compounds identified and quantified in samples of cheese analogues are given in Table 2.

In total, 23 aroma compounds were identified in Edam cheese of which ethanol ((195.86 ± 2.66) mg kg^{-1}), 2-methylpropan-1-ol ((3.32 ± 0.04) mg kg^{-1}), 2-methylbutan-1-ol ((2.59 ± 0.01) mg kg^{-1}), 3-methylbutan-1-ol ((4.36 ± 0.15) mg kg^{-1}), acetaldehyde ((61.13 ± 4.18) mg kg^{-1}), benzaldehyde ((6.11 ± 0.12) mg kg^{-1}), propan-2-one ((72.44 ± 0.37) mg kg^{-1}), 3-hydroxybutan-2-one ((13.98 ± 0.12) mg kg^{-1}), butan-2,3-dione ((3.62 ± 0.08) mg kg^{-1}), and acetic acid ((41.30 ± 0.61) mg kg^{-1}) were quantitatively the most important. All these compounds are regarded as important flavour components of Edam type cheese (Alewijn et al., 2007; Van Leuven et al., 2008).

In total, 18 and 17 aroma compounds were identified in butter and butter analogue, respectively. Quantitatively, the most important were ethanol ((5.96 ± 1.39) mg kg^{-1}), butan-1-ol ((1.71 ± 0.02) mg kg^{-1}), butan-2-ol ((1.15 ± 0.04) mg kg^{-1}), hexanal ((1.29 ± 0.15) mg kg^{-1}), acetaldehyde ((5.48 ± 0.13) mg kg^{-1}), propan-2-one ((31.23 ± 0.28) mg kg^{-1}), butan-2,3-dione ((6.61 ± 0.09) mg kg^{-1}),

Table 2. Volatile aroma compounds identified in processed cheese analogues produced with different types of fat

Aroma compounds	Aroma compound content per kg of cheese/mg				
	Butter	Butter oil	Coconut oil	Palm oil	Sunflower oil
Ethanol	$(258.47 \pm 12.08)^a$	$(221.12 \pm 0.20)^b$	$(227.42 \pm 3.77)^b$	$(198.49 \pm 15.05)^c$	$(240.58 \pm 17.58)^d$
Butan-1-ol	$(0.20 \pm 0.01)^a$	$(0.31 \pm 0.04)^a$	$(0.23 \pm 0.01)^a$	$(0.32 \pm 0.04)^a$	$(0.28 \pm 0.01)^a$
Butan-2-ol	$(16.72 \pm 0.36)^a$	$(1.98 \pm 0.03)^b$	$(131.08 \pm 2.34)^c$	nd	nd
Pentan-1-ol	$(2.31 \pm 0.11)^a$	$(1.49 \pm 1.06)^{b,c}$	$(1.74 \pm 0.08)^c$	nd	$(2.57 \pm 0.81)^a$
Hexan-1-ol	nd	$(0.01 \pm 0.01)^a$	nd	nd	$(0.01 \pm 0.01)^a$
Octan-1-ol	$(0.02 \pm 0.01)^a$	$(0.02 \pm 0.01)^a$	$(0.02 \pm 0.01)^a$	$(0.02 \pm 0.01)^a$	$(0.02 \pm 0.01)^a$
Decan-1-ol	$(0.01 \pm 0.01)^a$	$(0.01 \pm 0.01)^a$	$(0.02 \pm 0.01)^a$	$(0.01 \pm 0.01)^a$	$(0.01 \pm 0.01)^a$
3-Methylbutan-1-ol	$(0.43 \pm 0.05)^a$	$(0.12 \pm 0.01)^{b,c}$	$(0.42 \pm 0.13)^a$	$(0.03 \pm 0.01)^c$	$(0.67 \pm 0.06)^a$
In total: alcohols	278.16 ± 12.09	225.06 ± 1.08	360.93 ± 4.44	198.87 ± 15.05	244.14 ± 17.60
Acetaldehyde	$(82.89 \pm 4.18)^a$	$(86.11 \pm 8.15)^a$	$(33.95 \pm 8.32)^b$	$(21.01 \pm 2.50)^c$	$(6.42 \pm 1.33)^d$
Propanal	nd	nd	nd	nd	$(2.86 \pm 0.29)^a$
Pentanal	nd	$(0.02 \pm 0.01)^a$	nd	nd	nd
Hexanal	nd	$(0.01 \pm 0.01)^a$	nd	$(0.01 \pm 0.01)^a$	$(0.02 \pm 0.01)^a$
Heptanal	$(0.01 \pm 0.01)^a$	$(0.01 \pm 0.01)^a$	$(0.01 \pm 0.01)^a$	$(0.01 \pm 0.01)^a$	$(0.01 \pm 0.01)^a$
Octanal	nd	$(0.01 \pm 0.01)^a$	nd	$(0.02 \pm 0.01)^a$	$0.01 \pm 0.01)^a$
Benzaldehyde	nd	$(0.01 \pm 0.01)^a$	nd	nd	$(0.01 \pm 0.01)^a$
2-Phenylacetaldehyde	$(0.11 \pm 0.01)^a$	$(0.08 \pm 0.01)^a$	$(0.07 \pm 0.01)^a$	$(0.08 \pm 0.01)^a$	$(0.09 \pm 0.01)^a$
In total: aldehydes	(83.01 ± 4.18)	86.25 ± 8.15	34.03 ± 8.32	21.13 ± 2.50	9.42 ± 1.36
Propan-2-one	$(14.23 \pm 1.40)^a$	$(10.61 \pm 1.03)^b$	nd	$(4.39 \pm 1.07)^{c,d}$	$(6.69 \pm 0.31)^d$
Butan-2-one	$(0.10 \pm 0.01)^a$	$(0.07 \pm 0.01)^a$	$(0.16 \pm 0.01)^b$	$(0.06 \pm 0.01)^{a,b}$	$(0.12 \pm 0.01)^{a,b}$
Heptan-2-one	nd	nd	nd	nd	$(0.01 \pm 0.01)^a$
Undecan-2-one	$(0.05 \pm 0.01)^{a,c}$	$(0.09 \pm 0.01)^a$	$(0.07 \pm 0.01)^{b,c}$	$(0.08 \pm 0.01)^{a,b}$	$(0.08 \pm 0.01)^{a,b}$
4-Methylpentan-2-one	$(0.11 \pm 0.02)^a$	nd	$(0.17 \pm 0.01)^a$	$(0.11 \pm 0.02)^a$	$(0.21 \pm 0.02)^a$
3-Hydroxybutanone	$(0.14 \pm 0.01)^a$	$(0.20 \pm 0.03)^a$	$(0.25 \pm 0.01)^a$	$(0.24 \pm 0.01)^a$	$(0.18 \pm 0.01)^a$
In total: ketones	14.63 ± 1.40	10.97 ± 1.03	0.65 ± 0.02	4.88 ± 1.07	7.29 ± 0.31
Acetic acid	$(98.96 \pm 8.78)^a$	$(146.11 \pm 2.43)^b$	$(151.68 \pm 2.75)^{b,d}$	$(146.98 \pm 5.21)^b$	$(161.93 \pm 6.74)^{c,d}$
Butanoic acid	$(1.24 \pm 0.28)^a$	nd	nd	nd	$(2.22 \pm 0.21)^b$
In total: fatty acids	100.20 ± 8.78	146.11 ± 2.43	151.68 ± 2.75	146.98 ± 5.21	164.15 ± 6.74
Ethyl butanoate	nd	$(0.01 \pm 0.01)^a$	nd	$(0.14 \pm 0.01)^b$	$(0.21 \pm 0.04)^c$
Ethyl decanoate	nd	$(0.01 \pm 0.01)^a$	$(0.01 \pm 0.01)^a$	$(0.01 \pm 0.01)^a$	nd
Butyl acetate	nd	nd	nd	nd	$(0.01 \pm 0.01)^a$
In total: esters	nd	0.02 ± 0.01	0.01 ± 0.01	0.15 ± 0.01	0.22 ± 0.05
In total	476.00 ± 15.58	468.41 ± 8.63	547.30 ± 9.82	372.01 ± 16.16	425.22 ± 18.90

The results are expressed as mean ± SD ($n = 9$). Different letters in the same row indicate significant statistical differences ($p < 0.05$). nd – not detected.

acetic acid ((3.70 ± 0.09) mg kg^{-1}), and butanoic acid ((2.92 ± 0.12) mg kg^{-1}) in butter. Sweet cream butter, derived from unfermented cream, was used in this work, so the aroma compounds identified are in accordance with the findings of other authors (Peterson & Reineccius, 2003; Mallia et al., 2008). Ethanol ((258.47 ± 12.08) mg kg^{-1}), butan-2-ol ((16.72 ± 0.36) mg kg^{-1}), pentan-1-ol ((2.31 ± 0.11) mg kg^{-1}), acetaldehyde ((82.89 ± 4.18) mg kg^{-1}), propan-2-one ((14.23 ± 1.40) mg kg^{-1}), acetic acid ((98.96 ± 8.78) mg kg^{-1}), and butanoic acid ((1.24 ± 0.28) mg kg^{-1}) were quantified in butter analogue. The total content of aroma compounds was (45.19 ± 1.43) mg kg^{-1} and (476.00 ± 15.58) mg kg^{-1} in butter and butter analogue, respectively.

In total, 18 and 22 aroma compounds were identified in butter oil and butter oil analogue, respectively. Quantitatively, the most important were: ethanol ((22.09 ± 1.79) mg kg^{-1}), pentan-1-ol ((1.66 ± 0.01) mg kg^{-1}), butan-2,3-dione ((1.22 ± 0.03) mg kg^{-1}), acetic acid ((21.22 ± 0.96) mg kg^{-1}), propanoic acid ((1.74 ± 0.03) mg kg^{-1}), and butanoic acid ((1.14 ± 0.03) mg kg^{-1}) in butter oil, which is in accordance with the observations by Mallia et al. (2008). Ethanol ((221.12 ± 0.20) mg kg^{-1}), butan-2-ol ((1.98 ± 0.03) mg kg^{-1}), pentan-1-ol ((1.49 ± 1.06) mg kg^{-1}), acetaldehyde ((86.11 ± 8.15) mg kg^{-1}), propan-2-one ((10.61 ± 1.03) mg kg^{-1}), and acetic acid ((146.11 ± 2.43) mg kg^{-1}) were quantified in butter oil analogue. The total content of aroma compounds was (47.66 ± 2.03) mg kg^{-1} and (468.41 ± 8.63) mg kg^{-1} in butter oil and butter oil analogue, respectively.

In total, 13 and 16 aroma compounds were identified in coconut oil and coconut oil analogue, respectively. Quantitatively, the most important were ethanol ((35.97 ± 0.92) mg kg^{-1}) and acetic acid ((10.73 ± 0.91) mg kg^{-1}) in coconut oil and ethanol ((227.42 ± 3.77) mg kg^{-1}), butan-2-ol ((131.08 ± 2.34) mg kg^{-1}), pentan-1-ol ((1.74 ± 0.08) mg kg^{-1}), acetaldehyde ((33.95 ± 8.32) mg kg^{-1}), and acetic acid ((151.68 ± 2.75) mg kg^{-1}) in coconut oil ana-

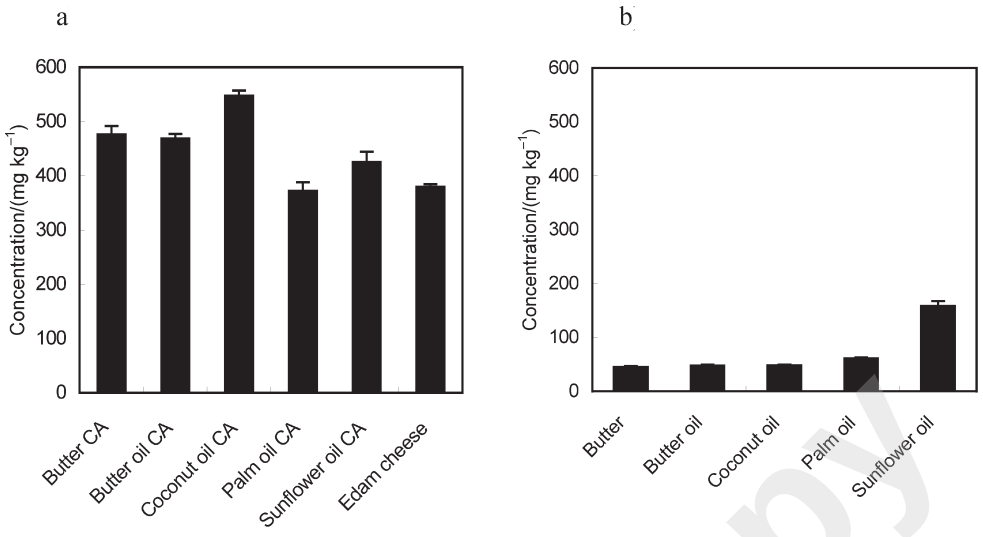

Fig. 1. Comparison of total content of aroma compounds in fats (a) and analogues (b). CA – cheese analogue.

logue. The total content of aroma compounds was (48.31 ± 1.29) mg kg^{-1} and (547.30 ± 9.82) mg kg^{-1} in coconut oil and coconut oil analogue, respectively.

In total, 19 and 18 aroma compounds were identified in palm oil and palm oil analogue, respectively. Quantitatively, the most important were ethanol ((28.78 ± 1.61) mg kg^{-1}), pentan-1-ol ((1.24 ± 0.04) mg kg^{-1}), 2-methylbutan-1-ol ((3.38 ± 0.07) mg kg^{-1}), acetic acid ((13.99 ± 0.78) mg kg^{-1}), and propanoic acid ((11.52 ± 0.18) mg kg^{-1}) in palm oil and ethanol ((198.49 ± 15.05) mg kg^{-1}), acetaldehyde ((21.01 ± 2.50) mg kg^{-1}), propan-2-one ((4.39 ± 1.07) mg kg^{-1}), and acetic acid ((146.98 ± 5.21) mg kg^{-1}) in palm oil analogue. The total content of aroma compounds was (61.11 ± 1.80) mg kg^{-1} and (372.01 ± 16.16) mg kg^{-1} in palm oil and palm oil analogue, respectively.

In total, 19 and 24 aroma compounds were identified in sunflower oil and sunflower oil analogue, respectively. Quantitatively, the most important were ethanol ((56.17 ± 4.85) mg kg^{-1}), propan-2-ol ((75.63 ± 7.09) mg kg^{-1}), acetic acid ((23.08 ± 1.33) mg kg^{-1}), and propanoic acid ((2.17 ± 0.08) mg kg^{-1}) in sunflower oil and ethanol ((240.58 ± 17.58) mg kg^{-1}), pentan-1-ol ((2.57 ± 0.81) mg kg^{-1}), acetaldehyde ((6.42 ± 1.33) mg kg^{-1}), propanal ((2.86 ± 0.29) mg kg^{-1}), propan-2-one ((6.69 ± 0.31) mg kg^{-1}), acetic acid ((161.93 ± 6.74) mg kg^{-1}), and butanoic acid ((2.22 ± 0.21) mg kg^{-1}) in sunflower oil analogue. The total content of aroma compounds was (158.66 ± 8.69) mg kg^{-1} and (425.22 ± 18.90) mg kg^{-1} in sunflower oil and sunflower oil analogue, respectively. Concentrations of other individual compounds identified in samples did not exceed 1 mg kg^{-1}.

Comparison of aroma profiles of samples

The contents of volatile aroma compounds in different types of fats and corresponding analogues are compared in Fig. 1 which expresses: i) the differences between the aroma profiles of various fats; ii) the differences between the aroma profiles of various analogues; iii) the differences between aroma profiles of fats and the corresponding analogues; iv) the probable contribution of Edam cheese to the aroma profile of analogues.

The highest content of aroma compounds ($p < 0.05$) was determined in sunflower oil ((158.66 ± 8.69) mg kg^{-1}). A significantly ($p < 0.05$) high concentration of ethanol ((56.17 ± 4.85) mg kg^{-1}) and propan-2-ol ((75.63 ± 7.09) mg kg^{-1}) contributed to this. Significantly ($p < 0.05$) high concentrations of acetaldehyde ((5.48 ± 0.13) mg kg^{-1}), propan-2-one ((31.23 ± 0.28) mg kg^{-1}), and butan-2,3-dione ((6.61 ± 0.09) mg kg^{-1}) were found in butter. These compounds are regarded as important components of butter aroma (Peterson & Reineccius, 2003; Mallia et al., 2008). The highest total content of aroma compounds in analogues was found in coconut oil analogue ((547.30 ± 9.82) mg kg^{-1}); significantly ($p < 0.05$) high concentrations of ethanol ((227.42 ± 3.77) mg kg^{-1}), butan-2-ol ((131.08 ± 2.34) mg kg^{-1}), and acetic acid ((151.68 ± 2.75) mg kg^{-1}) contribute to this fact. The lowest content of aroma compounds was in palm oil analogue ((372.01 ± 16.16) mg kg^{-1}).

Comparing the content of aroma compounds in fats with the corresponding analogues (Fig. 1), the analogues contain substantially higher concentrations of aroma compounds ($p < 0.05$). Villarino et al. (2007) found no perceptible aroma in coconut oil, so we assumed a generally rather low content of aroma compounds in fats. Taking into consideration the composition of the raw materials, we can conclude that the majority of aroma compounds identified in analogues could originate from Edam cheese, which contained

(379.62 ± 5.01) mg kg^{-1} of aroma compounds in total. In particular, alcohols and aldehydes were present in substantially ($p < 0.05$) low concentrations in fats compared with analogues. The significantly ($p < 0.05$) high concentrations of ethanol ((195.86 ± 2.66) mg kg^{-1}), acetaldehyde ((61.13 ± 4.18) mg kg^{-1}), and acetic acid ((41.30 ± 0.61) mg kg^{-1}) found in Edam cheese are probably transferred to analogues.

However, the content of fatty acids in analogues was significantly ($p < 0.05$) higher than in fats as well as in Edam, so some lipolysis can occur in the production of analogues as a consequence of heating. Liberated fatty acids are then the precursors of other compounds, alcohols and aldehydes among others (Liu et al., 2004; Alewijn et al., 2007).

The relatively high concentrations of ketones (especially propan-2-one) present in Edam cheese are absent in analogues, so they are probably decomposed or reduced to alcohols during heating (McSweeney & Sousa, 2000; McSweeney, 2004). The contribution of esters to the aroma of analogues appears to be negligible.

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Influence of volatile compounds on flavour of selected cultivars of gooseberry

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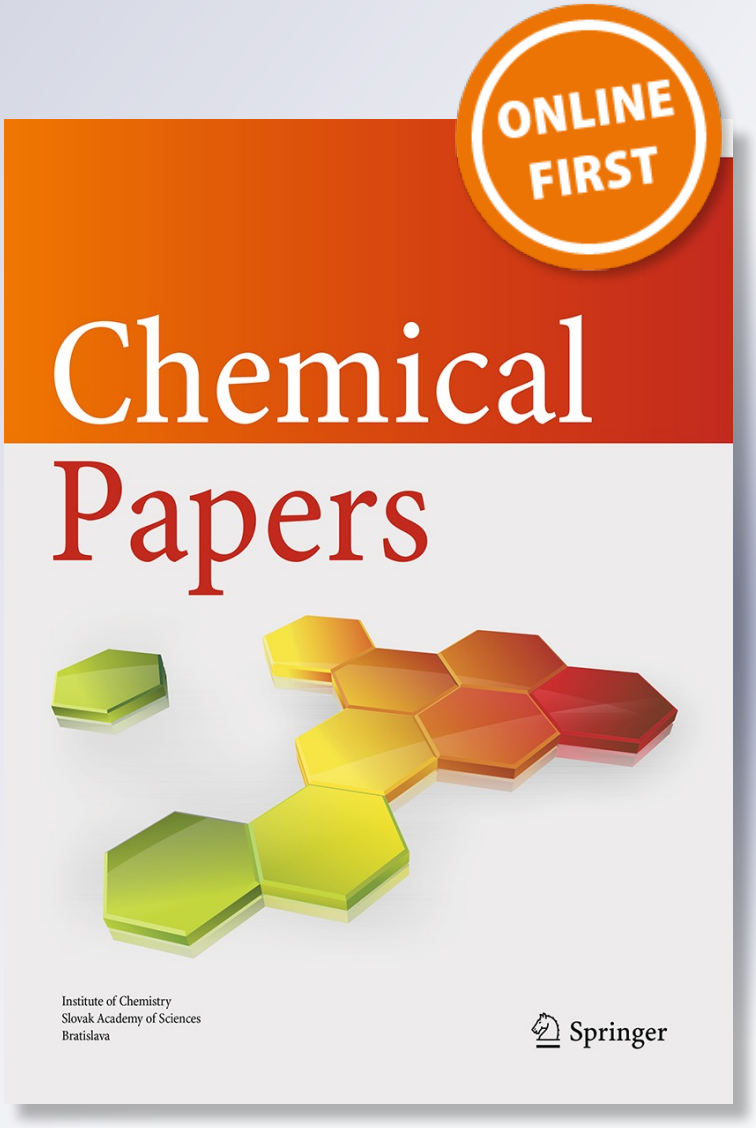

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ORIGINAL PAPER

Influence of volatile compounds on flavour of selected cultivars of gooseberry

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Abstract The flavour of seventeen red, yellow and green varieties of gooseberry (*Ribes grossularia* L.) was investigated in this study during two consequent years (2014–2015). Taste, odour, flavour descriptors (sweet, acid/sour, astringent) and off-flavour, together with appearance, colour, texture (firmness, crispiness) and overall acceptability were evaluated sensorially using line scale. Related volatile compounds were assessed by solid-phase microextraction coupled to gas chromatography–mass spectrometry. The significant differences ($p < 0.05$) in volatile compounds as well as in sensory properties were found between varieties. The differences between production years were small or not significant. Sensorially no obvious preference was found between red, yellow and/or green varieties. Red 'Karat' and yellow 'Darek' were considered to be the most acceptable with well evaluated all sensory properties. In total, 52 volatile compounds were identified in samples: 19 alcohols, 12 aldehydes, 8 ketones, 11 esters and 2 acids with quantitatively predominating alcohols and acids. (*Z*)-3-hexen-1-ol, (*Z*)-3-hexenal, heptan-2-one, methyl butanoate, ethyl butanoate, methyl acetate, ethyl acetate, ethanol and ethanal (with odour activity values >1) are considered to contribute to flavour/acceptability of gooseberry samples.

Keywords Gooseberry · Flavour · Volatiles · Sensory analysis · Solid-phase microextraction · Gas chromatography

Introduction

Gooseberries (*Ribes grossularia* L.) are berry-bearing deciduous shrubs, belonging to the genus *Ribes* L. Fruits are round, oval or pear shaped berries, with smooth or hairy skin, small to large sized (max about 2 cm) (Girard and Sinha 2006). Colour varies widely, fruits may be green, white, yellow, or shades of red from pink to purple to almost black. Their flavour is characteristic, mildly astringent, sweet and/or acidic (Harb and Streif 2004); size and shape, colour, firmness, taste and aroma of fruits depend mainly on variety and degree of maturity. Gooseberry fruits are rich in fibre, vitamins (C, E, B complex), minerals and many other nutritious components (flavonoids, phenolic acids, anthocyanins and tannins) (Heiberg and Maage 2003), albeit scarce information is available about compositional data on gooseberries (Maage 2002). Most of studies published deals with the measuring of phenolic compounds and antioxidant activity, e.g., Filipiak-Szok et al. (2012) and Chiang et al. (2013).

Although gooseberries are still considered a minor berry fruit, there is increasing interest of growers, processors and consumers, owing to their natural antioxidant activity (Kaplanova et al. 2016). Both immature (for preservation) and ripe (for direct consumption) gooseberries are practically used. Green gooseberries are firm and tart; they are used for production of wide range of various processed products, such as compotes, jams, juices, wines, liqueurs and/or vinegar; when fully mature, they are soft and several cultivars quite sweet (Harb and Streif 2004; Girard and

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Sinha 2006). So the practical use depends significantly on sensory quality. Ideally, fruit for direct consumption should be firm, bright, large, with the proper cultivar-specific colour, and free of decay, mechanical or insect injury. A long shelf-life with retention of both firmness and flavour is also desirable for the fruit market (Harb and Streif 2004; Girard and Sinha 2006).

This work is focused on aroma compounds, i.e., volatile compounds contributing to taste and aroma (flavour); most of them arise during fruit ripening (Harb and Streif 2004). The composition of aroma compounds of gooseberries and their contribution to aroma have not been comprehensively described so far; only Hempfling et al. (2013) and Nikfardjam et al. (2013) published recently results of volatile compounds in several gooseberry varieties, where they identified 122 and 27 volatiles, respectively. (*Z*)-3-hexenal, (*E*)-2-hexenal and methyl butanoate quantitatively predominated; other compounds occurred in relatively low concentrations. The above-mentioned aldehydes and esters, especially butanoates with predominating methyl esters, were considered to be characteristic for volatile profile of gooseberry (Hempfling et al. 2013), Nikfardjam et al. (2013) also identified (*Z*)-3-hexenal and ethyl acetate as responsible for gooseberry aroma. Harb and Streif (2004) evaluated sensory quality and acceptability of gooseberries depending on storage conditions. Firmness, sweetness/acidity balance and possible off-flavour were identified as the determining criteria of sensory quality of gooseberries.

Characterization of aroma profile of a fruits is now of great importance, since it enables to optimize and/or improve the quality of products. The objectives of the present study were (1) to identify and quantify volatile constituents in several varieties of gooseberry, (2) to evaluate flavour using sensory analysis, (3) to demonstrate the differences among samples, and (4) to investigate the contributions of compounds to the sensory quality and overall acceptability of samples. Volatile compounds were extracted by solid-phase microextraction (SPME), identified by gas chromatography–mass spectrometry (GC–MS) and quantified using gas chromatography with flame ionization detector (GC-FID). The descriptive sensory profiling was used for sensory analyses.

Experimental

Chemicals

All chemicals used as reference standards (listed in chapter Results and discussion) were of analytical grade purity; pentanal, hexanal, heptanal, (*Z*)-2-octenal, nonan-2-one, undecan-2-one, phenylacetaldehyde, benzaldehyde, 3-methylbutan-1-ol, (*Z*)-3-hexen-1-ol, and 1-octen-3-ol (Sigma-Aldrich, St. Louis, USA), and the remaining compounds were from Merck (Darmstadt, Germany).

Gooseberry samples

In total, 17 gooseberry varieties were analysed; 8 red-fruiting: 'Alan' (Al), 'Hinnonmaki Rot' (HR), 'Karat' (Kar), 'Karmen' (Ka), 'Krasnoslawjanskij' (Kr), 'Remarka' (Re), 'Rolonda' (Rol), 'Tamara' (Ta); 6 yellow-fruiting: 'Citronovy obři' (CO), 'Darek' (Da), 'Invicta' (In), 'Rodnik' (Rod), 'Zlaty fik' (ZF), 'Zebin' (Ze); 3 green-fruiting: 'Mucurines' (Mu), 'Prima' (Pr), 'Rixanta' (Rix). The varieties were grown in Research and Breeding Institute of Pomology Ltd. (Holovousy, Czech Republic).

The varieties were grown in the experimental orchard of the Research and Breeding Institute of Pomology Ltd., Holovousy. It has clay soil; the exact location of the orchard is: latitude 50°22′29″, longitude 15°34′38″, altitude 320 m. The mean temperature and precipitation in this area were 11.41 °C and 607 mm for 2014, while 11.28 °C and 569 mm for 2015, respectively.

The berries were handpicked in their full ripeness (evaluated based on colour and firm texture), during the seasons 2014–2015, immediately stored in the refrigerator at 5 °C and sensorially evaluated fresh within 2 days; all chemical analyses were performed within 7 days.

SPME-GC-FID/MS conditions

For analysis, 1 g of manually homogenized berries was placed into vial for SPME extraction; three samples of every cultivar were taken; every sample was analysed three times (number of repetitions, $n = 9$).

SPME extractions were carried out using Carboxen/Poly(dimethylsiloxane) (CAR/PDMS) fibre 85 μm (Supelco, Bellefonte, Pensylvania, USA) under the following conditions: extraction temperature 35 °C; equilibrium time 30 min; extraction time 20 min; desorption temperature 250 °C; desorption time 10 min.

Gas chromatograph TRACE GC (ThermoQuest, Milan, Italy) with capillary column DB-WAX (30 m × 0.32 mm × 0.5 μm; J. & W. Scientific, Santa Clara, California, USA) was used for GC-FID analyses under the following conditions: injector temperature 250 °C; split-less desorption 5 min; carrier gas N_2, flow rate 0.9 mL min^{-1}; flame ionization detector, temperature 220 °C; H_2 inlet 35 mL min^{-1}; air inlet 350 mL min^{-1}; make up N_2 30 mL min^{-1}. The oven ramp temperature was 40 °C for 1 min, then it was increased up to 200 °C at a rate of 5 °C min^{-1} and maintained at 200 °C for 7 min.

GC–MS analyses were performed on a gas chromatograph HP 6890 with an MS detector 5973 N and the Mass Spectral Library NIST 98 (Agilent, Santa Clara,

California, USA); capillary column ZB-5Sil MS (30 m × 0.25 mm × 0.25 μm; Phenomenex, Torrance, California, USA) was used with carrier gas He 0.9 mL min^{-1} and the oven temperature 50–250 °C at 3 °C min^{-1}. Other GC conditions were the same as described above. MS was operated in electron ionization (EI) mode at 70 eV with a scan range of *m/z* from 30 to 370.

The standard addition method was used for quantification of analytes to control the influence of the sample matrix. The standards were divided into groups consisting of five chemicals; these standard mixtures were gradually added directly into the sample and analysed in the same manner as the samples. Five content levels, in the range of 0.001–70 mg kg^{-1} (different for various standards, according to their content in the samples), for ethanol in the range of 0.01–220 mg kg^{-1} (due to its high content in the samples), were used to establish the calibration curves. Validation and the validation parameters of the used method were identical as previously described by Vitova et al. (2013, 2015). The repeatability was verified by repeated extractions ($n = 5$) of the above-mentioned standard mixtures (relative standard deviations <10%), detection and quantification limits were in the range of 0.001–0.50 mg kg^{-1}. Linearity was tested within the range of 0–220 mg kg^{-1}; correlation coefficients were all above 0.99.

Sensory analyses

The test panel consisted of 22 persons in both years (16 women and 6 men), selected from students and staff of the Department of Food Chemistry and Biotechnology, who were trained (including sensory profiling) for 3 months.

About 20 g of the samples was served in 50 ml glass covered containers, marked with 4-digit codes, in random order. Fresh water was provided to rinse mouth between samples.

The sensory attributes were evaluated using unstructured 100 mm line scale (0–100%), anchored from each end to identify the direction. The list of attributes comprised appearance, taste, odour and texture (ranging from unacceptable to excellent), colour (from atypical to typical, characteristic for red/yellow/green variety), three flavour characteristics (sweet, acid/sour, astringent, from weak to very strong), off-flavour (from imperceptible to very strong), two mouthfeel attributes encompassing firmness (from soft to firm) and crispiness (not crispy to very crispy), and overall acceptability (from unacceptable to delicious). These descriptors were determined in preliminary evaluations by panel of 3 experts (ISO 13299:2016), inspired by Harb and Streif (2004). Assessors were also asked to add comments for description of possible off-flavour.

Statistical evaluation

The results of instrumental analyses were treated using parametric one-way analysis of variance (ANOVA) followed by Duncan's test; they are expressed as mean ± standard deviation ($n = 9$). The results of sensory analyses were statistically evaluated by means of Kruskal–Wallis test followed by Nemenyi multiple comparison test; they are expressed as mean ± standard deviation (number of assessors $n = 22$).

Due to high number of experimental characteristics, the entire experimental dataset was processed by principal component analysis (PCA) to confirm differences among samples. A probability value of $p \leq 0.05$ was accepted for statistically significantly different results. All analyses were performed using Microsoft Excel 2010 (Microsoft, Redmond, Washington, USA) and Statistica 12 (StatSoft, Tulsa, Oklahoma, USA).

Results and discussion

SPME-GC-FID/MS assessment of volatile compounds

It is generally known that the content of volatiles and their contribution to flavour is an important characteristic of fruits (Girard and Sinha 2006). However, only two above, mentioned works (Hempfling et al. 2013; Nikfardjam et al. 2013) deal with the problematic of the volatiles in gooseberries. The main intention of this work was to identify and quantify volatiles in selected red/yellow/green gooseberry varieties grown in two consequent years (2014–2015), to compare their volatile profiles as well as the sensory characteristics and try to investigate which compounds could influence flavour. Simple and fast SPME as alternative to other long-lasting and/or expensive extraction methods was applied for assessment of volatile compounds; it has been previously successfully used by many authors to measure the volatiles of various foods (e.g., Serrano et al. 2009; Antalick et al. 2010; Panighel and Flamini 2014). Its limitations in quantification ability were mastered by in-depth quantifying process and keeping constant as many experimental conditions as possible.

In total, 52 volatile compounds were identified and quantified in gooseberry samples in this study; among them 19 alcohols: benzylalcohol, ethanol, propan-1-ol, propan-2-ol, butan-1-ol, butan-2-ol, pentan-1-ol, pentan-2-ol, hexan-1-ol, heptan-1-ol, heptan-2-ol, octan-1-ol, octan-2-ol, nonan-2-ol, decan-1-ol, (*Z*)-3-hexen-1-ol, 2-methylpropan-1-ol, 3-methylbutan-1-ol, 1-octen-3-ol; 12 aldehydes: phenylacetaldehyde, benzaldehyde, ethanal, propanal, pentanal, hexanal, heptanal, octanal, nonanal, (*E*)-2-

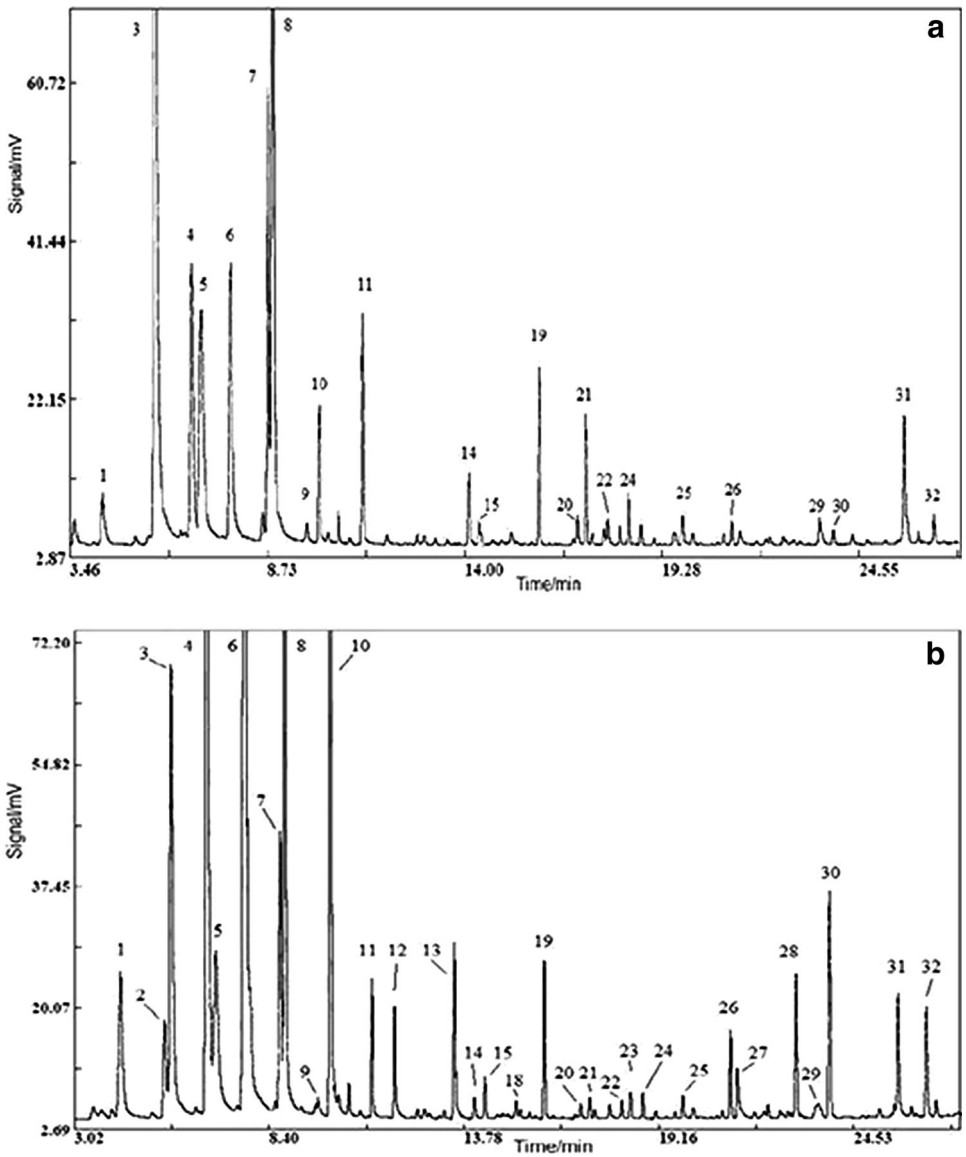

Fig. 1 Chromatograms of volatile compounds identified in two selected cultivars; **a** 'Karat'—red-fruiting; **b** 'Darek'—yellow-fruiting. Peak numbering: *1* ethanal, *2* propan-2-one, *3* methyl acetate, *4* ethyl acetate, *5* butan-2-one, *6* ethanol, *7* pentanal, *8* ethyl butanoate, *9* butan-2-ol, *10* propan-1-ol, *11* hexanal, *12* 2-methylpropan-1-ol, *13* butan-1-ol, *14* heptan-2-one, *15* heptanal, *16* 3-methylbutan-1-ol, *17* (Z)-2-hexenal, *18* ethyl hexanoate, *19* pentan-1-ol, *20* octanal, *21* 3-hydroxybutan-2-one, *22* heptan-2-ol, *23* ethyl heptanoate, *24* hexan-1-ol, *25* nonanal, *26* 1-octen-3-ol, *27* acetic acid, *28* nonan-2-ol, *29* benzaldehyde, *30* octan-1-ol, *31* ethyl decanoate, *32* 3-methylbutanoic acid

hexenal, (*Z*)-3-hexenal, (*Z*)-2-octenal; 8 ketones: propan-2-one, butan-2-one, heptan-2-one, nonan-2-one, decan-2-one, undecan-2-one, 3-hydroxybutan-2-one, tridecan-2-one; 11 esters: methyl acetate, methyl butanoate, ethyl acetate, propyl acetate, butyl acetate, ethyl propanoate, ethyl butanoate, ethyl pentanoate, ethyl hexanoate, ethyl heptanoate, ethyl decanoate; 2 acids: acetic and 3-methylbutanoic. Example of chromatograms of compounds identified in selected gooseberry varieties (red 'Karat' and yellow 'Darek' as sensorially the most acceptable, harvested in 2014) is given in Fig. 1.

Alcohols ethanol (9.0–228.6 mg kg^{-1}), butan-2-ol (1.2–4.7 mg kg^{-1}), 2-methylpropan-1-ol (0.01–3.1 mg kg^{-1}), 3-methylbutan-1-ol (0.5–7.0 mg kg^{-1}), octan-1-ol (0.01–7.1 mg kg^{-1}), acetic (0.01–33.4 mg kg^{-1}) and 3-methylbutanoic (3.1–95.4 mg kg^{-1}) acids, in other chemical groups aldehydes ethanal (1.1–6.4 mg kg^{-1}), (*E*)-2-hexenal (0.5–3.1 mg kg^{-1}), (*Z*)-2-octenal (1.3–1.9 mg kg^{-1}), ketones butan-2-one (0.3–2.3 mg kg^{-1}), esters ethyl acetate (0.04–7.9 mg kg^{-1}) and methyl acetate (0.05-14.4 mg kg^{-1}) were present in high concentrations >2 mg kg^{-1}. The content of other compounds identified did not exceed 1 mg kg^{-1}.

Comparison of volatiles in red, yellow and green fruiting varieties

To investigate the variability of volatile compounds in samples, two picking years for each cultivar were compared; then, the single cultivars were mutually compared, separately in 2014 and 2015. Similar to Hempfling et al.

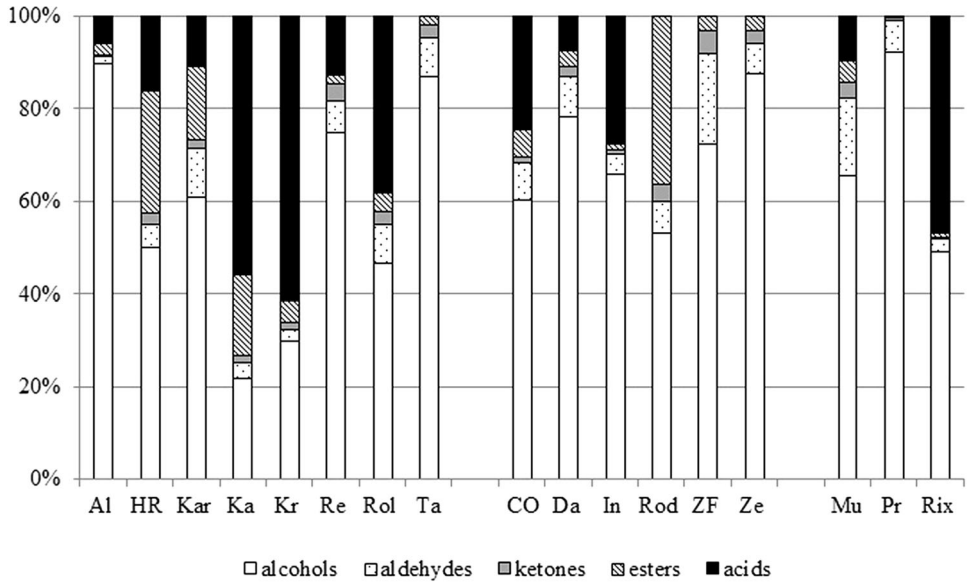

Fig. 2 Distribution of chemical groups of volatile compounds in gooseberry cultivars harvested in 2014. For sample labelling, see chapter Gooseberry samples

(2013) and Nikfardjam et al. (2013), significant differences ($p < 0.05$) were found between samples in the total content of compounds identified, as well as in the single chemical groups of compounds. The total content of compounds ranged from 21.9 mg kg^{-1} ('Remarka') to 263.4 mg kg^{-1} ('Alan') in red-fruiting, from 17.4 mg kg^{-1} ('Zlaty fik') to 139.2 mg kg^{-1} ('Invicta') in yellow-fruiting, and from 37.9 mg kg^{-1} ('Mucurines') to 202.9 mg kg^{-1} ('Rixanta') in green-fruiting varieties.

In contrast to Hempfling et al. (2013) and Nikfardjam et al. (2013), who found aldehydes and esters as dominating, alcohols and acids were quantitatively the most important in this study. Alcohols created 22–90% (w/w), 52–88% (w/w) and 49–93% (w/w), acids 1–62% (w/w), 1–28% (w/w) and 2–46% (w/w) in red/yellow/green fruiting varieties, respectively. Esters (1–36% w/w), aldehydes (1–18% w/w) and ketones (1–5% w/w) were mostly present at low quantity.

For illustrative purposes, the comparison of total content of single chemical groups of compounds identified in varieties from 2014 is shown in Fig. 2. As can be seen, content of alcohols predominate in most cultivars, and the content of ketones is very low in all varieties. The content of other groups is very variable. Generally, red-fruiting varieties had higher content of acids and esters, whilst yellow and green ones of aldehydes. Overall the composition of varieties was similar in both years, as confirmed by PCA analysis (see Fig. 3).

If we mutually compare single varieties within red-fruiting, 'Alan' contained significantly ($p < 0.05$) the highest total content of compounds owing to the especially high content of alcohols, namely ethanol, butan-2-ol and octan-1-ol. Conversely, 'Remarka' and 'Tamara' had the

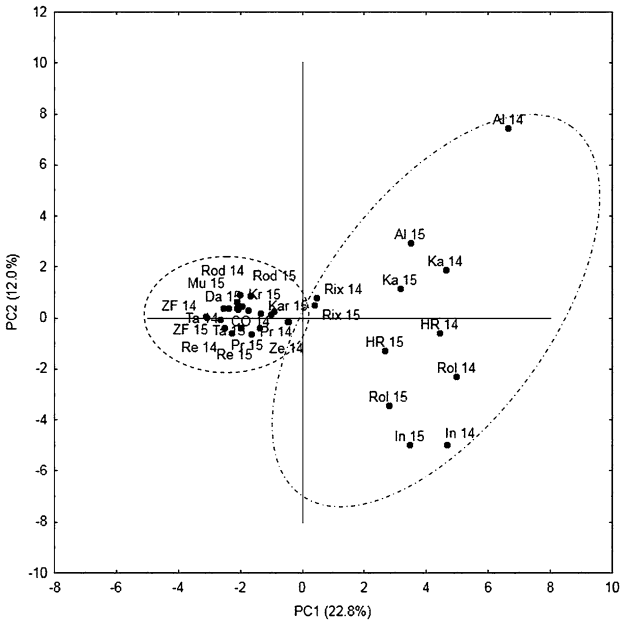

Fig. 3 PCA score plot of 17 gooseberry varieties harvested in 2014–2015; for sample labelling, see chapter Gooseberry samples

lowest, mainly because of low content of acids. In the case of yellow fruiting, 'Invicta' had the highest total content, caused by the high content of alcohols (ethanol, butan-2-ol, 2-methylpropan-1-ol and 3-methylbutan-1-ol) and acids (3-methylbutanoic); on the other hand, 'Rodnik' and 'Zlaty fik' had the lowest, which was caused by low content of alcohols and nearly absence of acids. Interestingly, 'Rodnik' contained quite high content of esters, comparable to red varieties. In the case of green fruiting, 'Rixanta' had the highest total content owing to the especially high content of acids (3-methylbutanoic); conversely, 'Mucurines' has

very low quantity of alcohols causing the lowest total content of compounds. The observed differences probably followed from many factors. According to the Girard and Sinha (2006), the content of fruit constituents could be influenced not only by cultivar, but also by environmental conditions as, e.g., climate, habitat, diseases and pest exposure. Provided that the storage and processing of samples were identical, in our case the differences between years of production (2014 vs. 2015) could be probably caused by the different climatic conditions in these years (see chapter Gooseberry samples).

To determine the statistically significant markers for characterization and differentiation of samples (red vs. yellow vs. green cultivars, and 2014 vs. 2015), PCA was performed using average concentrations of all volatile compounds identified in all 17 gooseberry varieties, representing the data matrix 34 × 52 (for 34 samples and 52 variables-compounds). The cumulative contribution of variance of the first four PCs was 54.3%. The first two components explain 34.8% of total variability, where PC1 (22.8%) rather explains the variance between cultivars (red/yellow/green varieties), whereas the PC2 (12.0%) explains the variability within cultivars during 2 years.

Although significant differences ($p < 0.05$) in contents of volatile compounds were found between cultivars, the differentiation of samples is ambiguous and unsatisfactory. As follows from score plot (Fig. 3), there is apparent cluster of samples laying very close together in the left part of the plot, correlating negatively with PC1. This cluster includes most of yellow- and green-fruiting varieties, which thus were judged to be similar in contents of compounds identified. Only yellow-fruiting 'Invicta' is placed separately (both years) in right lower part, closer to red varieties and being especially rich in ethanol (104.4 and 74.6 mg kg^{-1}), 2-methylpropan-1-ol (2.9 and 1.6 mg kg^{-1}), 3-methylbutan-1-ol (6.9 and 7.0 mg kg^{-1}) and 3-methylbutanoic acid (16.5 and 37.3 mg kg^{-1}). Values in parenthesis are mean content in 2014 and 2015, respectively. With regard to the red-fruiting varieties, four of them ('Alan', 'Hinnonmaki Rot', 'Karmen' and 'Rolonda') are well separated; they lay in the right part of the graph correlating positively with PC1 and creating the second cluster. The others ('Karat', 'Krasnoslawjanskij', 'Remarka' and 'Tamara') were rather different and more similar to yellow/green varieties, being placed close, even mixed with them. It is probably caused by very low amount (<0.08 mg kg^{-1}) of benzylalcohol, (*E*)-2-hexenal, propanal and acetic acid; in contrast to other red varieties, octan-1-ol, propan-1-ol and 3-methylbutan-1-ol were not detected in these varieties. We can consider that there could be possible to distinguish red from yellow/green varieties based on composition of compounds identified. Green- and yellow-fruiting varieties are not easy distinguishable in such way. Most of the varieties placed in right part of the plot showed detectable off-flavour, as mentioned later. On the other hand, the small (less significant) differences between picking years (2014 vs. 2015) are visible in the plot (Fig. 3); both years lay close to each other in most cultivars. This fact is especially clear in yellow/green varieties; in the case of red ones significant differences ($p < 0.05$) were found.

If we consider three significant PCs, the PC1 was highly correlated with benzylalcohol (0.89), propyl acetate (0.89), benzaldehyde (0.79), hexan-1-ol (0.72), propanal (0.66), 3-hydroxybutan-2-one (0.62), ethyl acetate (0.61); the PC2 was correlated with ethyl acetate (0.68), 3-hydroxybutan-2-one (0.65), 3-methylbutan-1-ol (−0.70), pentan-1-ol (−0,70); PC3 with acetic acid (0.74), propan-1-ol (0.73) and methyl acetate (0.69). The content of these 12 compounds is probably the most variable in samples and they could be considered to be the most important for differentiation between samples. The comparison of these selected volatiles (using ANOVA) in all varieties is given in Table 1.

Alcohols hexan-1-ol and pentan-1-ol were identified nearly in all varieties, mostly in quite low quantities (<50 μg kg^{-1}). Benzylalcohol and propan-1-ol were detected only in red varieties, with the exception of yellow 'Invicta', where about 400 μg kg^{-1} of benzylalcohol was found in both years. The content of benzylalcohol ranged from 32.3 μg kg^{-1} in 'Karat' to 694.4 μg kg^{-1} in 'Rolonda'. Propan-1-ol was found in 'Alan', 'Remarka' and 'Tamara' at low concentrations about 5–8 μg kg^{-1} and 'Karmen' at >100 μg kg^{-1}. 3-methylbutan-1-ol was identified only in five varieties: 'Rolonda' and 'Invicta' (about 2–7 μg kg^{-1}), 'Hinnonmaki Rot', 'Citronovy obři' and 'Mucurines' (>480 μg kg^{-1}). With regard to the aldehydes, benzaldehyde was identified in most varieties in range 3–60 μg kg^{-1}; propanal was present only in several varieties; its content was very variable (about 2–700 μg kg^{-1}). Also, 3-hydroxybutan-2-one was present in most varieties (about 2–60 μg kg^{-1}), which was significantly higher in red 'Alan' (>100 μg kg^{-1}). Esters methyl and ethyl acetate were present in all varieties, and their amounts were variable (2–1700 μg kg^{-1}); conversely, propyl acetate was present only in several varieties at very low quantity (2–15 μg kg^{-1}). Acetic acid was detected only in several varieties; significantly highest amount was in red 'Rolonda and 'Karmen' (7–33 mg kg^{-1}), the others about 10–20 μg kg^{-1}.

Another PCA was performed with these 12 compounds (data matrix 34 × 12), which resulted in three significant PCs accounting for 45.2, 21.0 and 17.2% of variance, respectively, which express satisfactory 83.4% of total variability. PCA score plot for the first two components

Table 1 Comparison of selected volatile compounds identified in gooseberry varieties

(a)

Gooseberry varieties	Picking year	Volatile compounds content/μg kg^{-1}					
		Benzylalcohol	Propan-1-ol	Hexan-1-ol	3-methylbutan-1-ol	Pentan-1-ol	Benzaldehyde
Red-fruiting							
Alan	14	$387.3 \pm 15.4A^{a}$	$4.9 \pm 0.3A^{a}$	$66.2 \pm 2.7A^{a}$	nd^{a}	$1.3 \pm 0.1A^{a}$	$41.2 \pm 2.2A^{a}$
	15	$407.9 \pm 17.2A_{a}$	$5.5 \pm 0.2A_{a}$	$14.6 \pm 1.1B_{a}$	nd_{a}	$1.9 \pm 0.1A_{ad}$	$10.2 \pm 0.7B_{ad}$
Hinnonmaki Rot	14	$503.1 \pm 24.4A^{b}$	nd^{c}	$42.3 \pm 1.8A^{b}$	$806.3 \pm 18.9A^{b}$	$3.8 \pm 0.3A^{b}$	$61.3 \pm 4.3A^{b}$
	15	$543.3 \pm 27.3A_{b}$	nd_{c}	$1.2 \pm 0.1B_{b}$	$989.7 \pm 21.5A_{bd}$	$1.7 \pm 0.1B_{ad}$	$24.4 \pm 1.0B_{b}$
Karat	14	$54.5 \pm 2.7A^{c}$	nd^{c}	nd^{h}	nd^{a}	nd^{f}	$12.3 \pm 0.8A^{d}$
	15	$32.3 \pm 1.5B_{c}$	nd_{c}	nd_{f}	nd_{a}	nd_{e}	$8.9 \pm 0.5A_{af}$
Karmen	14	$515.6 \pm 23.7A^{b}$	$168.2 \pm 12.0A^{b}$	$66.4 \pm 2.8A^{a}$	nd^{a}	$1.6 \pm 0.1A^{c}$	$61.1 \pm 3.7A^{b}$
	15	$535.8 \pm 19.8A_{b}$	$112.2 \pm 9.3A_{b}$	$10.9 \pm 8.8B_{ad}$	nd_{a}	$1.4 \pm 0.1A_{a}$	$14.2 \pm 0.8B_{dh}$
Krasnoslawjanskij	14	$86.2 \pm 3.5A^{c}$	nd^{c}	$6.6 \pm 0.3A^{d}$	nd^{a}	nd^{f}	nd^{g}
	15	$43.5 \pm 1.6B_{c}$	nd_{c}	$9.0 \pm 0.7B_{d}$	nd_{a}	nd_{e}	nd_{i}
Remarka	14	$97.9 \pm 3.8A^{d}$	$4.9 \pm 0.2A^{a}$	$8.6 \pm 0.4A^{cd}$	nd^{a}	$1.5 \pm 0.1A^{ac}$	nd^{g}
	15	$83.4 \pm 2.4B_{c}$	$6.5 \pm 0.2A_{a}$	$8.1 \pm 0.2A_{d}$	nd_{a}	$1.4 \pm 0.1A_{a}$	nd_{i}
Rolonda	14	$624.6 \pm 45.4A^{e}$	nd^{c}	$57.1 \pm 2.6A^{ae}$	$1.9 \pm 0.1*A^{c}$	$48.1 \pm 2.9A^{d}$	$47.5 \pm 2.7A^{a}$
	15	$694.4 \pm 34.8A_{d}$	nd_{c}	$9.6 \pm 0.5B_{d}$	$2.4 \pm 0.2*B_{c}$	$34.2 \pm 1.1A_{b}$	$8.4 \pm 0.8B_{af}$
Tamara	14	nd^{f}	$7.4 \pm 0.7A^{a}$	$11.2 \pm 1.0A^{c}$	nd^{a}	$1.2 \pm 0.1A^{a}$	$3.1 \pm 0.2A^{f}$
	15	nd_{e}	$8.1 \pm 0.6A_{a}$	$11.8 \pm 0.7A_{ad}$	nd_{a}	$1.3 \pm 0.1A_{a}$	$4.5 \pm 0.2A_{g}$
Yellow-fruiting							
Citronovy obří	14	nd^{f}	nd^{c}	$11.0 \pm 0.8A^{c}$	$487.6 \pm 25.6A^{b}$	$1.3 \pm 0.1A^{a}$	$7.2 \pm 0.4A^{e}$
	15	nd_{e}	nd_{c}	$9.7 \pm 0.8A_{d}$	$593.5 \pm 23.0A_{d}$	$1.7 \pm 0.1A_{a}$	$8.7 \pm 0.6A_{af}$
Darek	14	nd^{f}	nd^{c}	nd^{h}	nd^{a}	nd^{f}	$9.2 \pm 0.4A^{de}$
	15	nd_{e}	nd_{c}	nd_{f}	nd_{a}	nd_{e}	$6.0 \pm 0.2B_{fg}$
Invicta	14	$408.2 \pm 23.4A^{a}$	nd^{c}	$53.5 \pm 3.4A^{e}$	$6.9 \pm 0.3*A^{d}$	$39.2 \pm 1.7A^{e}$	$21.9 \pm 2.8A^{c}$
	15	$418.0 \pm 30.2A_{a}$	nd_{c}	$13.9 \pm 0.9B_{a}$	$7.0 \pm 0.3*A_{e}$	$52.5 \pm 3.7B_{c}$	$6.7 \pm 0.4B_{f}$
Rodnik	14	nd^{f}	nd^{c}	$9.9 \pm 0.6A^{c}$	nd^{a}	nd^{f}	$4.3 \pm 0.3A^{f}$
	15	nd_{e}	nd_{c}	$8.6 \pm 0.4A_{d}$	nd_{a}	nd_{e}	$6.3 \pm 0.4A_{fg}$
Zlaty fik	14	nd^{f}	nd^{c}	nd^{h}	nd^{a}	$1.2 \pm 0.1A^{a}$	$6.9 \pm 0.4A^{ef}$
	15	nd_{e}	nd_{c}	nd_{f}	nd_{a}	$1.7 \pm 0.1A_{a}$	$17.2 \pm 0.9B_{h}$
Zebin	14	nd^{f}	nd^{c}	$13.0 \pm 0.8A^{cf}$	nd^{a}	$1.5 \pm 0.1A^{ac}$	$25.0 \pm 1.9A^{c}$
	15	nd_{e}	nd_{c}	$15.3 \pm 1.2A_{a}$	nd_{a}	$1.3 \pm 0.1A_{a}$	$19.6 \pm 1.2A_{bh}$
Green-fruiting							
Mucurines	14	nd^{f}	nd^{c}	$17.8 \pm 1.3A^{f}$	$1652.7 \pm 132.5A^{c}$	nd^{f}	$12.1 \pm 1.0A^{d}$
	15	nd_{e}	nd_{c}	$14.7 \pm 0.9A_{a}$	$1252.7 \pm 109.9B_{b}$	nd_{e}	$8.7 \pm 0.6B_{af}$
Prima	14	nd^{f}	nd^{c}	nd^{h}	nd^{a}	$1.8 \pm 0.1A^{c}$	$8.4 \pm 0.6A^{ef}$
	15	nd_{e}	nd_{c}	nd_{f}	nd_{a}	$2.4 \pm 0.2A_{d}$	$5.7 \pm 0.2A_{fg}$
Rixanta	14	nd^{f}	nd^{c}	$23.6 \pm 1.6A^{g}$	nd^{a}	$1.5 \pm 0.1A^{ac}$	$12.3 \pm 0.8A^{d}$
	15	nd_{e}	nd_{c}	$25.4 \pm 1.5A_{e}$	nd_{a}	$1.9 \pm 0.1A_{a}$	$22.9 \pm 0.9B_{b}$

(b)

Gooseberry varieties	Picking year	Volatile compounds content/μg kg^{-1}					
		Propanal	3-hydroxybutan-2-one	Methyl acetate	Ethyl acetate	Propyl acetate	Acetic acid
Red-fruiting							
Alan	14	nd^{e}	$197.1 \pm 9.4A^{a}$	$6.2 \pm 0.3*A^{ad}$	$7.9 \pm 0.5*A^{a}$	$14.8 \pm 1.2A^{a}$	nd^{e}
	15	nd_{f}	$99.4 \pm 3.1B_{a}$	$1.9 \pm 0.1*B_{a}$	$4.4 \pm 0.3*B_{a}$	$9.6 \pm 0.4B_{a}$	nd_{g}

Table 1 continued

(b)

Gooseberry varieties	Picking year	Volatile compounds content/μg kg^{-1}					
		Propanal	3-hydroxybutan-2-one	Methyl acetate	Ethyl acetate	Propyl acetate	Acetic acid
Hinnonmaki Rot	14	$396.6 \pm 19.8A^{a}$	$57.9 \pm 2.6A^{b}$	$10.4 \pm 0.6*A^{b}$	$2.0 \pm 0.2*A^{b}$	$6.9 \pm 0.2A^{b}$	$10.8 \pm 0.1A^{a}$
	15	$236.1 \pm 11.3B_{a}$	$16.4 \pm 1.0B_{b}$	$9.5 \pm 0.4*A_{b}$	$1.2 \pm 0.1*B_{b}$	$9.1 \pm 0.5A_{ab}$	$16.9 \pm 0.1A_{af}$
Karat	14	$1.8 \pm 0.1A^{b}$	$38.6 \pm 2.5A^{c}$	$5.1 \pm 0.2*A^{d}$	$1374.5 \pm 74.7A^{d}$	$1.2 \pm 0.1A^{c}$	nd^{e}
	15	$1.6 \pm 0.1A_{c}$	$18.6 \pm 1.3B_{b}$	$4.9 \pm 0.2*A_{d}$	$1797.1 \pm 103.4B_{d}$	$1.9 \pm 0.1A_{c}$	nd_{g}
Karmen	14	$396.8 \pm 28.6A^{a}$	$21.4 \pm 2.6A^{d}$	$11.5 \pm 0.6*A^{b}$	$1448.7 \pm 94.5A^{d}$	$8.8 \pm 0.4A^{d}$	$33.3 \pm 1.0*A^{c}$
	15	$694.3 \pm 41.1B_{d}$	$45.2 \pm 2.8B_{c}$	$14.4 \pm 0.7*B_{e}$	$1296.1 \pm 98.1A_{b}$	$7.4 \pm 0.4A_{b}$	$26.1 \pm 0.9*A_{c}$
Krasnoslawjanskij	14	nd^{e}	$19.5 \pm 1.1A^{d}$	$3.1 \pm 0.2*A^{e}$	$281.8 \pm 18.4A^{c}$	nd^{f}	nd^{e}
	15	nd_{f}	$8.6 \pm 0.9A_{d}$	$3.2 \pm 0.2*A_{f}$	$238.1 \pm 20.5A_{cf}$	nd_{f}	nd_{g}
Remarka	14	nd^{e}	nd^{e}	$286.4 \pm 13.6A^{c}$	$36.4 \pm 5.6A^{eh}$	nd^{f}	$14.6 \pm 0.1A^{a}$
	15	nd_{f}	nd_{e}	$243.6 \pm 12.3A_{g}$	$49.8 \pm 3.9A_{e}$	nd_{f}	$21.8 \pm 0.1A_{af}$
Rolonda	14	$114.8 \pm 7.6A^{c}$	nd^{e}	$1270.0 \pm 93.2A^{f}$	$577.5 \pm 31.3A^{f}$	$10.0 \pm 0.8A^{d}$	$7.1 \pm 0.2*A^{d}$
	15	$252.5 \pm 12.8B_{a}$	nd_{e}	$1602.2 \pm 124.1B_{ac}$	$80.8 \pm 6.9B_{ef}$	$8.4 \pm 0.5A_{b}$	$8.1 \pm 0.3*A_{d}$
Tamara	14	nd^{e}	$2.3 \pm 0.1A^{f}$	$592.8 \pm 21.9A^{c}$	$105.8 \pm 8.7A^{e}$	nd^{f}	nd^{e}
	15	nd_{f}	$2.8 \pm 0.1A_{f}$	$659.2 \pm 21.6A_{gh}$	$130.5 \pm 12.1A_{ef}$	nd_{f}	nd_{g}
Yellow-fruiting							
Citronovy obří	14	$1.5 \pm 0.1A^{b}$	$2.1 \pm 0.1A^{f}$	$3.7 \pm 0.2*A^{e}$	$193.2 \pm 13.8A^{ce}$	$1.9 \pm 0.1A^{c}$	$18.4 \pm 0.1A^{a}$
	15	$1.8 \pm 0.1A_{c}$	$2.9 \pm 0.1A_{f}$	$3.54 \pm 0.2*A_{f}$	$169.3 \pm 12.6A_{f}$	$1.8 \pm 0.1A_{c}$	$12.8 \pm 0.1A_{e}$
Darek	14	nd^{e}	nd^{e}	$1674.8 \pm 103.6A^{f}$	$102.9 \pm 8.6A^{e}$	$1.5 \pm 0.1A^{c}$	$15.9 \pm 0.1A^{a}$
	15	nd_{f}	nd_{e}	$1425.6 \pm 94.8A_{ac}$	$130.2 \pm 10.6A_{ef}$	$1.4 \pm 0.1A_{c}$	$21.8 \pm 0.1A_{af}$
Invicta	14	$327.1 \pm 17.2A^{d}$	$17.1 \pm 0.9A^{g}$	$53.3 \pm 3.9A^{g}$	$131.3 \pm 9.2A^{e}$	$4.7 \pm 0.3A^{e}$	nd^{e}
	15	$414.5 \pm 21.7A_{e}$	$12.7 \pm 0.9A_{d}$	$809.1 \pm 34.7B_{gh}$	$834.2 \pm 18.9B_{g}$	$3.6 \pm 0.2A_{d}$	nd_{g}
Rodnik	14	nd^{e}	nd^{e}	$7.4 \pm 0.4*A^{a}$	$629.3 \pm 23.6A^{f}$	nd^{f}	$21.6 \pm 0.1A^{a}$
	15	nd_{f}	nd_{e}	$7.5 \pm 0.4*A_{b}$	$652.9 \pm 31.7A_{g}$	nd_{f}	$21.5 \pm 0.1A_{ae}$
Zlaty fik	14	nd^{e}	$2.2 \pm 0.1A^{f}$	$250.7 \pm 19.9A^{c}$	$37.7 \pm 3.4A^{h}$	nd^{f}	nd^{e}
	15	nd_{f}	$2.3 \pm 0.1A_{f}$	$225.0 \pm 21.2A_{g}$	$46.3 \pm 5.7A_{e}$	nd_{f}	nd_{g}
Zebin	14	$1.5 \pm 0.1A^{b}$	$2.5 \pm 0.1A^{f}$	$616.3 \pm 26.8A^{c}$	$208.1 \pm 14.7A^{c}$	$1.6 \pm 0.1A^{c}$	$21.9 \pm 0.1A^{a}$
	15	$1.6 \pm 0.1A_{c}$	$2.2 \pm 0.1A_{f}$	$601.6 \pm 18.9A_{gh}$	$192.2 \pm 12.3A_{f}$	$1.4 \pm 0.1A_{c}$	$22.2 \pm 0.1A_{f}$
Green-fruiting							
Mucurines	14	nd^{e}	$2.7 \pm 0.1A^{f}$	$1247.3 \pm 100.9A^{f}$	$218.8 \pm 16.3A^{ce}$	nd^{f}	nd^{e}
	15	nd_{f}	$3.5 \pm 0.1A_{f}$	$1437.6 \pm 98.6A_{ac}$	$231.8 \pm 19.3A_{f}$	nd_{f}	nd_{g}
Prima	14	nd^{e}	nd^{e}	$152.2 \pm 10.4A^{c}$	$123.5 \pm 9.5A^{e}$	$1.8 \pm 0.1A^{c}$	$15.6 \pm 0.1A^{a}$
	15	nd_{f}	nd_{e}	$185.2 \pm 9.8A_{g}$	$112.3 \pm 7.6A_{ef}$	$1.6 \pm 0.1A_{c}$	$17.5 \pm 0.1A_{ae}$
Rixanta	14	$1.5 \pm 0.1A^{b}$	$37.8 \pm 2.6A^{c}$	$540.3 \pm 18.9A^{c}$	$911.5 \pm 14.7A^{i}$	$4.1 \pm 0.2A^{e}$	nd^{e}
	15	$1.2 \pm 0.1A_{c}$	$29.8 \pm 2.7A_{b}$	$524.0 \pm 21.3A_{gh}$	$943.8 \pm 32.6A_{g}$	$5.9 \pm 0.3A_{e}$	nd_{g}

Values identified by an asterisk (*) mean content in mg kg^{-1}; the results are expressed as the mean ± standard deviation ($n = 9$); different capital letters in the same column indicate significant differences ($p < 0.05$) between the picking years (2014–2015) within the same cultivar; different small letters in superscript/subscript in the same column indicate significant differences ($p < 0.05$) between the cultivars in 2014/2015, respectively

nd not detected

(not presented) is very similar to previous one (see Fig. 3); two apparent clusters are distinguished here. First one, correlating negatively with PC1, includes yellow/green varieties, indicating their great similarity in composition of compounds identified. The second cluster is placed in the right part of the graph correlating positively with PC1. It includes most of the red varieties. The special position of yellow fruiting 'Invicta' was confirmed here, as it has the highest total content of all compounds identified.

Comparison of sensory characteristics in red, yellow and green fruiting varieties

Another partial aim of this study was to evaluate sensory quality of samples using descriptive sensory methods. The list of evaluated attributes comprised appearance and colour, texture supplemented with two mouthfeel attributes (firmness and crispiness), taste and odour with three flavour characteristics (sweet, acid/sour, astringent), possible off-flavour and overall acceptability. The results are summarized in Table 2. As in the case of volatile compounds in samples, two picking years for each cultivar were compared; then, the single cultivars were mutually compared, separately in 2014 and 2015. The similarities/differences of red vs. yellow vs. green varieties were also judged.

Appearance and colour are significant sensory properties creating the first impression of fruits. As mentioned before, the size, shape and colour of gooseberries depend mainly on variety (Girard and Sinha 2006); the larger fruits are preferred (Harb and Streif 2004). Appearance was evaluated taking into consideration mainly size and shape; large fruits of regular oval shape, with bright surface without injury and smooth skin with/without fine hairs, were considered as excellent. The colour of pulp is often identical with the skin, it becomes more intense during ripening and fruits reach their typical colour in full ripeness. Colour of samples was difficult to compare owing to three types of varieties evaluated; moreover, colour intensity was not uniform even in the same sample, as it is influenced by the location of the fruit in a shrub (Girard and Sinha 2006). For these reasons, colour was evaluated separately, from the hedonic point of view, using scale from atypical to typical for a given variety. Characteristic, intense, homogeneous colour was considered as excellent. While in the case of yellow and green varieties, pale colour is well evaluated; in red ones, the darker is better (Harb and Streif 2004).

In the case of red varieties, 'Karat' was evaluated as having the best appearance (94.4; 66.8%) as well as the colour (90.2; 71.4%). Values in parenthesis are mean evaluations in 2014 and 2015, respectively. This variety should have large, less usual pear shaped fruits; its colour varies from pink to red to purple (Hanč et al. 2013). In our case, the fruits were large with deep red colour, which was probably the reason of excellent evaluation. Conversely, red 'Karmen' was evaluated as having the worst (less good) appearance (28.5; 47.5%) and colour (39.1; 61.1%). This variety should have medium size and oval shape (Hanč et al. 2013). In our case, the fruits were large and oval; however, pale red colour was probably the reason of bad evaluation. In the case of yellow varieties, 'Darek' (77.7; 69.2%) and 'Rodnik' (77.7; 71.4%) were evaluated as the best in appearance as well as in colour ('Darek' 71.5; 64.3%, 'Rodnik' 73.2; 70.2%). Both these varieties have large fruits; 'Darek' is round shaped, yellow-green and 'Rodnik' of oval shape and deep yellow in full ripeness (Hanč et al. 2013). Conversely, 'Zebin' had the worst appearance (35.3; 28.5%), although it had very large fruits. Intense hairy skin was probably the cause of bad evaluation. The colour was yellow-green, evaluated as less good (38.9; 27.2%). The colour and appearance of 'Zebin' were the worst of all varieties. Appearance and colour of all three green varieties were evaluated similarly as good/very good (in range 50.4–83.3%), although only 'Mucurines' should have bright green colour. The other two varieties are ranked to specific group of yellow-green varieties according to their rather yellow-green colour (Girard and Sinha 2006; Hanč et al. 2013). However, in our case all three varieties were of medium size, yellow-green colour, with smooth skin practically without hairs. The difference in colour between green 'Mucurines' and other two ones was hardly perceptible and insignificant.

The texture of gooseberries depends mainly on variety. The pulp is soft in full ripeness; however, the texture is also related to skin firmness, which influences the overall firmness and crispiness of fruit. The skin becomes softer during ripening, which determines the use; fruits with softer skin are suitable for direct consumption; those with firmer skin are preferred for processing (Girard and Sinha 2006). The softening during storage was also observed by Harb and Streif (2004), which put emphasis on quickness of processing.

The texture was evaluated owing to the suitability for direct consumption, putting stress on soft pulp and firm skin, which keeps desirable crispiness. In red varieties texture of 'Karat' (86.1; 59.2%), 'Rolonda' (75.0; 71.4%) and 'Tamara' (72.2; 69.7%) were evaluated as very good, which was in accordance with the good evaluation of firmness and crispiness. In yellow varieties, 'Darek' (77.7; 71.4%) and 'Rodnik' (72.2; 71.4%) were best evaluated with firm and crispy fruits. Conversely, red 'Karmen' (28.5; 52.2%) and yellow 'Invicta' (57.1; 43.1%) and 'Zebin' (51.9; 42.8%) were evaluated as the worst; the fruits were too soft with no crispiness. In green varieties, 'Prima' had the best texture (66.6; 61.5%) with good firmness and crispiness; conversely, 'Rixanta' was evaluated very badly (38.8; 28.5%), the worst of all varieties. Also, its fruits were too soft. As can be seen, very soft texture is negatively perceived by assessors; too soft fruits were evaluated as unsatisfactory. That is in accordance with Harb and Streif (2004), who identified the fruit firmness as one of the main indicators of gooseberry quality.

The main attention was paid to taste and aroma (flavour) owing to the intended comparison with the volatile compounds identified. If we consider fully ripe fruits, provided that all samples were harvested and stored in the same way, these characteristics depend mainly on variety (Girard and

Table 2 Comparison of sensory characteristics of gooseberry varieties

(a)

Gooseberry varieties	Picking year	Sensory characteristics/%					
		Appearance	Colour	Texture	Mouthfeel		Overall acceptability
					Firmness	Crispiness	
Red-fruiting							
Alan	14	72.2 ± 5.3^{a}	68.4 ± 5.6^{a}	69.4 ± 4.4^{a}	69.4 ± 4.7^{a}	64.4 ± 5.7^{a}	50.7 ± 4.1^{a}
	15	$68.8 \pm 5.5_{a}$	$73.8 \pm 5.4_{a}$	$57.1 \pm 3.5_{a}$	$67.5 \pm 5.1_{a}$	$53.7 \pm 3.1_{a}$	$53.4 \pm 4.2_{a}$
Hinnonmaki Rot	14	80.5 ± 5.2^{a}	84.5 ± 4.7^{b}	69.4 ± 5.4^{a}	69.4 ± 5.3^{a}	48.3 ± 3.5^{b}	42.8 ± 3.5^{ac}
	15	$57.1 \pm 2.4_{ab}$	$59.4 \pm 3.2_{ab}$	$57.1 \pm 4.2_{a}$	$57.1 \pm 5.4_{ac}$	$69.4 \pm 5.6_{ac}$	$38.5 \pm 2.7_{b}$
Karat	14	94.4 ± 6.2^{b}	90.2 ± 7.4^{b}	86.1 ± 6.2^{b}	86.1 ± 7.2^{b}	80.3 ± 7.1^{d}	76.2 ± 5.8^{b}
	15	$66.8 \pm 4.7_{a}$	$71.4 \pm 6.8_{a}$	$59.2 \pm 4.8_{a}$	$65.1 \pm 4.6_{a}$	$64.5 \pm 4.7_{a}$	$77.1 \pm 5.4_{c}$
Karmen	14	28.5 ± 1.8^{c}	39.1 ± 2.8^{c}	28.5 ± 1.7^{c}	57.1 ± 4.4^{d}	42.8 ± 2.2^{b}	28.5 ± 1.2^{c}
	15	$47.5 \pm 2.1_{b}$	$61.1 \pm 5.1_{ab}$	$52.2 \pm 3.1_{a}$	$64.5 \pm 5.1_{a}$	$59.1 \pm 3.6_{a}$	$32.4 \pm 1.6_{b}$
Krasnoslawjanskij	14	62.6 ± 3.1^{ad}	68.3 ± 3.2^{a}	61.1 ± 5.3^{d}	66.8 ± 5.2^{a}	61.1 ± 4.0^{a}	37.9 ± 1.9^{c}
	15	$57.1 \pm 4.7_{ab}$	$67.8 \pm 5.3_{a}$	$64.2 \pm 4.9_{ab}$	$71.4 \pm 5.8_{ab}$	$42.8 \pm 2.7_{b}$	$28.5 \pm 1.7_{b}$
Remarka	14	50.0 ± 3.2^{d}	57.4 ± 2.8^{a}	63.8 ± 4.7^{d}	63.8 ± 5.7^{a}	68.3 ± 5.8^{ac}	64.8 ± 4.3^{b}
	15	$70.4 \pm 5.4_{a}$	$63.2 \pm 4.7_{a}$	$55.4 \pm 3.2_{a}$	$71.4 \pm 5.2_{ab}$	$42.8 \pm 3.1_{b}$	$57.1 \pm 5.4_{a}$
Rolonda	14	55.5 ± 4.6^{d}	60.2 ± 3.5^{a}	75.0 ± 5.1^{b}	75.0 ± 6.3^{ab}	59.1 ± 4.7^{a}	42.8 ± 3.5^{ac}
	15	$69.4 \pm 5.1_{a}$	$77.5 \pm 5.7_{a}$	$71.4 \pm 4.8_{b}$	$85.7 \pm 7.1_{b}$	$71.4 \pm 5.2_{c}$	$51.1 \pm 3.8_{a}$
Tamara	14	55.5 ± 3.7^{d}	57.9 ± 3.6^{a}	72.2 ± 5.2^{ab}	77.5 ± 5.2^{ab}	73.2 ± 6.3^{cd}	39.7 ± 2.5^{c}
	15	$50.2 \pm 3.4_{b}$	$53.8 \pm 3.2_{b}$	$69.7 \pm 4.4_{b}$	$69.9 \pm 4.6_{ab}$	$75.4 \pm 5.8_{c}$	$38.6 \pm 3.0_{b}$
Yellow-fruiting							
Citronovy obří	14	44.4 ± 2.4^{cd}	42.7 ± 2.4^{c}	66.6 ± 5.6^{d}	65.1 ± 5.6^{a}	66.6 ± 5.7^{ac}	33.2 ± 2.1^{c}
	15	$46.5 \pm 3.6_{b}$	$44.4 \pm 2.6_{b}$	$60.3 \pm 4.8_{ab}$	$71.3 \pm 5.4_{ab}$	$62.8 \pm 5.1_{a}$	$36.2 \pm 1.7_{b}$
Darek	14	77.7 ± 5.8^{a}	71.5 ± 5.7^{a}	77.7 ± 5.8^{b}	79.3 ± 6.7^{ab}	74.2 ± 6.9^{cd}	74.4 ± 5.4^{b}
	15	$69.2 \pm 5.3_{a}$	$64.3 \pm 4.3_{a}$	$71.4 \pm 4.3_{b}$	$70.5 \pm 6.1_{ab}$	$70.8 \pm 5.0_{ac}$	$71.6 \pm 5.9_{c}$
Invicta	14	66.6 ± 4.6^{ad}	69.5 ± 5.6^{a}	57.1 ± 2.2^{d}	42.8 ± 2.0^{c}	41.6 ± 3.8^{b}	42.8 ± 3.7^{ac}
	15	$42.8 \pm 3.8_{b}$	$49.5 \pm 3.7_{b}$	$43.1 \pm 2.6_{c}$	$41.6 \pm 3.3_{c}$	$57.1 \pm 4.4_{ad}$	$43.7 \pm 3.1_{ab}$
Rodnik	14	77.7 ± 5.7^{a}	73.2 ± 5.8^{a}	72.2 ± 5.1^{ab}	74.1 ± 6.2^{ab}	76.8 ± 6.2^{cd}	68.7 ± 5.8^{b}
	15	$71.4 \pm 4.2_{a}$	$70.2 \pm 5.8_{a}$	$71.4 \pm 5.2_{b}$	$71.4 \pm 5.2_{ab}$	$69.8 \pm 5.5_{ac}$	$57.1 \pm 4.4_{a}$
Zlaty fik	14	44.4 ± 2.7^{c}	47.4 ± 2.9^{c}	66.6 ± 5.3^{a}	69.2 ± 4.6^{a}	63.5 ± 4.6^{ac}	40.3 ± 3.2^{ac}
	15	$48.1 \pm 3.6_{ab}$	$43.2 \pm 3.7_{b}$	$67.9 \pm 4.0_{b}$	$54.3 \pm 4.7_{c}$	$59.0 \pm 5.1_{ad}$	$36.3 \pm 2.9_{b}$
Zebin	14	35.3 ± 2.2^{c}	38.9 ± 2.4^{c}	51.9 ± 3.9^{d}	59.6 ± 5.0^{d}	61.9 ± 5.1^{a}	48.1 ± 4.2^{a}
	15	$28.5 \pm 2.1_{c}$	$27.2 \pm 1.8_{c}$	$42.8 \pm 3.5_{c}$	$57.1 \pm 5.2_{c}$	$57.1 \pm 4.4_{ad}$	$42.8 \pm 3.6_{ab}$
Green-fruiting							
Mucurines	14	83.3 ± 5.3^{a}	78.4 ± 5.3^{ab}	55.5 ± 3.7^{d}	53.3 ± 3.9^{cd}	57.5 ± 4.5^{a}	69.5 ± 5.1^{b}
	15	$52.8 \pm 4.1_{ab}$	$50.4 \pm 4.2_{b}$	$42.8 \pm 3.5_{c}$	$46.7 \pm 4.1_{c}$	$48.5 \pm 3.7_{bd}$	$42.8 \pm 2.7_{ab}$
Prima	14	61.1 ± 5.2^{ad}	67.3 ± 3.1^{a}	66.6 ± 5.4^{a}	69.4 ± 5.6^{a}	65.5 ± 5.6^{ac}	65.2 ± 5.3^{b}
	15	$56.9 \pm 3.9_{ab}$	$59.9 \pm 3.7_{ab}$	$61.5 \pm 4.2_{ab}$	$62.5 \pm 5.3_{ac}$	$67.7 \pm 4.9_{ac}$	$57.4 \pm 3.4_{a}$
Rixanta	14	66.6 ± 4.6^{a}	69.6 ± 5.6^{a}	38.8 ± 1.7^{c}	42.6 ± 2.8^{c}	48.4 ± 3.8^{b}	49.0 ± 4.3^{a}
	15	$42.8 \pm 3.1_{b}$	$44.8 \pm 2.9_{b}$	$28.5 \pm 1.7_{d}$	$57.1 \pm 5.4_{c}$	$43.4 \pm 3.2_{b}$	$42.8 \pm 3.5_{ab}$

(b)

Gooseberry varieties	Picking year	Sensory characteristics/%					
		Taste	Odour	Flavour			Off-flavour
				Sweet	Acid/sour	Astringent	
Red-fruiting							
Alan	14	58.3 ± 5.3^{a}	49.3 ± 4.3^{ab}	57.1 ± 4.4^{ad}	71.4 ± 5.2^{a}	42.8 ± 3.5^{a}	14.2 ± 0.8
	15	$43.7 \pm 3.1_{a}$	$42.8 \pm 3.7_{a}$	$62.2 \pm 5.8_{ab}$	$52.1 \pm 4.5_{a}$	$28.5 \pm 1.7_{a}$	28.5 ± 0.7

Table 2 continued

(b)

Gooseberry varieties	Picking year	Sensory characteristics/%					
		Taste	Odour	Flavour			Off-flavour
				Sweet	Acid/sour	Astringent	
Hinnonmaki Rot	14	50.0 ± 4.4^{a}	57.1 ± 4.4^{b}	62.6 ± 5.3^{a}	51.6 ± 3.4^{b}	34.5 ± 2.2^{ad}	31.4 ± 1.2
	15	$28.5 \pm 1.7_{b}$	$42.8 \pm 3.2_{a}$	$64.3 \pm 4.7_{ab}$	$62.1 \pm 4.2_{ab}$	$70.4 \pm 5.2_{c}$	28.5 ± 0.7
Karat	14	68.8 ± 5.9^{b}	48.3 ± 3.8^{ab}	73.2 ± 5.9^{ab}	33.6 ± 2.7^{c}	38.1 ± 2.6^{a}	nd
	15	$57.1 \pm 4.4_{c}$	$42.8 \pm 3.7_{a}$	$72.4 \pm 6.2_{a}$	$42.8 \pm 3.1_{c}$	$45.7 \pm 4.1_{d}$	nd
Karmen	14	33.8 ± 2.6^{c}	42.8 ± 3.4^{ac}	65.7 ± 5.1^{a}	42.8 ± 3.5^{bc}	28.5 ± 1.7^{d}	nd
	15	$55.5 \pm 5.2_{c}$	$49.7 \pm 3.5_{a}$	$70.3 \pm 4.9_{a}$	$39.4 \pm 2.6_{c}$	$33.0 \pm 2.2_{a}$	nd
Krasnoslawjanskij	14	44.4 ± 4.3^{ac}	31.8 ± 2.9^{c}	73.6 ± 5.8^{ab}	41.8 ± 3.7^{bc}	35.1 ± 2.4^{ad}	21.3 ± 1.2
	15	$28.5 \pm 1.7_{b}$	$42.8 \pm 3.0_{a}$	$62.8 \pm 5.0_{ab}$	$47.1 \pm 4.4_{c}$	$42.8 \pm 3.5_{ad}$	44.8 ± 3.5
Remarka	14	50.2 ± 3.8^{a}	59.2 ± 5.1^{b}	54.2 ± 3.9^{d}	44.6 ± 4.2^{bc}	38.3 ± 2.4^{a}	nd
	15	$57.1 \pm 4.6_{c}$	$64.8 \pm 5.7_{b}$	$69.1 \pm 5.4_{a}$	$57.1 \pm 5.4_{a}$	$28.5 \pm 1.7_{a}$	nd
Rolonda	14	44.4 ± 3.5^{ac}	28.5 ± 2.4^{c}	67.4 ± 6.2^{a}	53.1 ± 4.1^{b}	28.5 ± 1.3^{d}	18.1 ± 0.4
	15	$42.8 \pm 3.5_{a}$	$47.1 \pm 3.2_{a}$	$57.1 \pm 4.4_{b}$	$62.8 \pm 5.7_{ab}$	$42.8 \pm 3.8_{ad}$	14.2 ± 0.8
Tamara	14	31.5 ± 1.9^{c}	37.6 ± 2.7^{c}	29.8 ± 1.9^{c}	44.6 ± 3.3^{bc}	43.8 ± 3.7^{ac}	nd
	15	$30.9 \pm 2.7_{b}$	$41.0 \pm 3.6_{a}$	$27.5 \pm 1.8_{c}$	$40.9 \pm 3.2_{c}$	$47.5 \pm 3.4_{d}$	nd
Yellow-fruiting							
Citronovy obří	14	37.1 ± 2.4^{c}	42.2 ± 3.8^{ac}	34.4 ± 2.6^{c}	72.4 ± 6.3^{a}	69.3 ± 5.6^{b}	nd
	15	$33.5 \pm 2.5_{b}$	$38.1 \pm 2.9_{a}$	$30.2 \pm 2.7_{c}$	$66.8 \pm 5.9_{b}$	$64.7 \pm 5.7_{b}$	nd
Darek	14	55.5 ± 3.6^{a}	58.7 ± 4.6^{b}	58.5 ± 4.3^{ad}	33.2 ± 2.5^{c}	28.6 ± 1.6^{d}	nd
	15	$61.5 \pm 4.7_{c}$	$67.4 \pm 5.9_{b}$	$64.0 \pm 5.2_{ab}$	$31.8 \pm 2.0_{c}$	$36.5 \pm 2.7_{a}$	nd
Invicta	14	42.8 ± 3.1^{ac}	28.5 ± 1.7^{c}	79.4 ± 6.5^{b}	57.1 ± 4.8^{b}	28.5 ± 2.1^{d}	14.2 ± 0.8
	15	$47.1 \pm 3.4_{ac}$	$41.6 \pm 3.6_{a}$	$71.4 \pm 5.8_{a}$	$42.8 \pm 3.2_{c}$	$31.2 \pm 1.8_{a}$	16.9 ± 1.2
Rodnik	14	50.3 ± 4.2^{a}	55.0 ± 4.5^{ab}	61.3 ± 4.7^{ad}	57.5 ± 4.9^{b}	53.2 ± 4.6^{c}	nd
	15	$42.8 \pm 3.5_{a}$	$57.1 \pm 5.4_{ab}$	$42.8 \pm 3.5_{d}$	$71.4 \pm 5.1_{b}$	$52.8 \pm 4.5_{b}$	nd
Zlaty fik	14	35.2 ± 2.8^{c}	38.6 ± 2.7^{c}	34.2 ± 2.8^{c}	58.6 ± 4.6^{b}	53.4 ± 4.9^{c}	nd
	15	$39.6 \pm 2.7_{ab}$	$37.1 \pm 2.8_{a}$	$38.9 \pm 2.7_{cd}$	$54.4 \pm 5.0_{a}$	$54.8 \pm 4.1_{b}$	nd
Zebin	14	46.3 ± 3.6^{ac}	50.2 ± 3.6^{ab}	61.2 ± 5.5^{ad}	44.1 ± 3.1^{bc}	46.3 ± 4.2^{ac}	nd
	15	$42.8 \pm 3.5_{a}$	$43.4 \pm 3.2_{a}$	$57.1 \pm 5.4_{b}$	$42.8 \pm 3.7_{c}$	$28.5 \pm 1.7_{a}$	nd
Green-fruiting							
Mucurines	14	50.6 ± 3.8^{a}	62.4 ± 5.5^{b}	59.2 ± 4.8^{ad}	27.6 ± 1.2^{c}	31.2 ± 1.8^{d}	nd
	15	$46.7 \pm 3.1_{ac}$	$57.1 \pm 5.4_{ab}$	$42.8 \pm 3.7_{d}$	$57.1 \pm 4.0_{a}$	$42.8 \pm 3.5_{ad}$	nd
Prima	14	37.7 ± 1.8^{c}	47.7 ± 3.7^{ab}	25.4 ± 1.9^{c}	75.9 ± 5.8^{a}	65.5 ± 5.1^{b}	nd
	15	$46.2 \pm 2.7_{ac}$	$38.5 \pm 2.3_{a}$	$31.4 \pm 2.6_{c}$	$68.4 \pm 5.7_{b}$	$60.1 \pm 5.2_{b}$	nd
Rixanta	14	39.0 ± 2.8^{c}	45.6 ± 3.8^{ac}	79.8 ± 6.4^{b}	35.5 ± 2.5^{c}	38.6 ± 2.4^{ad}	17.2 ± 0.6
	15	$57.1 \pm 4.4_{c}$	$42.8 \pm 3.5_{a}$	$71.4 \pm 5.2_{a}$	$57.1 \pm 5.2_{a}$	$28.5 \pm 1.7_{a}$	14.2 ± 0.8

The results are expressed as mean ± standard deviation ($n = 22$). Different small letters in superscript/subscript in the same column indicate significant differences ($p < 0.05$) between the cultivars in 2014/2015, respectively

nd not detected

Sinha 2006); the taste of gooseberries is characteristic mildly astringent because of tannins, sweet and/or acidic depending on the variety (Harb and Streif 2004). Characteristic, pleasant gooseberry taste/aroma without any off-flavour was considered as excellent. Because the taste of gooseberries could be either sweeter or more acidic (Harb and Streif 2004), the intensity of these descriptors were evaluated separately, similarly to astringency, which is characteristic for gooseberries, but in strong intensity could be unpleasant.

Red 'Karat' (68.8; 57.1%) had the best taste in both years. This variety should have sweet–sour taste (Hanč et al. 2013); in our case it was rather sweet (73.2; 72.4%) and the acidity was low (33.6; 42.8%). 'Tamara'

(31.5; 30.9%) had the worst taste with low sweetness (29.8; 27.5%) and higher astringency (43.8; 47.5%). No mention was found in the literature about typical taste of this variety. The odour of 'Remarka' (59.2; 64.8%) was best evaluated in both years; its odour used to be described as very aromatic (Hanč et al. 2013); conversely, 'Rolonda' had the worst odour (28.5; 47.1%) probably because of off-flavour. Between yellow varieties, 'Darek' had the best taste (55.5; 61.5%) as well as the odour (58.7; 67.4%) in both years. This variety should have sweet–sour taste (Hanč et al. 2013); in our case, it was rather sweet (58.5; 64.0%) with low acidity (33.2%; 31.8%). 'Citronovy obři' had the worst taste (37.1; 33.5%). This variety should also have sweet–sour taste (Hanč et al. 2013); however, in our case it was very acid (72.4; 66.8%) and astringent (69.3; 64.7%). Although 'Invicta' was quite sweet (79.4; 71.4%), its taste (42.8; 47.1%) and odour (28.5; 41.6%) were not well evaluated probably because of off-flavour. The taste of this variety is often described as sweet, but bland (Hanč et al. 2013). In the case of green varieties, 'Mucurines' could be labelled as having the best taste (50.6; 46.7%) and odour (62.4; 57.1%). According to the Nikfardjam et al. (2013), this variety showed strong grassy aroma, in accordance with (Hanč et al. 2013); its taste was mildly sweet (59.2; 42.8%) and sour (27.6; 57.1%). The evaluation of other two green varieties was worse; 'Prima' was found to be very acid (75.9; 68.4%) and astringent (65.5; 60.1%), while 'Rixanta' had off-flavour, although it was very sweet (79.8; 71.4%). As mentioned before, these two varieties do not have typical green colour, which could also influence evaluation of taste and aroma (Girard and Sinha 2006; Hanč et al. 2013).

The mild but obvious off-flavour was detected in red 'Alan' (14.2; 28.5%), 'Hinnonmaki Rot' (31.4; 28.5%), 'Krasnoslawjanskij' (21.3; 44.8%) and 'Rolonda' (18.1; 14.2%), yellow 'Invicta' (14.2; 16.9%) and green 'Rixanta' (17.2; 14.2%) varieties in both years, described as fermented, slightly bitter and/or acid. Although the samples were cold stored for a very short time, this fact is probably related to initiating fermentation process (Harb and Streif 2004) and seems to be connected with the high ethanol content. Nikfardjam et al. (2013) relate the possible overripe off-flavour to the ratio between (*Z*)-3-hexenal and ethyl acetate, while the pentan-2-one and heptan-2-one content are connected with the musty off-flavour; these defects were not detected in this study. As expected, the presence of off-flavour, although mild, negatively influenced the evaluation of taste and aroma. That is in accordance with Harb and Streif (2004), who identified the possible off-flavour as one of the main indicators of gooseberry quality.

Finally, the overall acceptability was evaluated taking into consideration all evaluated properties. Red 'Karat' (76.2; 77.1%) and yellow 'Darek' (74.4; 71.6%) could be labelled as the best of all varieties. They were well evaluated in both years with very good all of evaluated sensory properties. On the other hand, red 'Karmen' was concluded to be the least acceptable (28.5; 32.4%) as evaluated very badly in all characteristics. Contrary to Hempfling et al. (2013) who considered red fruiting varieties as the most interesting for commercial purposes, because they were the most preferred by consumers, no obvious preference was found between red, yellow and green types in this study.

The results of sensory evaluations were tested by PCA to investigate which sensory descriptors correlate to each other and which of them contribute to overall sensory quality and acceptability of samples. PCA was performed using average ratings of all sensory characteristics in all 17 gooseberry varieties in both years (data matrix 34 × 12). Three PCs were derived accounting for 34.0, 25.8 and 14.4% of the variance, respectively, which cumulatively explained satisfactory 74.2% of the total variability. The PC1 was highly correlated with appearance (−0.87), colour (−0.80), taste (−0.66), texture (−0.68), firmness (−0.60) and overall acceptability (−0.84). The PC2 was associated with sweet (0.87) and astringent (−0.84) tastes and crispiness (−0.65). As expected texture, firmness and crispiness highly correlate to each other, as well as the colour vs. appearance and taste vs. odour. Sweetness correlates negatively with acid and astringent tastes; sweeter and more astringent/acid samples are well divided along the PC2. The overall acceptability strongly correlates with PC1; the appearance, colour, taste and odour contribute the most to the overall acceptability. Harb and Streif (2004) identified the fruit firmness, sweet-acid taste balance and the possible off-flavour as the main indicators of sensory quality of gooseberries. In this study, PCA did not confirm the significance of texture and off-flavour in acceptability of gooseberries. However, it confirmed the importance of sweet/acid taste, as assessors preferred sweeter, less acidic gooseberries. Off-flavour was detected only in a few samples in low intensity and probably does not influence significantly acceptability of samples.

Although ANOVA indicates significant differences between varieties, there is no clear differentiation of samples according to the red/yellow/green varieties. PC1 correlates with the overall acceptability, PCA such confirmed red 'Karat' and yellow 'Darek' as the most acceptable varieties. The differences between years of production (2014 vs. 2015) were mostly small or not significant, as confirmed by ANOVA analysis.

The contributions of compounds identified to the sensory quality of samples

To better estimate which compounds could contribute to flavour/acceptability of samples, the PCA was performed using the instrumental and sensory data of 17 gooseberry varieties in both years (data matrix 34 × 64), and odour activity values (OAVs) were calculated by dividing contents of given compound in the sample by its odour threshold acquired from the literature (Jensen et al. 2000; Belitz et al. 2009; Sanchez-Palomo et al. 2010; Hempfling et al. 2013; Nikfardjam et al. 2013; Sádecká et al. 2014). Nikfardjam et al. (2013) identified in gooseberries 17 compounds as aroma active; similarly, Hempfling et al. (2013) identified 10 compounds with OAVs >1. Hempfling et al. (2013) characterize the typical gooseberry aroma as combination of green and fruity notes, where fruity aroma becomes stronger during ripening. (*Z*)-3-hexenal and short chain ethyl and/or methyl esters, especially methyl and ethyl butanoate, are considered as the carriers of this aroma. Nikfardjam et al. (2013) also identified (*Z*)-3-hexenal and ethyl acetate as responsible for the aroma of gooseberries. In accordance with these studies (*Z*)-3-hexen-1-ol (OAVs 1-3; geranium), 1-octen-3-ol (OAVs 6-14; mushroom-like), (*Z*)-3-hexenal (OAVs 794-5168; grassy), hexanal (OAVs 1-2; grassy, citrus-like), heptan-2-one (OAVs 1-2; fruity, sharp, herbaceous), methyl butanoate (OAVs 1-11; fruity, green), ethyl butanoate (OAVs 6-199; fruity, pineapple-like), methyl acetate (OAVs 1-8; fragrant, fruity) and ethyl acetate (OAVs 13-1589; fruity, pear like) were recognized as aroma active in this study. Their aroma description is in parenthesis. Moreover, another 7 compounds were proposed as possible contributors to gooseberry aroma as their OAVs were >1: ethanol (OAVs 1-2; alcoholic), octan-1-ol (OAVs 20-65; citrus, spicy, herbaceous), 3-methylbutan-1-ol (OAVs 1-2; burnt, alcohol), ethanal (OAVs 162-912; fruity, pungent), propanal (OAVs 1-5; fruity, pungent), acetic acid (OAVs 150-477; fruity-sour, vinegar) and 3-methylbutanoic acid (OAVs 95-2738; sweet, acid, rancid).

According to the PCA, between these compounds (*Z*)-3-hexen-1-ol, (*Z*)-3-hexenal, heptan-2-one, methyl butanoate, ethyl butanoate, methyl acetate, ethyl acetate, ethanol and ethanal correlate positively with flavour/acceptability of samples. As in Nikfardjam et al. (2013), (*Z*)-3-hexenal and ethyl acetate had the highest OAVs with the grassy and fruity aroma, respectively, while 1-octen-3-ol, hexanal, octan-1-ol, 3-methylbutan-1-ol, propanal, acetic and 3-methylbutanoic acid could negatively influence the flavour, as they correlate negatively with flavour/acceptability of samples. 3-methylbutanoic acid with its rancid aroma had the highest OAV.

Conclusions

Only a few studies on the volatile/aroma compounds of gooseberry have been published so far, as well as on their connection with the flavour of these fruits. This work is a complex study focused on selected varieties intended to be grown in the Czech Republic. The combination of the instrumental assessment of volatile compounds and sensory evaluation with the great emphasis on flavour was used for characterization of samples. In total, 52 volatile compounds were identified and quantified, with quantitatively predominating alcohols and acids. Based on PCA and calculation of OAVs, 9 of them were supposed to contribute to flavour of samples as having the OAVs >1 and correlating positively with sensory evaluation of flavour.

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Determination and quantification of volatile compounds in fruits of selected elderberry cultivars grown in Czech Republic

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Summary

The aim of this work was to identify and quantify the volatile aroma compounds in fruits of several elderberry cultivars and to find a cultivar with the highest content of them. Wild elder and sixteen cultivars of elderberries were analysed. Aroma compounds were extracted by solid phase microextraction, identified by gas chromatography-mass spectrometry and quantified by gas chromatography. In total, 102 volatile compounds were identified in all elderberry samples, among them 38 alcohols, 16 aldehydes, 10 ketones, 19 esters, 4 heterocycles, 6 hydrocarbons and 9 acids. Alcohols, aldehydes and esters were the most abundant, while significantly ($P < 0.05$) lower contents of heterocycles and hydrocarbons were found. Based on the literature, 36 compounds known as significant components of elderberry aroma, were chosen as markers of differences among cultivars. Owing to the highest total content of the selected compounds, cultivars Korsör (77.89 ± 3.57) mg·kg^{-1}, Pregarten (43.20 ± 7.14) mg·kg^{-1} and Samdal (67.85 ± 8.22) mg·kg^{-1} were recommended for practical use.

Keywords

elderberry; aroma compounds; solid-phase microextraction; gas chromatography; gas chromatography-mass spectrometry

Black elder (*Sambucus nigra* L.) is a plant with miscellaneous use due to its therapeutic effects in medicine, for its aroma and taste in the kitchen. It grows wild in several countries of Europe. Wild elderberry fruits and flowers are used mainly for the home-made production of marmalades, juices, syrups, teas, liqueurs and wines. Fruits are known by high contents of anthocyanin pigments and they are used for industrial production of natural colorants for various types of food products [1]. Czech food industry uses mainly imported frozen elderberries as a fruit component of yoghurts. However, elderberry contains many health-promoting substances [2–4], so its consumption is highly advisable. Novel food products based on elderberry, with a high antioxidative potential, could enrich the Czech consumer market.

Current research focuses on cultivated varieties, which offer higher yields, higher contents of valuable compounds, lower content of toxic sambunigrin, better sensory properties and other advantages compared to the wild plant. Elderberry is cultivated in some European countries, e.g. Austria, Denmark, Germany, Hungary. Several cultivars are grown in Slovakia in a small extent. The production of cultivated elderberries in Czech Republic is only at the beginning. No new varieties were bred in Czech region, but various foreign cultivars are tested for breeding here. The information about them is very scarce.

Successful commercialization of elderberry fruits depends on their sensory properties, in particular good taste and aroma, which are strongly associated with the content of volatile aroma-active substances [5]. The aroma of elderberry flowers [6–10] and fruits of elderberries [5, 11–13] were characterized by several authors, albeit not many articles were published. More than 100 volatiles were identified. Using GC-olfactometry, JENSEN et al. [12] divided the aroma compounds

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of elderberries into six odour classes: elderberry, flowery, fruity, grassy, agrestic and miscellaneous. Most of other authors adhered to this classification and came to similar conclusions [5, 13].

The aim of this work was to identify and quantify the volatile aroma compounds in fruits of several elderberry cultivars tentatively grown in Czech Republic. The volatile compounds identified were then compared in order to recommend the cultivars that would be suitable for growing in the Czech region on a large scale and would be suitable for following practical use. Aroma compounds were assessed by gas chromatography with flame ionization detector (GC-FID) and gas chromatography-mass spectrometry (GC-MS). Solid phase microextraction (SPME) as a modern sample preparation technique, saving preparation time, solvent needs and disposal costs, was used for their extraction. In headspace mode, the method is in particular suitable for the extraction of volatile and semi-volatile organic compounds [14] and was used by many authors to analyse volatile aroma compounds of food samples (reviewed by KATAOKA et al. [14]), including various fruits such as raspberries, apricots, plums, pineapples and others [15–23].

MATERIALS AND METHODS

Samples

Wild elder and sixteen cultivars of elderberries (grown by Research and Breeding Institute of Pomology, Holovousy, Czech Republic) were used for analysis: Albida, Allesö, Aurea, Bohatka, Dana, Haschberg, Korsör, Mammut, Pregarten, Riese aus Voßloch, Sambo, Sampo, Sambu, Samdal, Samyl and Weihenstephan.

The berries were picked in September 2011, frozen immediately after picking and stored in a freezer before processing. For analysis, 1 g of the de-frozen sample was mashed and placed into a vial for SPME extraction of aroma compounds. Three samples of every cultivar was taken, every sample was analysed three times ($n = 9$).

SPME-GC and SPME-GC-MS conditions

Volatile compounds in the elderberry samples were extracted by SPME, identified by GC-MS (based on mass spectra) and quantified using standards by GC-FID. The SPME conditions were: SPME fibre CAR/PDMS 85 μm (Supelco, Bellefonte, Pennsylvania, USA). Sample volume 1 ml, extraction temperature 35 °C, equilibrium time 30 min, extraction time 20 min, desorption temperature 250 °C, desorption time 5 min.

Gas chromatograph TRACE GC (ThermoQuest, Milan, Italy) was used, with a capillary column DB-WAX 30 m × 0.32 mm × 0.5 μm (J&W Scientific, Folsom, California, USA). GC conditions: injector 250 °C, splitless desorption 5 min, carrier gas N_2 0.9 ml·min^{-1}, FID at 220 °C, H_2 35 ml·min^{-1}, air 350 ml·min^{-1}, make up N_2 30 ml·min^{-1}. The oven temperature was 40 °C for 1 min, 40–200 °C at 5 °C·min^{-1}, 200 °C for 7 min.

GC-MS analyses were done by a gas chromatograph HP 6890 with MS detector 5973 N and Mass Spectral Library NIST 98 (Agilent, Santa Clara, California, USA). Capillary column ZB-5Sil MS 30 m × 0.25 mm × 0.25 μm (Phenomenex, Torrance, California, USA) was used, with carrier gas He 0.9 ml·min^{-1} and the oven temperature 50–250 °C at 3 °C·min^{-1}. Other GC conditions were the same as stated above. MS was operated in electron ionization (EI) mode at 70 eV with a scan range of m/z from 30 to 370.

A mixture of 20 standards was used for method validation. The intra-day repeatability was verified by repeated extraction ($n = 5$) of the mixture (relative standard deviation < 10%). The detection limit (*LOD*) and quantification limit (*LOQ*) were estimated by successive dilution of standards to achieve the lowest signal registered by the detector (*LOD*, $S/N = 3$; *LOQ*, $S/N = 10$). The detection limits varied in a range of 0.001–0.50 μg·ml^{-1}. Linearity was tested within the range of 0.001–200 μg·ml^{-1}, for ethanol and propan-2-ol in the range of 0.50–2 000 μg·ml^{-1}); based on linear regression analysis, correlation coefficients were all greater than 0.99.

Statistical evaluation

The results were treated using MS Excel 2010 (Microsoft, Redmond, Washington, USA) and were expressed as mean ± standard deviation ($n = 9$). Parametric one way analysis of variance and subsequently Duncan test was used for statistical evaluation of the results using Unistat version 5.5 (Unistat, London, United Kingdom).

RESULTS AND DISCUSSION

Identification of volatile aroma compounds in elderberries

In total, 102 volatile compounds were identified in all elderberry samples, among them:

– 38 alcohols: methanol, ethanol, propan-1-ol, propan-2-ol, butan-1-ol, pentan-1-ol, hexan-1-ol, heptan-1-ol, octan-1-ol, heptan-2-ol, benzyl alcohol, 2-phenylethyl alcohol,

1-penten-3-ol, (*E*)-2-hexen-1-ol, (*E*)-3-hexen-1-ol, (*Z*)-3-hexen-1-ol, 1-octen-3-ol, (*E*)-2-octenol, 2-methylpropan-1-ol, 2-methylbutan-1-ol, 3-methylbutan-1-ol, 4-methylpentan-1-ol, 4-methylhexan-2-ol, 2-ethylhexanol, butane-2,3-diol, butane-1,3-diol, linalool, hotrienol, nerol, citronellol, geraniol, borneol, menthol, α-terpineol, β-terpineol, hydroxycitronellol, eucalyptol, terpinen-4-ol;
- 16 aldehydes: ethanal, pentanal, hexanal, heptanal, octanal, nonanal, benzaldehyde, phenylacetaldehyde, 2-methylpropanal, 3-methylbutanal, 2-methylbutanal, (*E*)-2-heptenal, (*E*)-2-octenal, (*E*)-2-hexenal, geranial, citral;
- 10 ketones: butan-2-one, propan-2-one, pentan-2-one, hexan-2-one, heptan-2-one, octan-2-one, nonan-2-one, 3-hydroxybutan-2-one, camphor, β-damascenone;
- 19 esters: methyl acetate, ethyl acetate, propyl acetate, butyl acetate, hexyl acetate, octyl acetate, (*Z*)-3-hexenyl acetate, phenylethyl acetate, methyl butyrate, ethyl butyrate, butyl butyrate, butyl propanoate, ethyl valerate, methyl hexanoate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, methyl benzoate, ethyl benzoate;
- 4 heterocycles: (*Z*)-linalool oxide, (*E*)-linalool oxide, (*Z*)-rose oxide, nerol oxide;
- 6 hydrocarbons: (*Z*)-β-ocimene, limonene, α-terpinene, γ-terpinene, *o*-cymene, β-phellandrene; and
- 9 acids: acetic, propanoic, butanoic, hexanoic, octanoic, decanoic, 2-methylpropanoic, 3-methylbutanoic, 2-hydroxypropanoic acids.

Most of the compounds identified were previously detected in elderberries [5, 13].

As mentioned before, JENSEN et al. [12] divided the most important aroma compounds of the elderberries into six odour classes.

The characteristic elderberry odour is related to dihydroedulan, β-damascenone and ethyl-9-decenoate.

In the flowery group, (*Z*)- and (*E*)-rose oxide, nerol oxide, nonanal and hotrienol contribute significantly to elder flowery notes, whereas e.g. linalool, α-terpineol, benzyl alcohol, 2-phenylethyl alcohol and phenylacetaldehyde contribute to flowery notes [5, 12, 13].

The fruity odour of elderberry appears to be related to alcohols, aldehydes and esters of lower carboxylic acids and lower alcohols: in particular 2-methylpropan-1-ol, 2- and 3-methylbutan-1-ol, pentan-1-ol, pentanal, heptanal, octanal and methyl- and ethyl benzoate.

The grassy group is composed of aliphatic aldehydes and alcohols with typical odours of green grass: hexanal, hexan-1-ol, heptan-1-ol, octan-1-ol, (*E*)-2-hexen-1-al, (*Z*)-3-hexen-1-ol, (*E*)-3-hexen-1-ol, (*E*)-2-hexen-1-ol and (*E*)-2-octen-1-al [5, 13].

1-octen-3-one and 1-octen-3-ol with mushroom note and 3-hydroxybutan-2-one with creamy, buttery note belong to the agrestic group.

Benzaldehyde with its candy, sweet note could be placed to miscellaneous group, also lower carboxylic acids and ketones with creamy, oily or buttery odour.

Based on the olfactory evaluation compounds with characteristic elderberry odour are the most important, followed by fruity and flowery groups. The volatiles in the grassy group are important for the freshness of the elderberry [12].

On the basis of comparison to the literature (e.g. JENSEN et al. [12]; KAACK et al. [5]; KAACK [13]), compounds (*Z*)-3-hexen-1-ol, (*E*)-3-hexen-1-ol, (*E*)-2-hexen-1-ol, pentan-1-ol, hexan-1-ol, octan-1-ol, 2-phenylethyl alcohol, benzyl alcohol, 1-octen-3-ol, 2-methylbutan-1-ol, 3-methylbutan-1-ol, linalool, geraniol, α-terpineol, hotrienol, benzaldehyde, phenylacetaldehyde, pentanal, hexanal, heptanal, octanal, nonanal, (*E*)-2-hexenal, (*E*)-2-octenal, methyl acetate, ethyl acetate, butyl acetate, ethyl butyrate, ethyl octanoate, ethyl decanoate, (*Z*)-linalool oxide, (*Z*)-rose oxide, nerol oxide, (*Z*)-β-ocimene, β-damascenone and limonene were chosen as probably the most important components of the aroma of our samples, whose changes could express differences between cultivars. In particular these compounds were further monitored in samples of elderberry (Tab. 1A, 1B, 1C, 1D).

Comparison of volatile aroma compounds in elderberry cultivars

The volatile compounds of the samples were then compared in order to recommend the best cultivar with respect to the expected aroma properties. The comparison of the selected aroma compounds identified in cultivars is given in Tab. 1A, 1B, 1C, 1D, the comparison of the total content of compounds identified and the chemical groups of compounds is given in Tab. 2. The contents of the aroma compounds are expressed as micrograms (or milligrams) per kilogram of the sample. Differences among cultivars in the total content and chemical groups of aroma compounds, as well as in the case of single compounds, were observed in accordance with other authors [5, 7, 12, 13].

As can be seen from Tab. 2, the total content of aroma compounds was the significantly ($P < 0.05$)

Tab. 1A. Contents of selected aroma compounds in elderberry cultivars.

Aroma compounds [$\mu g \cdot kg^{-1}$]	Cultivar of elderberries			
	Albida	Allesö	Aurea	Bohatka
Alcohols				
2-Methylbutan-1-ol	587.67 ± 6.21^{a}	495.82 ± 106.88^{ac}	182.79 ± 7.09^{a}	7475.17 ± 609.56^{bg}
3-Methylbutan-1-ol	317.11 ± 2.08^{a}	nd	nd	523.24 ± 3.35^{b}
Pentan-1-ol	173.79 ± 1.84^{a}	57.62 ± 14.74^{b}	385.74 ± 17.79^{c}	151.90 ± 17.23^{ad}
Hexan-1-ol	751.27 ± 12.59^{a}	194.46 ± 8.27^{b}	435.86 ± 9.43^{c}	454.70 ± 24.47^{c}
(*E*)-2-hexen-1-ol	21.38 ± 0.57^{a}	nd	1.32 ± 0.02^{b}	nd
(*E*)-3-hexen-1-ol	nd	nd	10.53 ± 0.04^{a}	nd
(*Z*)-3-hexen-1-ol	61.49 ± 9.21^{a}	231.24 ± 26.00^{b}	115.51 ± 6.18^{cd}	77.94 ± 15.35^{a}
Octan-1-ol	193.07 ± 0.82^{a}	nd	nd	59.07 ± 0.15^{b}
1-Octen-3-ol	213.77 ± 9.03^{a}	nd	nd	160.47 ± 9.13^{b}
Benzyl alcohol	1.02 ± 0.01^{a}	nd	nd	2.02 ± 0.01^{b}
Phenylethyl alcohol	1.23 ± 0.01^{a}	nd	2.89 ± 0.07^{b}	nd
Linalool	2.79 ± 0.03^{a}	nd	1.18 ± 0.01^{a}	21.72 ± 0.73^{b}
Geraniol	1.05 ± 0.04^{a}	4.89 ± 0.08^{b}	nd	4.02 ± 0.10^{c}
α-Terpineol	213.56 ± 0.57^{a}	nd	nd	2699.56 ± 20.57^{b}
Hotrienol	4.09 ± 0.08^{a}	5.78 ± 0.12^{b}	nd	6.03 ± 0.99^{bcd}
Aldehydes				
Pentanal	176.71 ± 2.63^{a}	nd	nd	39.71 ± 5.43^{b}
Hexanal	67.55 ± 2.24^{a}	nd	nd	19.05 ± 2.54^{b}
(*E*)-2-hexenal	386.21 ± 5.52^{a}	72.29 ± 28.78^{b}	479.00 ± 106.83^{a}	155.21 ± 8.17^{c}
Heptanal	10.51 ± 0.08^{a}	nd	nd	6.61 ± 0.03^{b}
Octanal	3.09 ± 0.02^{a}	nd	nd	nd
(*E*)-2-octenal	1.05 ± 0.76^{a}	nd	nd	1.03 ± 0.04^{a}
Nonanal	5.16 ± 0.01^{a}	nd	nd	1.06 ± 0.01^{b}
Benzaldehyde	61.54 ± 3.76^{a}	57.95 ± 5.74^{a}	nd	16.92 ± 2.62^{b}
Phenylacetaldehyde	104.78 ± 9.88^{a}	nd	nd	108.68 ± 1.82^{a}
Ketones				
β-Damascenone	518.25 ± 56.19^{a}	429.39 ± 34.88^{ab}	400.12 ± 44.47^{b}	465.12 ± 62.44^{ab}
Esters				
Methyl acetate	253.56 ± 14.53^{a}	2717.87 ± 870.85^{be}	776.91 ± 64.87^{c}	4863.50 ± 370.49d
Ethyl acetate	42.27 ± 3.14^{a}	348.85 ± 29.98^{b}	349.05 ± 43.79^{b}	3397.35 ± 54.99c
Ethyl butyrate	3.02 ± 0.17^{a}	2.04 ± 0.75^{b}	nd	nd
Butyl acetate	369.13 ± 6.90^{a}	315.02 ± 30.00^{a}	521.09 ± 64.61^{b}	6.13 ± 0.90f
Ethyl octanoate	nd	3.14 ± 0.12^{a}	nd	nd
Ethyl decanoate	nd	5.02 ± 1.00^{a}	3.56 ± 0.08^{b}	1.02 ± 0.17c
Heterocycles				
(*Z*)-rose oxide	2.14 ± 0.06^{a}	1.68 ± 0.44^{a}	8.34 ± 0.95b	3.35 ± 0.01c
(*Z*)-linalool oxide	4.02 ± 0.12^{a}	nd	1.44 ± 0.04b	4.13 ± 0.70a
Nerol oxide	3.13 ± 0.90^{a}	5.09 ± 0.61^{bd}	4.12 ± 0.44b	nd
Hydrocarbons				
(*Z*)-β-ocimene	nd	7.87 ± 0.85^{ae}	3.68 ± 0.44b	5.85 ± 0.04c
Limonene	3.56 ± 0.02^{a}	8.85 ± 0.98^{bf}	nd	2.42 ± 0.21c

The results are expressed as mean ± standard deviation (n = 9). Different superscript letters in the same row indicate significant statistical differences ($P < 0.05$). nd – not detected.

Tab. 1B. Contents of selected aroma compounds in elderberry cultivars.

Aroma compounds [$\mu g \cdot kg^{-1}$]	Cultivar of elderberries			
	Dana	Haschberg	Korsör	Mammut
Alcohols				
2-Methylbutan-1-ol	199.79 ± 5.09a	533.98 ± 34.39^{ae}	3 699.03 ± 11.77^{df}	3 306.06 ± 96.92^{cef}
3-Methylbutan-1-ol	248.44 ± 6.32a	414.06 ± 5.16^{c}	nd	9.12 ± 1.36^{d}
Pentan-1-ol	148.63 ± 12.74^{ad}	32.70 ± 2.02^{b}	119.65 ± 19.25^{dg}	179.63 ± 29.78^{a}
Hexan-1-ol	328.41 ± 8.27^{d}	383.77 ± 18.83^{de}	471.71 ± 53.18^{ce}	230.37 ± 2.51^{b}
(*E*)-2-hexen-1-ol	11.24 ± 0.51^{c}	6.47 ± 0.33^{d}	1.44 ± 0.13^{b}	nd
(*E*)-3-hexen-1-ol	8.21 ± 0.04^{a}	nd	19.82 ± 0.79^{b}	nd
(*Z*)-3-hexen-1-ol	101.46 ± 7.24^{d}	170.56 ± 10.41^{e}	275.83 ± 15.08^{b}	34.91 ± 1.59^{f}
Octan-1-ol	5.02 ± 0.31^{c}	142.88 ± 6.25^{d}	nd	nd
1-Octen-3-ol	12.72 ± 1.24^{c}	198.77 ± 11.67^{a}	nd	nd
Benzyl alcohol	5.03 ± 0.23^{c}	2.06 ± 0.43^{b}	nd	nd
Phenylethyl alcohol	1.26 ± 0.02^{a}	3.64 ± 0.64^{b}	nd	7.10 ± 1.03^{c}
Linalool	13.12 ± 1.53^{c}	nd	1.65 ± 0.10^{a}	nd
Geraniol	2.35 ± 0.45^{d}	nd	7.21 ± 1.47^{e}	2.65 ± 0.54^{df}
α-Terpineol	104.29 ± 3.57^{c}	161.79 ± 2.06^{d}	nd	nd
Hotrienol	5.88 ± 0.47^{bd}	2.76 ± 0.03^{e}	nd	nd
Aldehydes				
Pentanal	nd	13.08 ± 1.96^{c}	nd	6.34 ± 1.08^{d}
Hexanal	14.49 ± 1.46^{b}	2.15 ± 0.06^{c}	7.91 ± 0.07^{d}	nd
(*E*)-2-hexenal	49.08 ± 6.74^{b}	115.35 ± 1.40^{d}	nd	nd
Heptanal	9.21 ± 0.06^{c}	4.87 ± 0.03^{d}	nd	nd
Octanal	nd	2.09 ± 0.01^{b}	nd	nd
(*E*)-2-octenal	nd	nd	nd	nd
Nonanal	nd	5.17 ± 0.01^{a}	12.84 ± 0.78^{c}	nd
Benzaldehyde	4.12 ± 0.47^{c}	19.88 ± 1.69^{b}	nd	nd
Phenylacetaldehyde	5.33 ± 0.05^{b}	143.78 ± 5.38^{c}	nd	nd
Ketones				
β-Damascenone	31.75 ± 6.08^{c}	394.62 ± 4.23^{b}	240.83 ± 53.23^{d}	471.92 ± 1.38^{a}
Esters				
Methyl acetate	86.51 ± 3.86a	787.20 ± 21.02^{c}	3796.46 ± 161.69^{e}	2 792.17 ± 355.56^{b}
Ethyl acetate	394.35 ± 14.45b	348.20 ± 75.27^{b}	69 229.85 ± 370.00^{d}	1 489.90 ± 271.26^{c}
Ethyl butyrate	nd	nd	nd	nd
Butyl acetate	nd	nd	nd	nd
Ethyl octanoate	15.02 ± 1.00b	nd	nd	nd
Ethyl decanoate	5.47 ± 0.78a	nd	nd	nd
Heterocycles				
(*Z*)-rose oxide	6.46 ± 0.69d	2.00 ± 0.23^{a}	nd	4.24 ± 0.17^{e}
(*Z*)-linalool oxide	9.85 ± 0.09c	nd	7.79 ± 0.94^{d}	13.11 ± 0.90^{e}
Nerol oxide	2.62 ± 0.21a	nd	nd	nd
Hydrocarbons				
(*Z*)-β-ocimene	1.79 ± 0.09d	nd	nd	7.79 ± 0.94^{a}
Limonene	2.44 ± 0.32c	nd	6.78 ± 0.84^{be}	nd

The results are expressed as mean ± standard deviation (n = 9). Different superscript letters in the same row indicate significant statistical differences ($P < 0.05$). nd – not detected.

Tab. 1C. Contents of selected aroma compounds in elderberry cultivars.

Aroma compounds [$\mu g \cdot kg^{-1}$]	Cultivar of elderberries				
	Pregarten	Riese aus Voβloch	Sambo	Sampo	Sambu
Alcohols					
2-Methylbutan-1-ol	3520.24 ± 1166.10^{df}	10071.90 ± 51.66^{g}	5576.26 ± 534.42^{bf}	32.62 ± 3.45^{a}	24.62 ± 1.40^{a}
3-Methylbutan-1-ol	402.14 ± 4.14^{c}	nd	nd	244.04 ± 13.27^{a}	4.87 ± 0.47^{d}
Pentan-1-ol	160.40 ± 41.19^{ad}	42.23 ± 9.17^{b}	120.41 ± 24.29^{dg}	536.94 ± 36.57^{e}	381.60 ± 97.17^{c}
Hexan-1-ol	236.92 ± 36.04^{b}	261.20 ± 4.32^{bd}	602.15 ± 7.55^{f}	104.69 ± 7.31^{g}	74.62 ± 1.40^{g}
(*E*)-2-hexen-1-ol	5.07 ± 0.06^{e}	2.57 ± 0.35^{b}	nd	nd	4.11 ± 0.24^{e}
(*E*)-3-hexen-1-ol	nd	25.22 ± 7.33^{b}	9.69 ± 0.48^{a}	11.75 ± 0.71^{a}	nd
(*Z*)-3-hexen-1-ol	55.97 ± 9.36^{a}	686.83 ± 47.44^{g}	133.01 ± 17.53^{hc}	62.40 ± 2.13^{a}	31.70 ± 9.72^{f}
Octan-1-ol	87.14 ± 2.83^{e}	nd	nd	121.40 ± 3.48^{df}	nd
1-Octen-3-ol	146.60 ± 5.27^{b}	nd	nd	393.94 ± 16.57^{d}	nd
Benzyl alcohol	nd	670.60 ± 35.11^{d}	nd	nd	nd
Phenylethyl alcohol	2.12 ± 0.51^{d}	nd	3.01 ± 0.43^{bd}	nd	nd
Linalool	9.75 ± 0.07^{c}	nd	nd	21.75 ± 0.71^{b}	nd
Geraniol	1.22 ± 0.03^{a}	1.48 ± 0.11^{a}	nd	nd	3.70 ± 0.72^{cf}
α-Terpineol	70.85 ± 2.36^{e}	nd	nd	264.89 ± 5.41^{f}	nd
Hotrienol	7.11 ± 0.89^{d}	4.57 ± 1.03^{ac}	nd	8.08 ± 1.36^{d}	nd
Aldehydes					
Pentanal	33.12 ± 2.08^{b}	nd	nd	81.72 ± 0.37^{e}	41.52 ± 2.37^{b}
Hexanal	81.33 ± 4.31^{e}	nd	nd	26.49 ± 0.74^{f}	nd
(*E*)-2-hexenal	24.06 ± 2.28^{e}	nd	nd	21.52 ± 0.13^{e}	nd
Heptanal	3.58 ± 0.89^{e}	nd	nd	7.15 ± 0.11^{f}	nd
Octanal	2.77 ± 0.86^{ab}	nd	nd	nd	nd
(*E*)-2-octenal	1.01 ± 0.03^{a}	nd	nd	1.02 ± 0.02^{a}	nd
Nonanal	1.38 ± 0.03^{d}	nd	nd	2.05 ± 0.21^{e}	nd
Benzaldehyde	12.75 ± 3.33^{b}	185.63 ± 30.21^{d}	17.94 ± 5.25^{b}	2.14 ± 0.02^{c}	nd
Phenylacetaldehyde	104.86 ± 3.46^{a}	nd	nd	152.14 ± 1.32^{c}	nd
Ketones					
β-Damascenone	282.41 ± 49.24^{d}	510.38 ± 44.82^{a}	nd	541.48 ± 74.92^{ae}	517.06 ± 44.82^{a}
Esters					
Methyl acetate	2738.63 ± 145.33^{b}	1031.04 ± 6.97^{c}	3239.29 ± 870.85^{be}	nd	9.49 ± 0.64^{f}
Ethyl acetate	33824.24 ± 239.17^{e}	2380.32 ± 437.86^{c}	147.80 ± 3.17^{b}	21.04 ± 0.03^{a}	nd
Ethyl butyrate	nd	nd	nd	nd	nd
Butyl acetate	1387.79 ± 48.94^{c}	nd	nd	169.15 ± 3.45^{d}	164.99 ± 3.45^{d}
Ethyl octanoate	nd	4.86 ± 0.47^{c}	nd	1.24 ± 0.02^{d}	4.74 ± 0.49^{c}
Ethyl decanoate	1.45 ± 0.01^{d}	nd	nd	1.08 ± 0.03^{c}	nd
Heterocycles					
(*Z*)-rose oxide	2.63 ± 0.03^{a}	6.70 ± 0.62^{bd}	nd	2.68 ± 0.16^{a}	2.93 ± 0.02^{ac}
(*Z*)-linalool oxide	nd	9.19 ± 0.85^{cd}	nd	2.32 ± 0.86^{b}	1.60 ± 0.31^{b}
Nerol oxide	1.15 ± 0.45^{c}	7.80 ± 1.17^{d}	6.15 ± 0.45^{d}	3.79 ± 0.94^{ab}	1.15 ± 0.22^{c}
Hydrocarbons					
(*Z*)-β-ocimene	1.04 ± 0.97^{d}	nd	1.44 ± 0.02^{d}	nd	9.92 ± 0.24^{e}
Limonene	2.32 ± 0.86^{c}	4.79 ± 0.54^{d}	6.08 ± 0.03^{e}	nd	nd

The results are expressed as mean ± standard deviation ($n = 9$). Different superscript letters in the same row indicate significant statistical differences ($P < 0.05$). nd – not detected.

Tab. 1D. Contents of selected aroma compounds in elderberry cultivars.

Aroma compounds [$\mu g \cdot kg^{-1}$]	Cultivar of elderberries			
	Samdal	Samyl	Weihenstephan	Wild elder
Alcohols				
2-Methylbutan-1-ol	5220.53 ± 1348.57^{bf}	64.44 ± 3.81^{a}	101.16 ± 9.14^{a}	nd
3-Methylbutan-1-ol	nd	287.54 ± 23.47^{a}	260.16 ± 4.12^{a}	nd
Pentan-1-ol	33.40 ± 1.18^{b}	721.07 ± 60.96^{f}	28.79 ± 0.28^{b}	96.75 ± 8.22^{g}
Hexan-1-ol	457.29 ± 141.13^{cf}	181.37 ± 9.51^{b}	212.12 ± 36.04^{b}	161.25 ± 26.04^{b}
(*E*)-2-hexen-1-ol	nd	nd	nd	nd
(*E*)-3-hexen-1-ol	16.12 ± 4.07^{ab}	259.84 ± 17.12^{c}	nd	nd
(*Z*)-3-hexen-1-ol	153.97 ± 44.22^{cde}	406.40 ± 34.23^{i}	169.63 ± 26.04^{eh}	33.65 ± 5.13^{f}
Octan-1-ol	nd	98.04 ± 3.53^{ef}	39.23 ± 2.47^{b}	nd
1-Octen-3-ol	nd	141.65 ± 5.53^{be}	121.13 ± 6.14^{e}	nd
Benzyl alcohol	nd	nd	2.18 ± 0.29^{b}	nd
Phenylethyl alcohol	nd	2.07 ± 0.37^{d}	nd	nd
Linalool	nd	128.89 ± 5.22^{d}	22.53 ± 6.01^{b}	nd
Geraniol	5.02 ± 0.45^{b}	nd	3.45 ± 0.28^{f}	nd
α-Terpineol	nd	236.97 ± 4.37^{g}	380.05 ± 2.64^{h}	nd
Hotrienol	nd	2.56 ± 0.47^{e}	6.87 ± 1.37^{bd}	nd
Aldehydes				
Pentanal	nd	18.11 ± 2.56^{f}	19.05 ± 0.26^{f}	38.17 ± 2.16^{b}
Hexanal	nd	31.58 ± 1.36^{g}	14.30 ± 1.78^{b}	nd
(*E*)-2-hexenal	nd	22.68 ± 1.27^{e}	110.82 ± 9.14^{d}	111.47 ± 8.22^{d}
Heptanal	nd	7.17 ± 1.22^{bf}	3.13 ± 0.18^{e}	nd
Octanal	nd	nd	1.05 ± 0.08^{c}	4.97 ± 0.03^{d}
(*E*)-2-octenal	nd	2483.48 ± 171.16^{b}	nd	nd
Nonanal	nd	8.32 ± 1.26^{f}	5.01 ± 0.02^{a}	nd
Benzaldehyde	14.63 ± 0.87^{b}	15.69 ± 2.62^{b}	17.20 ± 3.67^{b}	90.42 ± 4.16^{e}
Phenylacetaldehyde	nd	68.13 ± 2.82^{d}	110.02 ± 4.58^{a}	nd
Ketones				
β-Damascenone	414.04 ± 113.83^{ab}	647.95 ± 97.40^{e}	329.73 ± 91.94^{bd}	329.04 ± 110.33^{bd}
Esters				
Methyl acetate	4448.73 ± 1404.30^{de}	18.25 ± 0.70^{a}	98.96 ± 4.84^{a}	597.24 ± 14.53^{c}
Ethyl acetate	57082.61 ± 1368.42^{f}	12.93 ± 4.02^{a}	31.44 ± 4.82^{a}	116.93 ± 4.11^{b}
Ethyl butyrate	nd	1.15 ± 0.22^{c}	nd	nd
Butyl acetate	nd	992.92 ± 27.24^{e}	180.24 ± 4.78^{d}	nd
Ethyl octanoate	nd	nd	nd	nd
Ethyl decanoate	nd	1.25 ± 0.23^{c}	1.65 ± 0.08^{d}	nd
Heterocycles				
(*Z*)-rose oxide	3.92 ± 0.24^{e}	6.29 ± 0.67^{d}	2.44 ± 0.82^{ac}	nd
(*Z*)-linalool oxide	nd	4.92 ± 0.24^{a}	7.20 ± 0.67^{d}	8.32 ± 0.12^{cd}
Nerol oxide	1.21 ± 0.20^{c}	nd	1.02 ± 0.58^{c}	3.04 ± 0.33^{a}
Hydrocarbons				
(*Z*)-β-ocimene	7.85 ± 1.06^{ae}	1.55 ± 0.25^{d}	9.32 ± 0.12^{e}	nd
Limonene	2.24 ± 0.61^{c}	9.92 ± 0.24^{f}	3.04 ± 0.33^{a}	7.32 ± 0.12^{b}

The results are expressed as mean ± standard deviation (n = 9). Different superscript letters in the same row indicate significant statistical differences ($P < 0.05$). nd – not detected.

Tab. 2. Contents of chemical groups of compounds in elderberry cultivars.

Elderberry cultivar	Aroma compounds							
	Alcohols [mg·kg^{-1}]	Aldehydes [mg·kg^{-1}]	Ketones [mg·kg^{-1}]	Esters [mg·kg^{-1}]	Acids [mg·kg^{-1}]	Heterocycles [μg·kg^{-1}]	Hydrocarbons [μg·kg^{-1}]	Total [mg·kg^{-1}]
Albida	3854.56 ± 204.63^{aA}	5.15 ± 0.54^{aB}	1.08 ± 0.11^{aC}	0.66 ± 0.02^{aC}	14.38 ± 4.38^{adD}	9.03 ± 0.06^{aE}	3.56 ± 0.02^{aE}	3876.69 ± 204.67^{a}
Allesö	1006.63 ± 120.65^{bjA}	38.66 ± 3.12^{bB}	1.15 ± 0.18^{aC}	3.38 ± 0.20^{bC}	1.21 ± 0.08^{beC}	6.77 ± 0.44^{bD}	16.72 ± 0.80^{bD}	1048.08 ± 122.86^{b}
Aurea	82.26 ± 2.14^{cA}	52.81 ± 5.31^{cfB}	0.71 ± 0.02^{bC}	1.66 ± 0.57^{cC}	2.03 ± 0.05^{bC}	13.9 ± 0.44^{cD}	3.68 ± 0.44^{adD}	136.04 ± 5.75^{c}
Bohatka	561.28 ± 47.24^{dA}	11.00 ± 1.64^{dB}	2.04 ± 0.56^{cfC}	8.26 ± 0.43^{dB}	1.76 ± 0.03^{bC}	7.48 ± 0.60^{bD}	8.27 ± 0.10^{cD}	585.67 ± 47.64^{d}
Dana	1.19 ± 0.02^{eA}	0.08 ± 0.01^{eB}	0.03 ± 0.01^{dC}	0.51 ± 0.03^{agD}	13.08 ± 0.45^{aE}	18.93 ± 0.20^{dC}	4.23 ± 0.13^{dF}	14.82 ± 0.02^{e}
Haschberg	71.43 ± 1.14^{cA}	60.15 ± 6.95^{fA}	1.08 ± 0.02^{aB}	1.14 ± 0.60^{acB}	2.38 ± 0.15^{bB}	2.06 ± 0.23^{eC}	nd	136.15 ± 7.07^{c}
Korsör	271.67 ± 27.27^{fA}	9.24 ± 0.02^{gB}	0.61 ± 0.02^{eC}	73.08 ± 0.53^{eD}	3.47 ± 0.28^{cE}	7.79 ± 0.94^{bfF}	6.78 ± 0.84^{eF}	358.04 ± 29.13^{f}
Mammut	1112.29 ± 83.66^{bgjA}	26.08 ± 7.98^{hiB}	2.04 ± 0.58^{cfC}	4.28 ± 0.63^{bC}	3.79 ± 0.02^{cC}	17.35 ± 0.50^{gD}	7.79 ± 0.94^{ceD}	1146.60 ± 84.24^{b}
Pregarten	1197.00 ± 29.22^{gA}	40.24 ± 14.89^{bcfB}	1.48 ± 0.14^{cC}	36.58 ± 0.39^{fB}	17.38 ± 3.32^{dD}	3.78 ± 0.14^{hE}	3.36 ± 0.82^{adE}	1294.05 ± 36.02^{g}
Riese aus Voßloch	823.27 ± 41.07^{hA}	18.60 ± 2.87^{hB}	1.75 ± 0.05^{fC}	3.45 ± 0.45^{bC}	3.14 ± 0.54^{cC}	23.69 ± 0.95^{iD}	4.79 ± 0.54^{dD}	849.03 ± 41.49^{h}
Sambo	713.15 ± 55.08^{iA}	5.47 ± 0.63^{aB}	0.45 ± 0.04^{gC}	3.41 ± 1.87^{bBD}	1.35 ± 0.24^{beD}	6.15 ± 0.45^{bkE}	7.52 ± 0.02^{eE}	721.17 ± 55.31^{i}
Sampo	1043.40 ± 108.30^{bjA}	2.29 ± 0.10^{iB}	0.86 ± 0.12^{hC}	0.16 ± 0.01^{gC}	3.81 ± 0.03^{cB}	8.79 ± 0.86^{afD}	nd	1051.08 ± 108.31^{b}
Sambu	1044.33 ± 118.87^{bjA}	3.18 ± 0.43^{iB}	0.71 ± 0.02^{bC}	0.17 ± 0.01^{gC}	1.29 ± 0.04^{beC}	5.68 ± 0.22^{jkD}	9.92 ± 0.24^{fD}	1047.57 ± 118.89^{b}
Samdal	680.52 ± 47.11^{iA}	9.11 ± 0.88^{dgB}	1.89 ± 0.04^{cfC}	61.53 ± 2.77^{hD}	1.30 ± 0.84^{eC}	5.13 ± 0.21^{jE}	10.09 ± 0.90^{fE}	754.21 ± 46.15^{i}
Samyl	1182.31 ± 108.56^{bgA}	6.55 ± 0.70^{aB}	1.10 ± 0.06^{aC}	1.03 ± 0.03^{cC}	12.89 ± 0.71^{aD}	11.21 ± 0.32^{lE}	11.47 ± 0.23^{gE}	1204.74 ± 108.73^{bg}
Weihenstephan	1009.23 ± 48.44^{jA}	28.69 ± 5.29^{iB}	0.71 ± 0.08^{beC}	0.13 ± 0.04^{gC}	4.17 ± 2.08^{bcD}	10.66 ± 0.62^{lE}	12.36 ± 0.24^{hE}	1041.83 ± 48.77^{b}
Wild elder	2392.32 ± 95.67^{kA}	44.27 ± 6.22^{bcB}	0.72 ± 0.07^{bC}	0.71 ± 0.02^{aC}	2.25 ± 0.23^{bD}	11.36 ± 0.12^{lE}	7.32 ± 0.12^{eE}	2437.48 ± 96.14^{j}

The results are expressed as mean ± standard deviation (n = 9). Different superscript letters in the same column (capital letter in the same row) indicate significant statistical differences ($P < 0.05$). nd – not detected.

highest in Albida cultivar and in wild elder, while the lowest in Aurea, Bohatka, Dana, Haschberg and Korsör cultivars.

Alcohols contributed the most to the aroma profile of all cultivars concerning the quantity (except Dana cultivar); they mostly created about 60–95% of the total content of aroma compounds. Albida cultivar and wild elder contained the significantly ($P < 0.05$) highest content of alcohols, which was in particular caused by quite high contents (> 10 mg·kg^{-1}) of methanol, ethanol, propan-2-ol and 2-methylpropan-1-ol. Although present in higher contents, these compounds are not considered as typical and important for characteristic elder aroma [12]. Aurea, Bohatka, Dana, Haschberg and Korsör cultivars had the lowest ($P < 0.05$) contents of alcohols.

Aldehydes were the second most abundant group of compounds; they contributed significantly ($P < 0.05$) to the aroma profile of Aurea and Haschberg cultivars. The high content of aldehydes was also found in cultivars Allesö, Pregarten and Weihenstephan, and in wild elder. This fact was caused by a particularly high content (> 20 mg·kg^{-1}) of ethanal, which is, however, also not considered as important for the elder aroma [12, 13].

Esters were the third important group, in particular in Korsör, Samdal and Pregarten cultivars. The contents of methyl- and ethyl acetate, and moreover butyl acetate in the case of Pregarten cultivar, were the highest (> 1 mg·kg^{-1}). Many esters were identified in various elder samples so far and they are known as important components of the elderberry aroma, contributing more precisely to its fruity note [5, 12, 13].

From the quantitative point of view, acids contributed particularly to the aroma profile of

Dana cultivar, because a surprisingly low content of alcohols was found in this cultivar. Further, Albida, Pregarten and Samyl cultivars contained higher contents of acids, in particular acetic acid (> 10 mg·kg^{-1}). This compound is also not considered as important for the elder aroma [12, 13].

Significantly ($P < 0.05$) lower contents of ketones, heterocycles and hydrocarbons, compared to the above mentioned groups, were found in all cultivars (Tab. 2). Bohatka, Mammut, Riese aus Voßloch and Samdal cultivars had the highest contents of ketones, in particular propan-2-one (> 0.2 mg·kg^{-1}), which is not important for the elder aroma, and β-damascenone (> 0.4 mg·kg^{-1}), which is related to the characteristic elderberry odour [5, 12, 13]. (*Z*)-β-ocimene and limonene were the most abundant hydrocarbons, present in a range 1–10 μg·kg^{-1}, except Haschberg and Sampo cultivars, where no hydrocarbons were identified. These two compounds were also identified by several authors as important in elderberries [5]. (*Z*)-rose oxide, (*Z*)-linalool oxide and nerol oxide were the most abundant heterocycles, present in a range 1–14 μg·kg^{-1}. (*Z*)-rose oxide and nerol oxide are considered to contribute to elder aroma with elder flower notes [5, 12, 13].

The most surprising finding was that wild elder had the second highest content of aroma compounds, which was similar to Kaack [9], who compared extracts from wild and cultivated elder flowers. Wild elderberries have markedly bitter, sour and astringent taste and a mild, not intense aroma. They are cultivated to reach, among others, better taste and aroma. Therefore, a much richer and more varied aroma profile could be expected in the cultivated types. However, we should take into consideration that several volatile compounds identified were present in relatively high contents but probably are not so necessary for the typical aroma of elderberries. On the other hand, compounds that are known as significant components of the elderberry aroma, were found in very low contents. This is in accordance with the well established concept of aroma value of the compound, which is calculated by dividing the content of the compound in a food by its odour threshold (e.g. acquired from the literature). Compounds with higher aroma values (lower threshold values) are considered as contributing to the aroma [24]. The higher total content of volatile aroma compounds does not necessary mean a more intense aroma of the sample.

Concerning single selected aroma compounds (Tab. 1), only pentan-1-ol, hexan-1-ol and (*Z*)-3-hexen-1-ol were present in all cultivars.

β-damascenone as a contributor to the typical elderberry aroma [5, 13] was identified in all cultivars except Sambo. Dihydroedulan and ethyl-9-decenoate, also known as components of the elderberry aroma [5, 13], were not present in our samples.

In the group of compounds with elder flower note [12], (*Z*)-rose oxide, nerol oxide, nonanal, hotrienol and (*Z*)-linalool were compared, and their contents in various cultivars were found to be in the range about 5–10 μg·kg^{-1}.

From the flowery group [12], (*Z*)-β-ocimene, geraniol, benzyl alcohol and 2-phenylethyl alcohol were present in contents 1–8 μg·kg^{-1}, with the exception of Riese aus Voβloch cultivar, which contained a significantly ($P < 0.05$) high amount of benzyl alcohol (670.60 ± 35.11) μg·kg^{-1}. Linalool was present at contents 5–15 μg·kg^{-1}, except for Samyl cultivar (128.89 ± 5.22) μg·kg^{-1}. Phenylacetaldehyde (100–120 μg·kg^{-1}) and α-terpineol (200–300 μg·kg^{-1}) were the most abundant in this group, in particular cultivar Bohatka contained a significantly ($P < 0.05$) high amount of α-terpineol (2.69 ± 0.02) mg·kg^{-1}.

Esters are mainly responsible for the fruity note of elderberry [12]. Several esters were followed (Tab. 1), methyl acetate (1–4 mg·kg^{-1}) and ethyl acetate, in particular in Korsör (69.23 ± 0.37) mg·kg^{-1}, Pregarten (33.82 ± 0.24) mg·kg^{-1} and Samdal (57.08 ± 1.37) mg·kg^{-1} cultivars, were the most important. Several aldehydes from this group were identified, heptanal and octanal (3–8 μg·kg^{-1}) and pentanal (20–40 μg·kg^{-1}) with significantly high ($P < 0.05$) content in Albida (176.71 ± 2.63) μg·kg^{-1}. Pentan-1-ol (100–400 μg·kg^{-1}), 3-methylbutan-1-ol (200–500 μg·kg^{-1}) and 2-methylbutan-1-ol (100–3 000 μg·kg^{-1}) with significantly ($P < 0.05$) high content in cultivars Bohatka (7.48 ± 0.61) mg·kg^{-1} and Riese aus Voβloch (10.07 ± 0.05) mg·kg^{-1}, were the most important alcohols in this group.

Regarding the important aldehydes from grassy group [12], hexanal was present mainly in cultivars Albida (67.55 ± 2.24) μg·kg^{-1} and Pregarten (81.33 ± 4.31) μg·kg^{-1}; (*E*)-2-hexen-1-al in cultivars Albida (386.21 ± 5.52) μg·kg^{-1} and Aurea (479.00 ± 106.83) μg·kg^{-1}. The important alcohols from grassy group [12] hexan-1-ol, octan-1-ol and (*Z*)-3-hexen-1-ol were present in the range about 100–500 μg·kg^{-1}, (*E*)-3-hexen-1-ol in particular in Samyl cultivar (259.84 ± 17.12) μg·kg^{-1}.

The last two groups of compounds are probably not necessary for the typical aroma of elderberry [5, 12, 13]. 1-octen-3-ol belonging to the agrestic group was present in contents about 150–400 μg·kg^{-1}, 1-octen-3-one, albeit known as

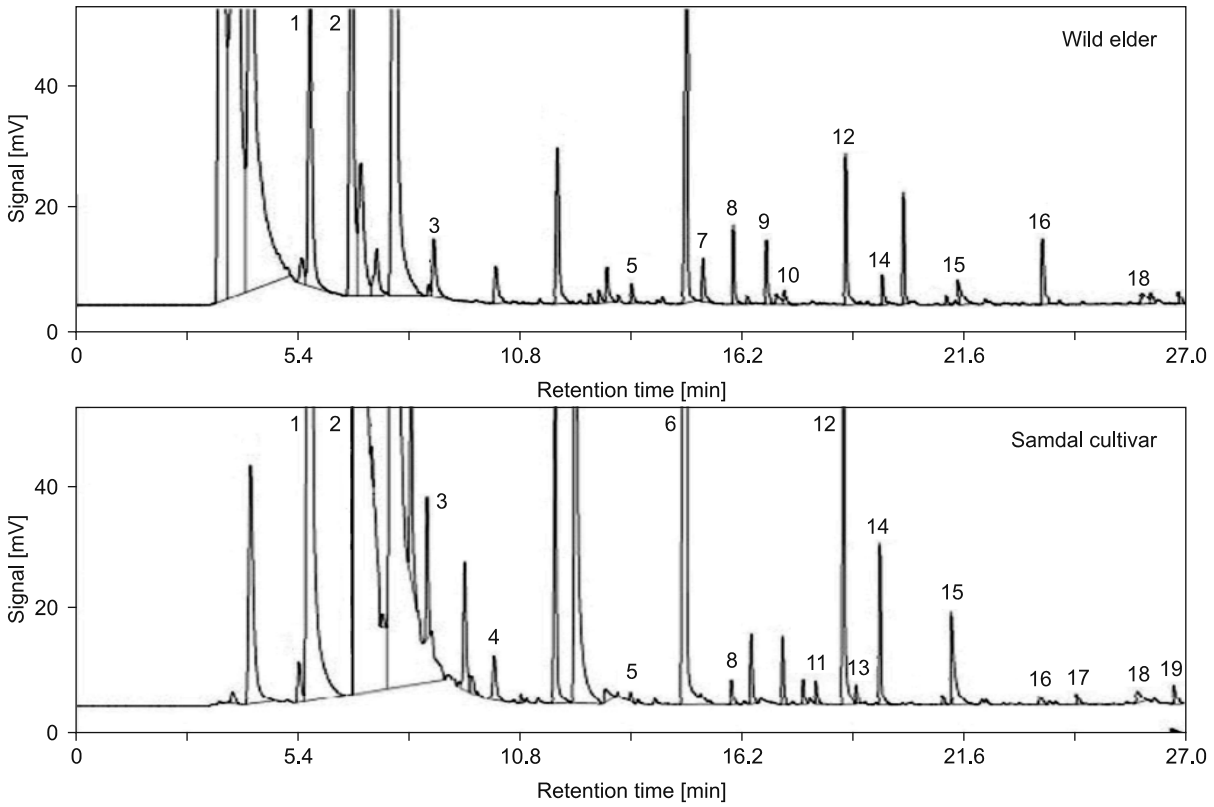

Fig. 1. Chromatograms of selected aroma compounds in wild elder (upper) and Samdal cultivar (lower).

Peak designation: 1 – methyl acetate, 2 – ethyl acetate, 3 – pentanal, 4 – ethyl butyrate, 5 – limonene, 6 – 2-methylbutan-1-ol, 7 – (*E*)-2-hexenal, 8 – pentan-1-ol, 9 – octanal, 10 – (*Z*)-linalool oxide, 11 – (*Z*)-rose oxide, 12 – hexan-1-ol, 13 – (*E*)-3-hexen-1-ol, 14 – (*Z*)-3-hexen-1-ol, 15 – nerol oxide, 16 – benzaldehyde, 17 – geraniol, 18 – β-damascenone, 19 – (*Z*)-β-ocimene.

the member of this group [5, 12], was not identified in our samples. Benzaldehyde, placed to miscellaneous group [12], was present in contents about 20–50 $\mu g \cdot kg^{-1}$, except for the cultivar Riese aus Voβloch (185.63 ± 30.21) $\mu g \cdot kg^{-1}$.

Taking into consideration the highest total content of the selected compounds, which are known to be involved in the aroma of elderberry [5, 12, 13], we could recommend Korsör (77.89 ± 3.57) $mg \cdot kg^{-1}$, Pregarten (43.20 ± 7.14) $mg \cdot kg^{-1}$ and Samdal (67.85 ± 8.22) $mg \cdot kg^{-1}$ cultivars for possible practical use.

For illustration, the chromatogram of compounds identified in Samdal cultivar is presented in Fig. 1, compared to a chromatogram of wild elderberry. Although the total content of volatile compounds in wild elder was high (Tab. 2), a lower number of selected compounds was present in lower quantities, with the lowest ($P < 0.05$) total content (1.59 ± 0.03) $mg \cdot kg^{-1}$ of them.

CONCLUSION

Although several articles about aroma profile of elder berries have been published up to now, this work is focused on selected cultivars intended to be grown in Czech Republic. One-hundred-and-two volatile compounds were identified; alcohols, aldehydes and esters being the most abundant. Thirty-six from the identified compounds were followed as possible components of the aroma. Taking into consideration the highest contents of these compounds, three promising cultivars (Korsör, Pregarten and Samdal) from the sixteen tested were recommended for growing on a large scale.

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COMPARISON OF FLAVOUR AND VOLATILE FLAVOUR COMPOUNDS OF MIXED ELDERBERRY JUICES

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Abstract

VÍTOVÁ EVA, SŮKALOVÁ KATEŘINA, MAHDALOVÁ MARTINA, BUTOROVÁ LENKA, BABÁK LIBOR, MATĚJÍČEK ALEŠ. 2015.Comparison of Flavour and Volatile Flavour Compounds of Mixed Elderberry Juices. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis,* 63(1): 147–152.

The aim of this work was to find the best composition for fruit drink based on elderberries with optimal flavour characteristics. For this purpose elderberry juice was mixed with various fruit juices (grape, black currant, apple, orange, carrot) in various ratios, flavour was evaluated sensorially and instrumentally as the content of aroma compounds.
Five flavour characteristics (sweet, acid/sour, bitter, astringent, characteristic elderberry), off-flavour, odour, texture (mouth-feel), colour and overall acceptability were evaluated sensorially using scale. Aroma compounds were extracted by solid phase microextraction and assessed by gas chromatography with flame ionization detection and gas chromatography-mass spectrometry. The significant differences ($P < 0.05$) in flavour were found between samples, which could be explained by differences in their volatile profiles. In total 57 compounds were identified in fruit juices and included 20 alcohols, 10 aldehydes, 8 ketones, 7 acids, 7 esters and 5 other compounds. Alcohols were quantitatively the most important group of all juices. The grape-elderberry juice, in optimum ratio 7:3 (70% v/v of elderberry), was proposed for practical use owing to the pleasant sweetish, elderberry flavour, and excellent other sensory characteristics.

Keywords: elderberry, flavour, aroma compounds, GC, SPME, sensory analysis

INTRODUCTION

The popularity of functional beverages led to the search for other sources of raw materials that provide great taste and functionality to consumers. Black elder (*Sambucus nigra* L.) is a plant with miscellaneous use, for its therapeutic effects in medicine, for its aroma and taste in the cuisine. Wild elderberry fruits and flowers are used mainly for the home made production of marmalades, juices, syrups, teas, liqueurs and wines. Fruits are known by high content of anthocyanine pigments and they are used for production of natural colorants (Karovicova *et al.*, 1990). The production of cultivated elderberries in Czech Republic is only at the beginning, Czech food industry uses only imported frozen elderberries as fruit component of yoghurts. However, elderberry contains many health promoting substances (Christensen *et al.*, 2008; Veberic *et al.*, 2009), which, in the form of appropriate modern food products, could enrich the consumer market. Successful commercialization of elderberry fruits depends especially on good flavour, which is related to the content of sugars and acids, and aroma, which is strongly associated to the content of volatile aroma active substances (Kaack *et al.*, 2005).

The aroma of elder flowers (Velisek *et al.*, 1981; Jørgensen *et al.*, 2000; Kaack and Christensen, 2008) and elder berries (Davidek *et al.*, 1982; Jensen *et al.*, 2000) have been characterized before in detail by several authors and more than 100 volatiles have been identified. Most of them are well known

aroma constituents of fruit products. Several authors also dealt with sensory evaluation of flavour of elderberries. They mostly evaluated selected descriptors, e.g. sweetness, sourness, fruitness, freshness, flowerness and bitterness using scale: 1-the lowest intensity ⇒ 5-the highest intensity (e.g. Kaack, 2008a; Kaack, 2008b). The others used preference test using scale: 0-without elderberry flavour to 10-intensive elderberry flavour (e.g. Kaack *et al*., 2005; Kaack *et al*., 2006).

The aim of this work was to identify and quantify the volatiles in elderberry juice mixed with various types of fruit juices in various ratios in order to find the best composition for attractive and healthy fruit drink based on elderberries with optimal flavour. Aroma compounds were extracted by solid phase microextraction (SPME) and assessed by GC-FID and GC-MS, the flavour was evaluated sensorially. SPME is very useful alternative to other tedious or expensive extraction methods and have been used by many authors to measure the volatile compounds of food samples (reviewed by Kataoka *et al*., 2000) including various fruit and vegetable juices (Riu-Aumatell *et al*., 2004; Soria *et al*., 2008; Aprea *et al*., 2009; Li *et al*., 2010; Schmarr and Bernhardt, 2010) and wines (Verzera *et al*., 2008; Olivero and Trujillo, 2010). Many works about sensory evaluation of various types of fruit and fruit juices were also published (Jain *et al*., 2003; Cliffe-Byrnes and O'Beirne, 2007; Perez-Cacho *et al*., 2008; Altisent *et al*., 2011; Baroň and Kumšta, 2012).

MATERIALS AND METHODS

Samples

Six types of fruit juices were analysed: grape, black currant, apple, orange, carrot and elderberry (standard). The various mixtures of elderberry with other fruit juices were prepared in a ratio 3:7 (30% v/v of elderberry). Then the mixture elderberry-grape was tested in various proportions (50–90% v/v of elderberry). Sample labeling: E: pure elderberry, G: pure grape, E-gr: elderberry-grape, E-bc: elderberry-black currant, E-ap: elderberry-apple, E-or: elderberry-orange, E-ca: elderberry-carrot. E-gr 50 to E-gr 90: elderberry-grape in various proportions.

Elderberry juice was acquired by pressing of fresh berries (Breeding institute of Faculty of Agriculture, Mendel University in Brno, Lednice, Czech Republic, picked in September 2012), pasteurized (78 °C 30 s) (Pasteur EHA 18, Germany) and immediately cooled to 6 °C until analysis. Grape, black currant, apple, orange and carrot juices were bought on the Czech market.

SPME-GC-FID/MS Conditions

Volatile compounds were extracted by solid phase microextraction (SPME), identified by gas chromatography-mass spectrometry (GC-MS) and quantified using standards by GC-FID. For analysis 1 ml of juice was placed into vial for SPME extraction, three samples of every juice was taken, every sample was analysed three times.

The SPME conditions were: SPME fiber CAR™/PDMS 85 µm (Supelco). Sample volume 1 ml, extraction temperature 35 °C, equilibrium time 30 min., extraction time 20 min., desorption temperature 250 °C, desorption time 5 min.

Gas chromatograph used was TRACE™ GC (ThermoQuest, I), capillary column DB-WAX (30 m × 0.32 mm × 0.5 µm). GC conditions: injector 250 °C, splitless desorption 5 min., carrier gas N_2 0.9 ml.min^{-1}, flame ionization detector (FID) at 220 °C, H_2 35 ml.min^{-1}, air 350 ml.min^{-1}, make up N_2 30 ml.min^{-1}. The oven temperature was 40 °C for 1 min, 40–200 °C at 5 °C/min, 200 °C for 7 min. GC-MS analyses were performed on GC 8000 (Carlo Erba, I) with MS TRIO 1000 (Fisons Instruments, USA). Carrier gas He, GC column and conditions were the same as described above. The validation parameters of SPME-GC-FID/MS method were published previously (Vítová *et al*., 2006; Vítová *et al*., 2007).

Sensory Analysis

The sensory analysis was carried out in a sensory laboratory (ISO 8589: 2008). The assessors were selected, then trained (including sensory profiling) and monitored for six months in accordance with (ISO 8586-1: 2002). Fifteen assessors (9 women and 6 men) were then used for evaluation of the samples in different sessions. 30 ml of the samples were served in 50 ml glass beakers, marked with 4-digit codes, in random order. Tap water was used between the samples.

The sensory attributes were evaluated using unstructured 10 cm line scale, anchored from each end to identify the direction. The list of attributes comprised one term for colour (characteristic elderberry, ranging from atypical to typical deep purple), odour (characteristic elderberry, from imperceptible to very strong), five flavour characteristics (sweet, acid/sour, bitter, astringent, characteristic elderberry, from weak to very strong), off-flavour (from imperceptible to very strong), texture and mouth-feel attribute encompassing viscosity (from thin to viscous), and overall acceptability (from unacceptable to delicious) (ISO 13299: 2003).

Statistical Evaluation

The results of sensory analyses were statistically evaluated by means of Kruskall-Wallis test and then by Nemenyi multiple comparison test; they are expressed as mean (n = 15). The results of instrumental analyses were treated using parametric one way analysis of variance and subsequently by Duncan test; they are expressed as mean ± SD (n = 9). All these analyses were performed at $p < 0.05$ using Unistat version 5.5 (Unistat, London, United Kingdom).

RESULTS AND DISCUSSION

Sensory Evaluation of the Fruit Juices

The main intention of this work was to find the best composition of fruit juices for fruit drink based on elderberries with optimal flavour. The flavour should be pleasant-tasting and simultaneously retaining noticeable characteristic elderberry note. Kaack (2008a; 2008b) dealt with similar idea of new elderberry products in his works. Recent research found that elderberries are concentrated sources of anthocyanins that appear to benefit health in several ways owing to their powerful antioxidant capacity (Christensen *et al.*, 2008). So the processing of elderberry to new food products and their increased consumption is highly advisable. However, the pure elderberry juice has bitter, sour, astringent taste, not suitable for direct consumption. It is possible to assume that these negative properties could be improved by mixing of elderberry with other fruit juice. For this reason the several partial aims were set:

i) the samples of selected types of fruit juices, in various ratios mixed with elderberry juice, were prepared;
ii) the samples were sensorially evaluated to find the optimum sensory quality;
iii) the content of aroma compounds was determined in these samples to compare the aroma profiles;
iv) the OAVs (odour activity values) were calculated for compounds identified to estimate their contribution to aroma and flavour quality.

At first various types of juices, in various ratios combined with elderberry, were preliminary evaluated by panel of 6 experts (results are not included). On the base of sensory properties, and also market price taking into consideration, grape, black currant, apple, orange and carrot juices were chosen and were further tested in recommended ratio 30% elderberry : 70% other fruit (v/v) (i.e. combined mixed fruit juices). The pure elderberry juice was evaluated simultaneously as comparative standard. The results are given in Tab. I.

The emphasis was put on preservation of characteristic deep purple colour, odour and flavour of elderberry. The mixture black currant-elderberry had the highest ($P < 0.05$) sensory ratings in colour, odour and characteristic flavour, similar to pure elderberry. The colour of carrot- and orange-elderberry juices was evaluated as unsatisfactory owing to their atypical, brownish shade. The texture (viscosity) of all juices was well evaluated, the mixtures with black currant and grape were the nearest to standard. Flavour and odour were considered as the most important characteristics. The mixtures with black currant and grape were evaluated as tastiest ($P < 0.05$), with the highest ratings of overall acceptability. Currant with more acid/sour (piquant) taste, grape was sweeter, very pleasant. Both of them maintained characteristic elderberry flavour in sufficient intensity, bitterness and astringency were suppressed and almost imperceptible. Carrot-elderberry was found as the worst ($P < 0.05$) with strong unpleasant earthy off-flavour.

Finally the grape-elderberry juice was chosen for practical use owing to the pleasant sweetish taste; good price and accessibility of grapes in the market were also taken into consideration. This mixture was further investigated; the others were excluded from this study because of low practical utility. So the various blends grape-elderberry with various contents (50–90% v/v) of elderberry juice were prepared to find the optimum ratio with the best flavour (Tab. I). All of these samples (including the most diluted by grape juice) kept the deep purple colour and good texture. Significant ($P < 0.05$) differences were found in odour and flavour. As expected, juices with higher addition of grape had higher ratings in sweet flavour and overall acceptability, juices with predominance of elderberry had stronger elderberry flavour. Finally the compromise was selected, the mixture with 70% (v/v) of elderberry, which was evaluated as very good, sweet with adequately strong characteristic elderberry aroma.

Comparison of Aroma Profiles of Juices

The content of volatile aroma compounds and their contribution to flavour is one of the important characteristics of fruits. The flavour of fruits is made up of a great number of volatile compounds, among which may be a number of alcohols, esters, acids, terpenes, carbonyl compounds, phenols and lactones (Rosillo *et al.*, 1999). These aroma compounds are produced during ripening, harvest, post-harvest and storage depending on many factors related to the species, variety and type of technological treatment. Their concentrations are very low and vary between varieties (Kataoka *et al.*, 2000). Despite the developments in flavour research, most biochemical pathways determining this quality trait are still unknown (Song and Forney, 2008).

In total 57 compounds were identified in samples including 20 alcohols: ethanol, propan-1-ol, propan-2-ol, butan-1-ol, butan-2-ol, pentan-2-ol, pentan-1-ol, hexan-1-ol, heptan-2-ol, octan-1-ol, octan-2-ol, decan-1-ol, decan-2-ol, (E)-3-hexenol, oct-1-en-3-ol, 2-methylpropanol, 2-methylbutan-1-ol, 3-methylbutan-1-ol, phenylmethanol, phenylethanol, 10 aldehydes: pentanal, hexanal, heptanal, octanal, nonanal, (E)-2-hexenal, (E)-2-octenal, 3-methylbutanal, benzaldehyde, phenylethanal, 8 ketones: butan-2-one, pentan-2-one, heptan-2-one, nonan-2-one, decan-2-one, undecan-2-one, 4-methylpentan-2-one, 3-hydroxybutan-2-one, 7 acids: acetic, propanoic, octanoic, decanoic, 2-methylpropanoic, 3-methylbutanoic, 2-hydroxypropanoic, 7 esters: methyl acetate, ethyl acetate, propyl acetate, butyl acetate, ethyl butanoate, ethyl octanoate, ethyl decanoate and 5 other compounds: limonene, β-damascenon, α-terpineol,

I: *Colour, odour, flavour, texture and mouthfeel and overall acceptability ratings (in %) of mixed elderberry juices*

Sensory characteristics		Type of fruit juice										
		E	E-gr	E-bc	E-ap	E-or	E-ca	E-gr 50	E-gr 60	E-gr 70	E-gr 80	E-gr 90
Colour	Characteristic elderberry	92.1^{aAC}	63.1^{bc}	87.9^{a}	73.2^{bc}	44.2^{c}	31.1^{c}	75.5^{B}	78.9^{B}	81.3^{BC}	87.6^{C}	89.4^{AC}
Odour	Characteristic elderberry	93.4^{aA}	69.5^{ab}	82.4^{ab}	64.3^{bc}	47.3^{cd}	38.4^{d}	74.8^{B}	74.9^{B}	81.2^{BC}	88.2^{AC}	90.3^{AC}
Flavour	Sweet	21.5^{aA}	84.8^{bc}	51.3^{ac}	72.8^{bc}	69.3^{c}	55.4^{ac}	79.6^{B}	69.5^{BC}	61.5^{BCD}	42.4^{AC}	35.3^{AD}
	Acid/sour	84.4^{aA}	32.4^{bc}	78.6^{ac}	44.3^{bc}	42.4^{c}	42.8^{c}	40.1^{B}	45.6^{BC}	59.4^{BCD}	62.7^{AC}	79.3^{AD}
	Bitter	89.3^{aA}	41.5^{bc}	54.6^{c}	42.2^{bc}	43.4^{c}	44.1^{c}	50.2^{B}	59.1^{BC}	64.3^{CD}	77.9^{D}	86.4^{A}
	Astringent	78.1^{aA}	37.2^{bc}	53.8^{c}	36.9^{b}	38.3^{bc}	41.8^{c}	44.5^{B}	58.6^{BC}	62.4^{CD}	71.4^{D}	75.8^{A}
	Characteristic elderberry	95.4^{aA}	61.2^{b}	84.4^{ab}	59.3^{b}	42.3^{bc}	39.4^{c}	72.5^{B}	76.5^{BC}	82.4^{CD}	88.2^{DE}	91.6^{AE}
	Off-flavour	11.8^{aA}	36.4^{bc}	33.2^{b}	41.5^{bc}	43.2^{c}	87.6^{d}	32.2^{B}	25.8^{BC}	15.4^{CD}	13.8^{AD}	12.6^{A}
Texture and mouthfeel	Viscosity	81.3^{aA}	71.0^{b}	74.3^{c}	68.9^{b}	66.4^{c}	69.6^{bc}	77.3^{B}	78.1^{BC}	78.3^{BC}	79.2^{AC}	81.2^{A}
Overal acceptability		72.3^{acA}	86.2^{b}	85.6^{b}	81.5^{ac}	64.0^{ac}	35.1^{c}	87.4^{B}	87.2^{B}	83.7^{BC}	79.8^{CD}	75.4^{AD}

The results are expressed in % as mean (n = 15). Different small letters in the same row indicate significant differences ($p < 0.05$) among combined mixed fruit juices, different capital letters in the same row indicate significant differences ($p < 0.05$) among elderberry-grape juices. Sample labeling: E: pure elderberry, E-gr: elderberry-grape, E-bc: elderberry-black currant, E-ap: elderberry-apple, E-or: elderberry-orange, E-ca: elderberry-carrot. E-gr 50 to E-gr 90: elderberry-grape in various proportions (50–90% v/v of elderberry).

II: *Comparison of chemical groups of compounds identified in mixed elderberry juices*

Aroma compounds ($\mu g.ml^{-1}$)	Type of fruit juice					
	E	E-gr	E-bc	E-ap	E-or	E-ca
Alcohols	1450.6 ± 13.93^{aA}	1006.2 ± 19.91^{b}	760.5 ± 21.69^{c}	1255.5 ± 14.42^{ab}	842.2 ± 17.59^{c}	1169.3 ± 17.83^{ab}
Aldehydes	0.6 ± 0.22^{aA}	0.1 ± 0.01^{b}	0.2 ± 0.02^{c}	0.5 ± 0.08^{a}	0.1 ± 0.01^{c}	1.5 ± 0.32^{d}
Ketones	1.2 ± 0.04^{aA}	2.4 ± 0.05^{b}	2.4 ± 0.09^{b}	2.3 ± 0.15^{b}	4.3 ± 0.85^{c}	3.0 ± 0.16^{bc}
Acids	230.1 ± 8.69^{aA}	87.2 ± 11.09^{b}	139.2 ± 13.88^{c}	96.3 ± 34.09^{b}	189.4 ± 6.68^{ac}	31.7 ± 1.72^{d}
Esters	11.8 ± 2.27^{aA}	7.1 ± 0.54^{b}	7.4 ± 0.41^{b}	7.0 ± 0.29^{b}	3.6 ± 0.11^{c}	6.1 ± 0.33^{b}
Others	0.7 ± 0.02^{aA}	1.0 ± 0.01^{b}	nd	0.5 ± 0.01^{c}	nd	nd
In total AC	1694.4 ± 16.58^{aA}	1102.8 ± 21.59^{b}	909.1 ± 32.09^{c}	1361.7 ± 49.03^{ab}	1039.2 ± 24.80^{bc}	1211.4 ± 11.36^{b}
	E-gr 50	**E-gr 60**	**E-gr 70**	**E-gr 80**	**E-gr 90**	**G**
Alcohols	1118.1 ± 12.32^{B}	1202.9 ± 14.54^{B}	1326.5 ± 21.34^{AB}	1396.4 ± 13.34^{A}	1351.0 ± 18.48^{AB}	661.0 ± 4.06^{C}
Aldehydes	0.6 ± 0.03^{A}	0.3 ± 0.02^{B}	0.2 ± 0.02^{B}	0.4 ± 0.08^{B}	0.2 ± 0.04^{BC}	0.1 ± 0.02^{C}
Ketones	1.1 ± 0.01^{A}	1.1 ± 0.01^{A}	1.0 ± 0.01^{A}	1.0 ± 0.01^{A}	1.0 ± 0.01^{A}	0.1 ± 0.01^{B}
Acids	200.2 ± 5.36^{A}	132.7 ± 5.36^{B}	150.5 ± 10.12^{B}	105.0 ± 1.59^{C}	114.2 ± 1.80^{C}	166.1 ± 2.84^{AC}
Esters	7.1 ± 1.04^{B}	9.2 ± 0.32^{C}	10.8 ± 0.18^{AC}	12.9 ± 0.42^{A}	12.1 ± 0.86^{AC}	0.5 ± 0.01^{D}
Others	0.4 ± 0.02^{B}	1.1 ± 0.05^{C}	1.6 ± 0.03^{D}	1.4 ± 0.07^{D}	1.3 ± 0.04^{CD}	1.5 ± 0.12^{D}
In total AC	1327.3 ± 14.89^{B}	1347.4 ± 5.47^{B}	1490.3 ± 21.34^{AB}	1517.3 ± 9.59^{A}	1480.2 ± 13.03^{AB}	828.9 ± 4.96^{C}

The results are expressed as mean ± SD (n = 9). Different small letters in the same row indicate significant differences ($p < 0.05$) among combined mixed fruit juices, different capital letters in the same row indicate significant differences ($p < 0.05$) among elderberry-grape juices. nd – not detected. AC – aroma compounds. Sample labeling: E: pure elderberry, G: pure grape, E-gr: elderberry-grape, E-bc: elderberry-black currant, E-ap: elderberry-apple, E-or: elderberry-orange, E-ca: elderberry-carrot. E-gr 50 to E-gr 90: elderberry-grape in various proportions (50–90% v/v of elderberry).

(Z)-rose oxide, linalool. Most of them are known as important aroma components and were found by many authors in various types of fruits (e.g. Rocha *et al.*, 2007; Soni *et al.*, 2008; Fraternale *et al.*, 2011; Selli and Kelebek, 2011).

The comparison of chemical groups of compounds is given in Tab. II. Alcohols were the most important group of all juices forming about 80–90% of all compounds quantified. Aldehydes and ketones were quantitatively the least ($P < 0.05$) important; their concentrations were minimal in all fruit juices. The highest total content of compounds was found in pure elderberry. In spite of good sensory rating, the grape juice contained the lowest total content of aroma compounds, especially caused by low concentration of alcohols. It gradually increased in

grape-elderberry mixtures with increasing portion of elderberry (Tab. II). In spite of significant ($P < 0.05$) differences in sensory evaluation of flavour, the total content of aroma compounds in samples E-gr 50 to E-gr 90 was similar and was created by combination of aroma compounds in pure grapy and elderberry juices.

Both sensory studies and instrumental analysis confirm the importance of volatiles production in fruit and their contribution to eating quality. The calculation of OAV, which is defined as "the ratio of concentration in food to threshold concentration in the same matrix", is the possibility how to predict which compound could contribute to aroma. Odour threshold concentrations were acquired from the literature (Rocha *et al*., 2007; Verzera *et al*., 2008; Sanchez-Palomo *et al*., 2010); the calculated OAVs suggest that ethanol (mild alcoholic), propan-1-ol (sweet alcoholic), propan-2-ol (buttery), 2-methylbutan-1-ol (fruity), hexanal (fruity, grassy), heptanal (fruity), phenylethanal (flowery), nonan-2-one (fruity, flowery), undecan-2-one (fruity, flowery), 4-methylpentan-2-one (fruity), 3-methylbutanoic acid (oily), β-damascenon (woody, elderberry) and α-terpineol (flowery) could be the contributors to aroma of samples in this study, which is in accordance with other authors (Sanchez-Palomo *et al*., 2010; Selli and Kelebek, 2011). Theoretically, the remaining compounds did not directly contribute (OAVs < 1), they can only enhance some notes because of synergistic effects.

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ORIGINAL PAPER

Comparison of selected aroma compounds in cultivars of sea buckthorn (*Hippophae rhamnoides* L.)

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Thirteen cultivars of sea buckthorn (*Hippophae rhamnoides* L.) berries: Aromat, Botanicky, Buchlovicky, Hergo, Krasavica, Leicora, Ljubitelna, Pavlovsky, Peterbursky, Sluničko, Trofinovsky, Vitaminnaja and Velkoosecky, were tested for the content of volatile aroma compounds using gas chromatography with the solid phase microextraction method during two consequent years (2012–2013). In total, 69 volatile compounds were identified: 26 alcohols, 12 aldehydes, 11 ketones, 9 acids and 11 esters. Based on principal component analysis, 18 most relevant compounds, best representing the variability of the whole system and suitable for the discrimination of the samples, were selected from all compounds identified. These compounds were then compared using the analysis of variance to confirm differences between the samples. Significant ($p < 0.05$) differences were found in the varieties in both years, Krasavica and Sluničko cultivars were found to be quite different from other varieties, being rich in the compounds identified and containing most of the selected compounds. Variability within the cultivars (between picking years) was low or not significant.

Keywords: sea buckthorn, aroma compounds, flavour, GC, SPME

Introduction

Sea buckthorn (*Hippophae rhamnoides* L.) is a hardy, deciduous, spiny shrub or tree belonging to the family *Elaeagnaceae*. The fruits are yellow-orange, drupe-like, consisting of a single seed surrounded by a fleshy edible pulp (Li & Schroeder, 1996; Yang & Kallio, 2002). Their mass varies in the range of 4–60 g per 100 of fruit being 5–11 mm in size, and the shape varies from flattened spherical, cylindrical, ovate or elliptic to various irregular shapes. The fruit is considered ripe when it becomes bright yellow, orange or red in colour. The combination of fruit colour, shape, and size contributes to the variability among varieties (Li & Beveridge, 2003). It originates in Asia, where it has been used for centuries for food, therapeutic, and pharmaceutical purposes. It is also naturally distributed in Europe, e.g. in France, Denmark, Netherlands, Germany, Poland, Sweden, Norway (Li & Beveridge, 2003). During the last decade, sea buckthorn has attracted considerable attention of researchers around the world, mainly for its nutritional and medicinal value. All parts of this plant are considered to be a good source of a large number of bioactive substances like vitamins, carotenoids, phytosterols, organic acids, polyunsaturated fatty acids and some essential amino acids (Beveridge et al., 1999; Suryakumar & Gupta, 2011). Its fruits are among the most nutritious and vitamin-rich of all berries, with an exceptionally high content of antioxidants (Zeb, 2004). These bioactive components are investigated by many researchers (Cakir, 2004; Zeb, 2004; Dulf, 2012).

Besides their nutritional value, sensory quality of berries (especially flavour) is important from the consumers' point of view. However, literature dealing with flavour of sea buckthorn is scarce. Tang et al.

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(2001) found astringency, sourness and bitterness as the salient sensory attributes that characterize the flavour of sea buckthorn; the aroma was described as e.g. strawberry, peach (Tang et al., 2001); passion fruit and pineapple (Li & Beveridge, 2003); exotic soft fruit or citrus fruit (Tiitinen et al., 2005). Despite its highly acidic nature and exotic flavour, sea buckthorn berries posses high potential for the production of various products like juice, tea, jam, jelly and many others (Chauhan et al., 2003). How the chemical composition affects flavour of sea buckthorn still remains to be explored. It is generally known that flavour of fruits is influenced by volatile aroma compounds; those of sea buckthorn were firstly studied by Hirvi and Honkanen (1984) who identified a total of 60 components; esters, particularly ethyl-, 3-methylbutyl- and *cis*-3-hexen-1-yl esters, were present in the highest amounts. The most important compunds were: ethyl hexanoate, 3-methylbutyl 3-methylbutanoate, 3-methylbutanoic acid, 3-methylbutyl hexanoate, 3-methylbutyl benzoate and 3-methylbutyl octanoate. Other compound groups identified were terpenes, alcohols, phenols, aldehydes, ketones and organic acids. Cakir (2004) identified more than 80 compounds in *H. rhamnoides* subsp. *sinensis Rousi* grown in China including alkanes, alkenes, aldehydes, acetals, ketones, esters and terpenoids. The major components were ethyl dodecenoate, ethyl octanoate, decanol, ethyl decanoate and ethyl dodecanoate. Headspace volatiles from frozen berries of sea buckthorn were also studied by Tiitinen et al. (2006). A total of 45 compounds were identified; esters of branched or straight chain aliphatic alcohols and acids, such as methyl, ethyl and propyl esters of 2-methylpropanoic, 2-methylbutanoic and 3-methylbutanoic acids, are the main compounds found. Other common compounds are alcohols, terpene hydrocarbons, aldehydes and ketones. Ethyl 2-methylbutanoate, ethyl 3-methyl butanoate, ethyl hexanoate, 3-methylbutyl 3-methylbutanoate, ethyl octanoate and 3-methylbutyl hexanoate are the most abundant compounds. Wang et al. (2011) used headspace SPME for the extraction of volatile compounds characterizing sea buckthorn raw juice and other products. The extracted compounds comprised higher alcohols, ethyl esters, acetates, fatty acids, and carbonyl compounds. Recently, Socaci et al. (2013) used in-tube extraction for the isolation of volatile components to compare wild and cultivated sea buckthorn varieties. Similarly to other authors, they found the most abundant derivatives to be ethyl esters of 2-methylbutanoic, 3-methylbutanoic, hexanoic, octanoic, butanoic and benzoic acids, as well as 3-methylbutyl 3-methylbutanoate and 3-methylbutyl 2-methylbutanoate.

The aim of this work was to compare selected volatile aroma compounds of several new cultivars of sea buckthorn berries grown in the Czech Republic. Compounds were identified and quantified using gas chromatography-mass spectrometry (GC-MS) with solid-phase microextraction (SPME). Differences between the aroma profiles of the studied cultivars were expressed, thus contributing to better understanding of the sea buckthorn flavour.

Experimental

The sea buckthorn varieties were grown in the breeding institute of the Mendel University in Brno (Lednice, CZ). In total, 13 cultivars were analysed: two red-coloured: Aromat (large-sized berries, sweet/sour flavour), Krasavica (middle-sized, sweet/sour); five orange-coloured: Buchlovicky (middle-sized, sweet/sour), Leicora (large-sized, sweet/sour), Trofinovsky (small-sized, acidulous), Vitaminnaja (middle-sized, acidulous), Velkoosecky (small-sized, acidulous) and six yellow-orange-coloured: Botanicky (large-sized, acidulous), Hergo (small-sized, sweet/sour/astringent), Ljubitelna (small-sized, acidulous), Pavlovsky (small-sized, acidulous), Peterbursky (small-sized, acidulous), Sluničko (small-sized, sweet/sour). The berries were handpicked fully ripe (evaluated by experienced agronomists) during the seasons of 2012–2013, immediately frozen at –15 ℃ and stored until analysis. For analysis, 1 g of defrozen, manually homogenised berries was placed into a vial for SPME extraction; three samples of every cultivar were taken, every sample was analysed three times (number of experiments, $n = 9$).

SPME extractions were carried out using a 85 μm Carboxen/Poly(dimethylsiloxane) fibre (CAR/PDMS) (Supelco, Bellefonte, Pennsylvania, USA) under the following conditions: extraction temperature of 35 ℃, equilibrium time of 30 min, extraction time of 20 min, desorption temperature of 250 ℃, desorption time of 5 min. The following conditions were used for gas chromatographic analyses using a gas chromatograph TRACE GC (ThermoQuest, Milan, Italy) with a capillary column DB-WAX (30 m × 0.32 mm × 0.5 μm) (J&W Scientific, Folsom, CA, USA): injector temperature of 250 ℃, split-less desorption of 5 min, carrier gas N_2, flow-rate of 0.9 mL min^{-1}, flame ionisation detector (FID) set at 220 ℃, H_2 inlet of 35 mL min^{-1}, air inlet of 350 mL min^{-1}, make up N_2 flow of 30 mL min^{-1}. The oven ramp temperature was 40 ℃ for 1 min, and it was increased up to 200 ℃ at the rate of 5 ℃ min^{-1} and maintained at 200 ℃ for 7 min. GC-MS analyses were performed on a gas chromatograph HP 6890 with an MS detector 5973 N and the Mass Spectral Library NIST 98 (Agilent, Santa Clara, CA, USA). Helium was used as the carrier gas. GC column and the conditions were as described above.

The standard addition method was used for the quantification of analytes to control the influence of the sample matrix. The mixture of standards was divided into groups consisting of five chemicals which were gradually added (1 mL) directly into the sample.

These standard mixtures were analysed in the same manner as the samples. Five concentration levels, in the range of 0.001–200 mg kg^{-1} (different for various standards), were used to establish the calibration curves. Validation and the validation parameters of the SPME-GC-FID/MS method were published previously (Vítová et al., 2013). Repeatability was verified by repeated extractions (number of experiments, $n = 5$) of the standard mixtures (relative standard deviation < 10 %), detection limits were in the range of 0.001–0.50 mg kg^{-1}. Linearity was tested within the range of 0.001–200 mg kg^{-1}; correlation coefficients were all above 0.99 (Vítová et al., 2013).

The following chemicals were used as standards: pentanal, hexanal, heptanal, (*E*)-oct-2-enal, propan-2-one, nonan-2-one, decan-2-one, undecan-2-one, phenylacetaldehyde, benzaldehyde, 2-methylbutan-1-ol, dodecan-1-ol, heptadecan-1-ol, heptadecan-2-ol, hexadecan-2-ol, hex-3-en-1-ol, oct-3-en-1-ol (Sigma–Aldrich, Germany); acetic, propanoic, butanoic, hexanoic, benzoic, 2-hydroxypropanoic, 2-methylpropanoic, 2-methylbutanoic, 3-methylbutanoic acids, acetaldehyde, propanal, octanal, nonanal, (*E*)-hex-2-enal, methyl acetate, ethyl acetate, ethyl butanoate, ethyl hexanoate, ethyl heptanoate, ethyl octanoate, ethyl decanoate, ethyl dodecanoate, propyl acetate, butyl acetate, phenylethyl acetate, ethanol, propan-1-ol, propan-2-ol, butan-1-ol, butan-2-ol, pentan-1-ol, pentan-2-ol, hexan-1-ol, heptan-2-ol, octan-1-ol, octan-2-ol, nonan-2-ol, decan-1-ol, 2-methylpropan-1-ol, 3-methylbutan-1-ol, benzyl alcohol, phenylethanol, pentan-2-one, heptan-2-one, tridecan-2-one, 4-methylpentan-2-one, 3-hydroxybutan-2-one, butane-2,3-dione (Merck, Germany); methanol, butan-2-one (Lachema, Czech Republic); oct-1-en-3-ol, 3-methylbutanal (Fluka, Switzerland). All the chemicals were of chemically pure grade.

The results were treated using MS Excel 2010 (Microsoft Corporation, Redmond, Washington, USA) and are expressed as the mean ± standard deviation ($n = 9$). Principal component analysis (PCA) was used to reduce the original data set to the most relevant compounds. The differences between the cultivars and the picking years were statistically treated with the parametric one way analysis of variance (ANOVA) followed by the Duncan test at $p < 0.05$. These analyses were performed using Unistat version 5.5 (Unistat, London, UK).

Results and discussion

SPME-GC-FID/MS assessment of volatile aroma compounds

The content of volatile aroma compounds and their contribution to flavour is an important characteristic of fruits (Schmarr & Bernhardt, 2010). However, very few information about aroma compounds of sea buckthorn has been published so far. A total of 60 components were firstly identified by Hirvi and Honkanen (1984), also Cakir (2004), Tiitinen et al. (2006) and Wang et al. (2011) identified 30, 45 and about 40 compounds, respectively, which may contribute to the typical flavour of sea buckthorn berries. Esters of branched or straight chain aliphatic alcohols and acids were the major compounds found.

In total, 13 sea buckthorn cultivars were investigated in this study during the seasons of 2012–2013. Volatile aroma compounds were extracted by SPME, identified by GC-MS (based on mass spectra), confirmed using retention times of standards, and quantified by GC-FID using standards. In total, 69 volatile compounds were identified in all samples, among them: 26 alcohols: methanol, ethanol, propan-1-ol, propan-2-ol, butan-1-ol, butan-2-ol, pentan-1-ol, pentan-2-ol, hexan-1-ol, heptan-2-ol, octan-1-ol, octan-2-ol, nonan-2-ol, decan-1-ol, dodecan-1-ol, heptadecan-1-ol, heptadecan-2-ol, hexadecan-2-ol, hex-3-en-1-ol, oct-3-en-1-ol, 2-methylpropan-1-ol, 2-methylbutan-1-ol, 3-methylbutan-1-ol, oct-1-en-3-ol, benzyl alcohol, phenylethanol; 12 aldehydes: acetaldehyde, propanal, pentanal, hexanal, heptanal, octanal, nonanal, 3-methylbutanal, (*E*)-hex-2-enal, (*E*)-oct-2-enal, benzaldehyde, phenylacetaldehyde; 11 ketones: propan-2-one, butan-2-one, pentan-2-one, heptan-2-one, nonan-2-one, decan-2-one, undecan-2-one, tridecan-2-one, 3-hydroxybutan-2-one, 4-methylpentan-2-one, butane-2,3-dione; 11 esters: methyl acetate, ethyl acetate, propyl acetate, ethyl butanoate, ethyl hexanoate, ethyl heptanoate, ethyl octanoate, ethyl decanoate, ethyl dodecanoate, butyl acetate, phenylethyl acetate; and 9 acids: acetic, propanoic, butanoic, hexanoic, benzoic, 2-hydroxypropanoic, 2-methylpropanoic, 2-methylbutanoic, 3-methylbutanoic. In contrast to other authors, namely Cakir (2004) and Tiitinen et al. (2006), alcohols were the most numerous compounds; among them methanol, ethanol, propan-1-ol, propan-2-ol, butan-2-ol, pentan-1-ol, pentan-2-ol, 2-methylpropan-1-ol, 2-methylbutan-1-ol, 3-methylbutan-1-ol, benzyl alcohol and phenylethanol were present in highest amounts. Aldehydes: acetaldehyde, heptanal, octanal and (*E*)-oct-2-enal, ketones: tridecan-2-one and 3-hydroxybutan-2-one, and acetic, 2-methylpropanoic and 3-methylbutanoic acids were also present in significant quantities. The content of other compounds identified did not exceed 1 mg kg^{-1}. An example of the chromatogram of compounds identified in the Krasavica cultivar (harvested in 2013) is given in Fig. 1.

According to Zeb (2004), the composition of sea buckthorn varies mainly with the origin and climate. To assess the differences between the cultivars, PCA was performed using the data of 13 sea buckthorn varieties and the content of all compounds identified (data matrix 26 × 69 for 26 samples and 69 compounds). The scatter plot of PCA scores of all sea

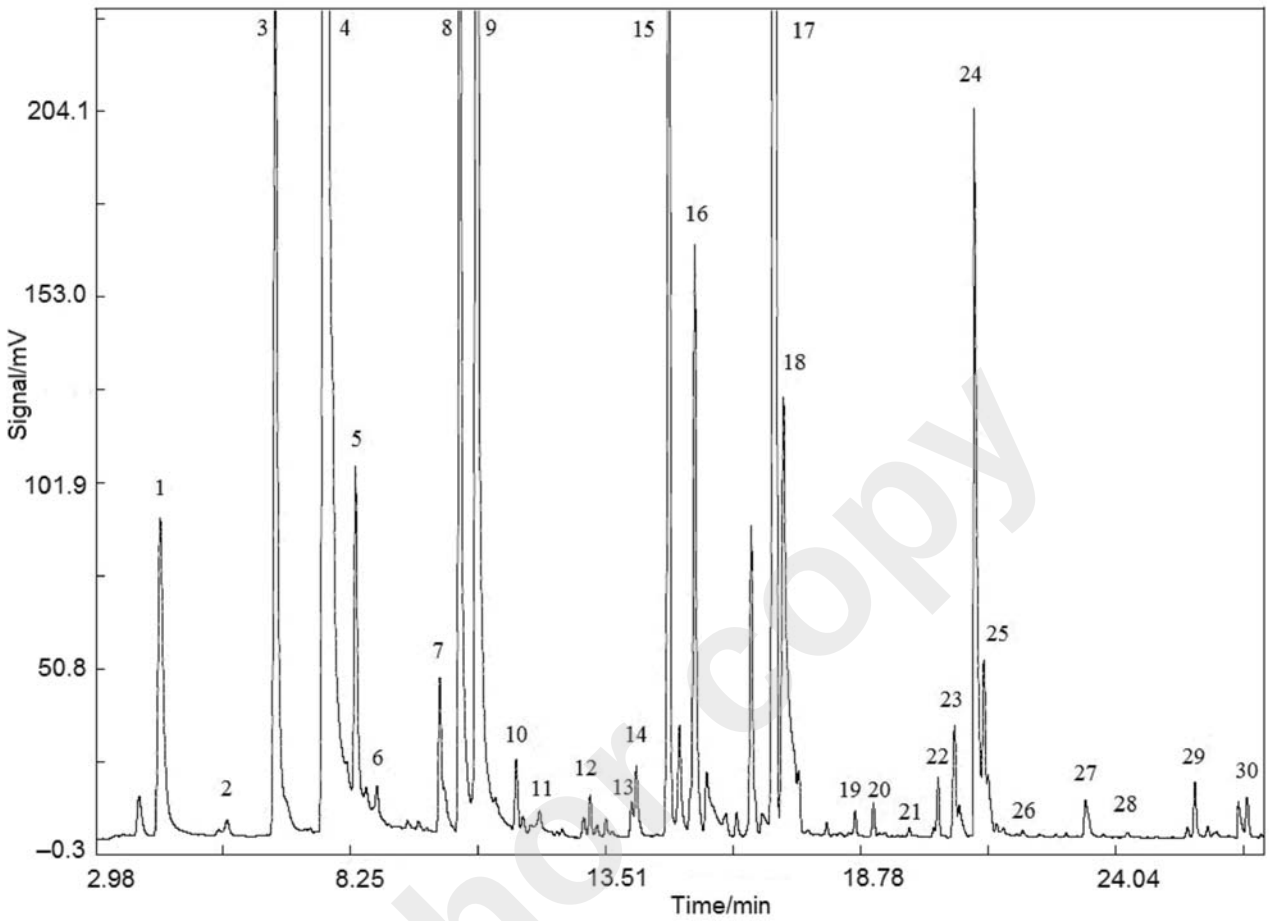

Fig. 1. Chromatogram of compounds identified in the Krasavica cultivar (harvested in 2013). Peak numbering: 1 – acetaldehyde, 2 – methyl acetate, 3 – ethyl acetate, 4 – ethanol, 5 – pentan-2-one, 6 – pentanal, 7 – ethyl butanoate, 8 – propan-1-ol, 9 – butyl acetate, 10 – 2-methylpropan-1-ol, 11 – pentan-2-ol, 12 – butan-1-ol, 13 – heptanal, 14 – heptan-2-one, 15 – 3-methylbutan-1-ol, 16 – ethyl hexanoate, 17 – octanal, 18 – 3-hydroxybutan-2-one, 19 – heptan-2-ol, 20 – hexan-1-ol, 21 – nonanal, 22 – octan-2-ol, 23 – ethyl octanoate, 24 – oct-1-en-3-ol, 25 – acetic acid, 26 – nonan-2-ol, 27 – linalool (3,7-dimethylocta-1,6-dien-3-ol), 28 – octan-1-ol, 29 – undecan-2-one, 30 – benzyl alcohol.

buckthorn varieties is shown in Fig. 2. Similarly to Tiitinen et al. (2006), the differences were found among the studied varieties in both years. However, the differentiation of cultivars is ambiguous; the cluster in the left bottom part includes most of the samples, correlating negatively with PC1 and PC2, which means that these varieties are more similar in the content of aroma compounds. Only Krasavica and Sluničko are located individually in this graph, which indicates significant differences compared to other samples. The Krasavica variety is located in the bottom right part, correlating positively with PC1. Sluničko is located in the top part, correlating positively with PC2. Both these varieties are rich in ethanol, 2-methylpropan-1-ol, 3-methylbutan-1-ol, acetaldehyde, 3-hydroxybutan-2-one and acetic acid. With the exception of Trofinovsky and Aromat, composition in the two studied years (indexes 12, 13 in Fig. 2) within the same variety were very close to each other, indicating that differences in the composition between the picking years are small.

The cumulative contribution of the first three principal components to total variance was 67.47 %. PC1 (34.96 %) explains the differences between the cultivars, whereas PC2 (22.69 %) explains the differences between the growing seasons. PC3 represents 9.82 % of the remaining differences. Octan-1-ol (0.977), hexan-1-ol (0.954), 2-methylpropan-1-ol (0.953), nonan-2-ol (0.941), undecan-2-one (0.924), acetic acid (0.851), benzyl alcohol (0.839), linalool (0.839), 3-hydroxybutan-2-one (0.834), pentanal (0.785), nonanal (0.769), propan-1-ol (0.762) and ethyl butanoate (0.703) were the most significant parameters for PC1 construction. The important role of ethyl octanoate (0.840), propanoic acid (0.803), pentan-2-one (0.803), propanal (0.803) and ethyl butanoate (0.691) was proven by PC2 and that of ethyl heptanoate (0.824) by PC3. These 18 compounds can be considered as the most important representing the variability of the system and were therefore taken into account when reducing the extensive original data set.

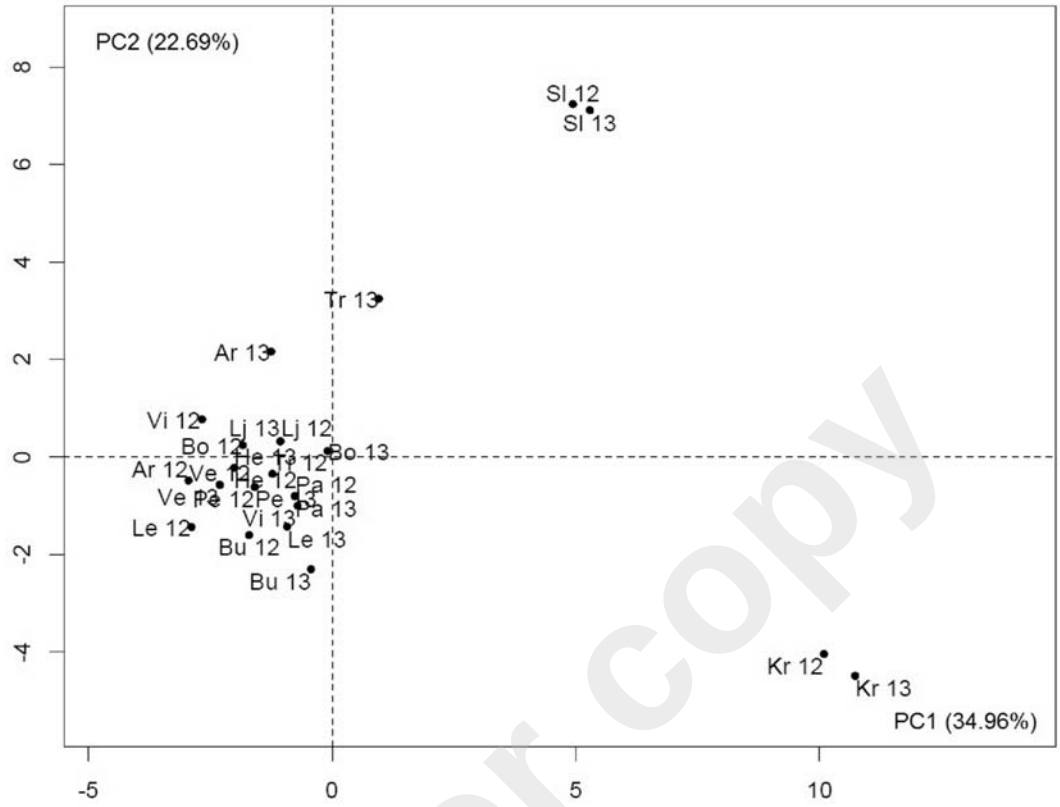

Fig. 2. PCA score plot of 13 sea buckthorn cultivars harvested in 2012–2013: Aromat (Ar), Botanicky (Bo), Buchlovicky (Bu), Hergo (He), Krasavica (Kr), Leicora (Le), Ljubitelna (Lj), Pavlovsky (Pa), Peterbursky (Pe), Sluničko (Sl), Trofinovsky (Tr), Vitaminnaja (Vi), Velkoosecky (Ve).

Comparison of selected aroma compounds identified in sea buckthorn cultivars

Based on PCA, the most relevant compounds, best suitable for the discrimination of the samples, were selected from all compounds identified. These selected aroma compounds were subjected to a further PCA analysis (data matrix: 26 × 18 for 26 samples and 18 compounds) and were also compared using ANOVA to better express the differences between the cultivars and the picking years. In this case, the cumulative contribution of the first three PCs to total variance was 91.20 %. Such high percentage is very good to represent the variability of samples. PC1 represented 58.33 %, PC2 24.62 % and PC3 8.24 % of the total variance. The score plot (not presented) was similar to that in Fig. 2, confirming the above mentioned dissimilarity of the Krasavica and Sluničko varieties; they contain most of the selected compounds (see Table 1). The comparison of 18 selected compounds using ANOVA is given in Table 1. Two picking years for each cultivar were compared; then, the single cultivars were mutually compared, separately in 2012 and 2013. Propan-1-ol was detected in the Buchlovicky, Krasavica, Leicora, Ljubitelna, Peterbursky and Vitaminnaja cultivars occurring in various amounts ranging from (18.1 ± 1.1) μg kg^{-1} in Vitaminnaja to (3.4 ± 0.2) mg kg^{-1} in Krasavica. 2-Methylpropan-1-ol was detected in Aromat, Botanicky, Krasavica, Sluničko, Trofinovsky, Vitaminnaja and Velkoosecky. Its concentration ranged from (2.3 ± 0.1) mg kg^{-1} in Velkoosecky to (763.1 ± 41.2) mg kg^{-1} in Krasavica. Octan-1-ol was detected in Aromat, Krasavica, Ljubitelna, Sluničko, Trofinovsky and Vitaminnaja in quite low concentrations, ranging from (1.2 ± 0.1) μg kg^{-1} in Aromat and Vitaminnaja to (43.9 ± 2.5) μg kg^{-1} in Krasavica. Hexan-1-ol was detected in most cultivars except for Hergo, Pavlovsky and Velkoosecky. Its amount was in the range of (2.1 ± 0.1) μg kg^{-1} in Leicora to (5.0 ± 0.3) mg kg^{-1} in Krasavica. Nonan-2-ol was present only in Krasavica, Pavlovsky and Sluničko in very low amounts, about tens of μg kg^{-1}. Linalool, about 2 μg kg^{-1}, was detected only in Hergo, Krasavica and Velkoosecky. Benzyl alcohol was detected in Aromat, Buchlovicky, Krasavica, Pavlovsky and Vitaminnaja. Its amount was in the range of (5.6 ± 0.3) μg kg^{-1} to (22.6 ± 1.4) μg kg^{-1}, with the exception of Krasavica containing (3.7 ± 0.23) mg kg^{-1}. Propanal was detected in Botanicky, Hergo, Sluničko and Velkoosecky in low concentrations ranging from (2.1 ± 0.1) μg kg^{-1} in Hergo to (5.4 ± 0.3) μg kg^{-1} in Sluničko. Pentanal was detected in Botanicky, Krasavica and Vitaminnaja also in very low concentrations

Table 1. Comparison of selected volatile aroma compounds identified in sea buckthorn cultivars (picking years 2012–2013)

Sea buckthorn cultivar	Picking year	Selected aroma compounds content/(μg kg^{-1})					
		Propan-1-ol	2-Methylpropan-1-ol	Hexan-1-ol	Nonan-2-ol	Linalool	Octan-1-ol
Aromat	2012	nd	18.2 ± 0.8*A_a	13.2 ± 1.1A_{ae}	nd	nd	1.5 ± 0.1A_a
	2013	nd	21.8 ± 1.9*A^a	31.3 ± 1.7B^a	nd	nd	1.2 ± 0.1A^a
Botanicky	2012	nd	23.7 ± 1.2*A_a	20.9 ± 1.8A_a	nd	nd	nd
	2013	nd	20.2 ± 1.9*A^a	41.1 ± 2.7B^a	nd	nd	nd
Buchlovicky	2012	373.3 ± 17.8A_a	nd	22.4 ± 2.1A_a	nd	nd	nd
	2013	526.1 ± 28.6A^a	nd	17.1 ± 0.8A^b	nd	nd	nd
Hergo	2012	nd	nd	nd	nd	2.6 ± 0.1A_a	nd
	2013	nd	nd	nd	nd	2.8 ± 0.1A^a	nd
Krasavica	2012	2.8 ± 0.2*A_b	763.1 ± 41.2*A_b	4.3 ± 0.2*A_b	29.3 ± 1.5A_a	2.3 ± 0.1A_a	43.9 ± 2.5A_b
	2013	3.4 ± 0.2*A^b	521.3 ± 35.4*B^b	5.0 ± 0.3*A^c	34.3 ± 2.1A^a	1.8 ± 0.1A^a	35.6 ± 2.4A^b
Leicora	2012	436.5 ± 26.8A_a	nd	2.1 ± 0.1A_c	nd	nd	nd
	2013	198.5 ± 11.0B^c	nd	22.8 ± 2.1B^b	nd	nd	nd
Ljubitelna	2012	1.3 ± 0.1*A_c	nd	39.6 ± 1.8A_d	nd	nd	1.9 ± 0.1A_{ae}
	2013	1.6 ± 0.1*A^d	nd	68.7 ± 3.4B^d	nd	nd	5.1 ± 0.5B^c
Pavlovsky	2012	nd	nd	nd	18.3 ± 1.7A_b	nd	nd
	2013	nd	nd	nd	12.1 ± 0.8B^b	nd	nd
Peterburský	2012	414.0 ± 26.6A_a	nd	11.1 ± 0.6A_e	nd	nd	nd
	2013	330.4 ± 18.8A^e	nd	8.1 ± 0.5A^e	nd	nd	nd
Sluničko	2012	nd	195.1 ± 14.6*A_c	3.6 ± 0.2*A_b	22.6 ± 1.7A_{ab}	nd	27.6 ± 1.8A_c
	2013	nd	265.1 ± 19.0*A^c	3.7 ± 0.2*A^c	28.9 ± 1.3A^c	nd	18.5 ± 0.6B^d
Trofinovsky	2012	nd	5.1 ± 0.4*A_d	128.0 ± 6.3A_f	nd	nd	3.2 ± 0.2A_d
	2013	nd	9.0 ± 0.6*B^d	81.8 ± 5.8B^d	nd	nd	1.4 ± 0.1B^a
Vitaminnaja	2012	532.2 ± 29.7A_d	8.0 ± 0.7*A_e	52.4 ± 3.6A_d	nd	nd	2.2 ± 0.1A_e
	2013	18.1 ± 1.1B^f	5.7 ± 0.3*A^e	63.3 ± 4.5A^d	nd	nd	1.2 ± 0.1B^a
Velkoosecky	2012	nd	3.2 ± 0.1*A_d	nd	nd	1.3 ± 0.1A_a	nd
	2013	nd	2.3 ± 0.1*A^f	nd	nd	2.8 ± 0.1A^a	nd

		Selected aroma compounds content/(μg kg^{-1})					
		Benzyl alcohol	Propanal	Pentanal	Nonanal	Ethyl octanoate	Ethyl butanoate
Aromat	2012	13.5 ± 0.9A_{ac}	nd	nd	3.9 ± 0.1A_a	0.9 ± 0.1*A_a	96.4 ± 7.2A_a
	2013	8.2 ± 0.3A^{ad}	nd	nd	3.8 ± 0.2A^a	1.2 ± 0.1*A^a	31.7 ± 2.4B^a
Botanicky	2012	nd	2.3 ± 0.2A_a	4.9 ± 0.3A_a	1.3 ± 0.1A_b	279.5 ± 9.5A_b	64.8 ± 4.7A_{ae}
	2013	nd	3.0 ± 0.2B^a	7.3 ± 0.5B^a	1.5 ± 0.1A^b	521.5 ± 18.3B^b	59.8 ± 4.2A^b
Buchlovicky	2012	9.3 ± 0.8A_a	nd	nd	nd	133.2 ± 9.6A_c	10.4 ± 0.9A_b
	2013	22.6 ± 1.4B^b	nd	nd	nd	252.9 ± 19.7B^c	8.1 ± 0.5A^c
Hergo	2012	nd	2.5 ± 0.2A_a	nd	nd	112.1 ± 7.6A_c	4.2 ± 0.2A_c
	2013	nd	2.1 ± 0.1A^b	nd	nd	132.1 ± 8.5A^{dg}	3.1 ± 0.1A^d
Krasavica	2012	3.7 ± 0.2*A_b	nd	12.2 ± 0.8A_b	3.4 ± 0.2A_{ac}	399.0 ± 27.6A_d	161.8 ± 7.8A_d
	2013	2.8 ± 0.2*A^c	nd	14.3 ± 0.7A^b	3.9 ± 0.2A^a	315.0 ± 26.2A^{ch}	184.1 ± 9.7A^e
Leicora	2012	nd	nd	nd	nd	97.6 ± 6.3A_c	5.6 ± 0.3A_c
	2013	nd	nd	nd	nd	271.8 ± 8.8B^c	7.1 ± 0.5A^c
Ljubitelna	2012	nd	nd	nd	nd	110.8 ± 7.5A_c	41.4 ± 2.2A_e
	2013	nd	nd	nd	nd	162.8 ± 10.4A^{dg}	52.8 ± 3.4A^b
Pavlovsky	2012	18.8 ± 1.3A_c	nd	nd	nd	5.1 ± 0.4A_e	1.3 ± 0.1A_f
	2013	6.4 ± 0.3B^a	nd	nd	nd	2.2 ± 0.1B^e	1.2 ± 0.1A^f
Peterburský	2012	nd	nd	nd	nd	15.2 ± 0.9A_f	3.3 ± 0.1A_c
	2013	nd	nd	nd	nd	23.1 ± 1.7B^f	4.2 ± 0.2A^d
Sluničko	2012	nd	3.5 ± 0.1A_b	nd	2.3 ± 0.1A_d	101.2 ± 8.8A_c	397.2 ± 19.7A_g
	2013	nd	5.4 ± 0.3B^c	nd	2.5 ± 0.1A^c	137.2 ± 5.8A^g	450.1 ± 12.0A^g
Trofinovsky	2012	nd	nd	nd	2.8 ± 0.1A_c	129.1 ± 7.3A_c	148.3 ± 7.9A_d
	2013	nd	nd	nd	1.3 ± 0.1B^b	413.2 ± 18.8B^h	67.3 ± 4.6B^b
Vitaminnaja	2012	5.6 ± 0.3A_d	nd	5.1 ± 0.1A_a	nd	29.3 ± 1.6A_g	10.8 ± 0.5A_b
	2013	11.7 ± 0.7A^d	nd	8.0 ± 0.4B^a	nd	146.1 ± 8.4B^g	32.6 ± 1.8B^a
Velkoosecky	2012	nd	3.5 ± 0.1A_b	nd	nd	58.7 ± 2.6A_h	3.7 ± 0.2A_c
	2013	nd	2.4 ± 0.1B^b	nd	nd	68.5 ± 3.4A^i	6.2 ± 0.2A^c

Table 1. (continued)

Sea buckthorn cultivar	Picking year	Selected aroma compounds content/(μg kg^{-1})					
		Ethyl heptanoate	Pentan-2-one	3-Hydroxybutan-2-one	Undecan-2-one	Acetic acid	Propanoic acid
Aromat	2012	2.0 ± 0.2A$_a$	nd	nd	nd	nd	5.4 ± 0.2A$_a$
	2013	5.2 ± 0.4B^a	nd	nd	nd	nd	2.7 ± 0.2B^a
Botanicky	2012	3.5 ± 0.1A$_b$	2.3 ± 0.1A$_a$	717.3 ± 25.6*A$_{ag}$	nd	70.2 ± 4.5*A$_a$	nd
	2013	8.9 ± 0.3B^b	4.6 ± 0.3B^a	837.3 ± 31.2*A^a	nd	63.2 ± 3.8*A^a	nd
Buchlovicky	2012	1.7 ± 0.1A$_a$	nd	585.1 ± 36.4*A$_a$	nd	174.8 ± 8.5*A$_b$	3.8 ± 0.1A$_b$
	2013	3.0 ± 0.1B^c	nd	605.5 ± 34.2*A^b	nd	193.2 ± 9.9*A^b	8.9 ± 0.5B^b
Hergo	2012	nd	nd	14.7 ± 1.0A$_b$	nd	103.2 ± 6.4*A$_c$	nd
	2013	nd	nd	8.4 ± 0.6B^c	nd	97.2 ± 5.6*A^c	nd
Krasavica	2012	nd	2.1 ± 0.1A$_a$	881.3 ± 42.5*A$_c$	2.3 ± 0.1A$_a$	365.9 ± 19.4*A$_d$	nd
	2013	nd	4.4 ± 0.2B^a	798.3 ± 38.4*A^a	3.7 ± 0.1A^a	481.8 ± 32.5*B^d	nd
Leicora	2012	2.7 ± 0.2A$_a$	nd	34.4 ± 1.7A$_d$	nd	92.8 ± 6.4*A$_{ac}$	6.2 ± 0.4A$_a$
	2013	5.6 ± 0.3B^a	nd	88.4 ± 5.6B^d	nd	71.8 ± 5.9*A^a	4.3 ± 0.3A^c
Ljubitelna	2012	nd	nd	126.1 ± 9.4*A$_e$	nd	nd	nd
	2013	nd	nd	186.3 ± 11.2*A^e	nd	nd	nd
Pavlovsky	2012	nd	3.6 ± 0.1A$_a$	953.5 ± 42.5*A$_c$	nd	nd	3.43 ± 0.2A$_b$
	2013	nd	1.3 ± 0.1B^b	813.2 ± 44.8*A^a	nd	nd	3.12 ± 0.2Aa^c
Peterburský	2012	nd	nd	306.7 ± 18.7*A$_f$	nd	nd	nd
	2013	nd	nd	401.4 ± 22.9*A^f	nd	nd	nd
Sluničko	2012	nd	16.0 ± 0.6A$_b$	249.9 ± 16.3*A$_f$	2.1 ± 0.1A$_a$	168.5 ± 4.2*A$_b$	545.9 ± 24.2A$_c$
	2013	nd	18.2 ± 0.7A^c	253.4 ± 16.8*A^g	2.42 ± 0.1A^a	188.3 ± 14.4*A^b	765.9 ± 31.3B^d
Trofinovsky	2012	2.7 ± 0.1A$_a$	nd	830.5 ± 46.7*A$_g$	nd	199.1 ± 9.5*A$_b$	nd
	2013	3.7 ± 0.1B^c	nd	670.6 ± 39.4*A^b	nd	200.7 ± 14.3*A^b	nd
Vitaminnaja	2012	3.4 ± 0.1A$_c$	nd	251.6 ± 13.2*A$_f$	nd	30.9 ± 1.8*A$_e$	12.3 ± 0.9A$_d$
	2013	8.1 ± 0.5B^b	nd	87.0 ± 6.3*B^h	nd	108.2 ± 8.3*B^c	6.4 ± 0.3B^e
Velkoosecky	2012	nd	nd	nd	nd	nd	nd
	2013	nd	nd	nd	nd	nd	nd

Values identified by an asterix (*) mean content in mg kg^{-1}; the results are expressed as the mean ± standard deviation ($n = 9$); different capital letters in the same column indicate significant differences ($p < 0.05$) between the picking years (2012–2013) within the same cultivar; different small subscript and superscript letters in the same column indicate significant differences ($p < 0.05$) between the cultivars in 2012 and 2013, respectively; nd – not detected.

ranging from (4.9 ± 0.3) μg kg^{-1} in Botanicky to (14.3 ± 0.7) μg kg^{-1} in Krasavica. Nonanal was detected in Aromat, Botanicky Krasavica, Sluničko and Trofinovsky, also in very low concentrations ranging from (1.3 ± 0.1) μg kg^{-1} in Botanicky and Trofinovsky to (3.9 ± 0.1) (3.9 ± 0.2) μg kg^{-1} in Aromat (Krasavica), respectively. Ethyl butanoate was detected in all analysed cultivars. Its amount was very variable in the range from (1.2 ± 0.1) μg kg^{-1} in Pavlovsky to (450.1 ± 12.0) μg kg^{-1} in Sluničko. Ethyl octanoate was also detected in all analysed cultivars also in very variable amounts in the range from (2.2 ± 0.1) μg kg^{-1} in Pavlovsky to (1.2 ± 0.1) mg kg^{-1} in Aromat. Ethyl heptanoate was detected in Aromat, Botanicky, Buchlovicky, Leicora, Trofinovsky and Vitaminnaja in very low concentrations ranging from (1.7 ± 0.1) μg kg^{-1} in Buchlovicky to (8.9 ± 0.3) μg kg^{-1} in Botanicky. Pentan-2-one was detected in Botanicky, Krasavica, Pavlovsky and Sluničko in quite low concentrations ranging from (1.3 ± 0.1) μg kg^{-1} in Pavlovsky to (18.2 ± 0.7) μg kg^{-1} in Sluničko. 3-Hydroxybutan-2-one was detected in all analysed cultivars with the exception of Aromat and Velkoosecky. Its content was quite high in most cultivars, from (87.0 ± 6.3) mg kg^{-1} in Vitaminnaja to (953.5 ± 42.5) mg kg^{-1} in Pavlovsky, however, Hergo and Leicora contained only tens of μg kg^{-1} of this compound. Undecan-2-one was present only in Krasavica and Sluničko in very low concentrations, from (2.1 ± 0.1) μg kg^{-1} to (3.7 ± 0.1) μg kg^{-1}. Acetic acid was detected in Botanicky, Buchlovicky, Hergo, Krasavica, Leicora, Sluničko, Trofinovsky and Vitaminnaja. Its content was high in all cultivars, ranging from (30.9 ± 1.8) mg kg^{-1} in Vitaminnaja to (481.8 ± 32.5) mg kg^{-1} in Krasavica. Propanoic acid was detected in Aromat, Buchlovicky, Leicora, Pavlovsky, Sluničko and Vitaminnaja. Its content varied from (2.7 ± 0.2) μg kg^{-1} in Aromat to (765.9 ± 31.3) μg kg^{-1} in Sluničko. ANOVA confirmed significant differences ($p < 0.05$) between the cultivars in both years, the differences within cultivars (in 2012–2013) were mostly small or not significant (see Table 1).

Conclusions

Only a few studies on the aroma compounds present in sea buckthorn berries have been published so far; this work is a complex study focused on selected cultivars intended to be grown in the Czech Republic. In total, 69 volatile compounds were identified,

several of them, e.g. 2-methylpropan-1-ol and/or 2-methylbutan-1-ol, were found in sea buckthorn for the first time. Based on PCA, 18 relevant compounds (see Table 1) from all those identified, which could be used to discriminate the samples, were selected. The small variability of the selected aroma compounds within a variety and large variability among varieties were found. Especially the Krasavica and Sluničko cultivars were found to be significantly different from the other cultivars studied, probably owing to the rich content of aroma compounds. The differences between the picking years (2012 and 2013) were small or not significant ($p \geq 0.05$) for most cultivars.

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Comparison of volatiles identified in *Aronia melanocarpa* and *Amelanchier alnifolia* using solid-phase microextraction coupled to gas chromatography-mass spectrometry

LENKA BUTOROVÁ – EVA VÍTOVÁ – MARTIN POLOVKA

Summary

The volatile constituents of two chokeberry (*Aronia melanocarpa)* and five saskatoon berry *(Amelanchier alnifolia)* cultivars were evaluated by solid-phase microextraction (SPME) coupled to gas chromatography with flame ionization detector (GC-FID) and gas chromatography-mass spectrometry (GC-MS) during three seasons (2011–2013). Altogether, 39 and 31 volatile compounds were identified in chokeberries and saskatoon berries, respectively. Similarities were found between chokeberry and saskatoon berry composition of volatiles, in both of them, alcohols and aldehydes were the most abundant, esters, ketones, acids and terpenoids created the minor part. From all the identified volatiles, fourteen compounds were recognized as being responsible for overall aroma of the berries under study (with odour activity values ≥ 1) with their typical fruity, sweet, grassy and/or floral aroma. Based on the results of principal component analysis, 24 compounds were selected and compared using analysis of variance to investigate differences among samples. Statistical analysis confirmed that there were significant differences among varieties and the year of production, but also some similarities were found. These differences/similarities were probably influenced by climatic conditions, habitat and/or degree of maturity/ripening.

Keywords

Aronia melanocarpa; Amelanchier alnifolia; volatiles; solid-phase microextraction; gas chromatography-mass spectrometry

Nowadays, increasing attention of consumers is oriented toward less known fruits, as they are rich sources of natural antioxidants responsible for their health benefit properties [1–5].

Aronia melanocarpa, also known as black chokeberry, is a shrub or tree native to North America, belonging to rose family (Rosaceae). Its dark berries are similar to black currant with a very astringent flavour. They have been used both as food and in traditional medicine for treatment of e.g. cold [6]. Chokeberry products are accepted as nutritional supplements, and are also processed into juices, wines, jams etc. [7].

Amelanchier alnifolia (saskatoon berry) is a shrub native to North America. The fruit is a pome fruit belonging to the rose family (Rosaceae) [8]. The red or dark-purple pomes are sweet and edible. Saskatoon berries are consumed fresh, processed into jams, spreads, juices, syrups, wines etc. [9].

Besides the nutritional value, the sensory quality is important from the consumers' point of view. Characterization of aroma profile of a plant is of great importance, since it enables to optimize and/or improve the quality of products and to develop new products for the market [10, 11]. It is generally known that the volatile aroma compounds are responsible for the typical flavour

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of berries [12, 13]. However, scarce information is available about volatile compounds of chokeberries and saskatoon berries.

In case of chokeberries, Hirvi and Honkanen [14] firstly identified 48 volatile compounds by gas chromatography-mass spectrometry (GC-MS), with benzaldehyde derivatives identified as major components. The presence of these compounds is probably related to the hydrolysis of cyanogenic precursors (amygdalin). Dolezal et al. [15] identified 17 volatile aroma compounds in chokeberries. The degradation products of cyanogenic precursors and aromatic amino acids, mainly benzaldehyde, benzylalcohol, benzylesters were dominant. Kraujalytė et al. [16] identified in total 74 volatile compounds, the majority of them being degradation products of fatty acids or amino acids. Typical aroma of chokeberries, described as almond, fruity, sour and/or green, was influenced mainly by the presence of aldehydes, alcohols and terpenoids. Other common groups of volatiles with a lower abundance were ketones, esters and acids. The branched esters were major aroma-active compounds with fruity notes [16].

A lack of information is available about the aroma profile of saskatoon berry. Only the study of Mazza and Hodgins [17] dealt with assessment of benzaldehyde as the major volatile aroma compound in 7 varieties of saskatoon berries.

The aim of this work was (i) to identify and quantify volatile compounds of chokeberries and saskatoon berries in selected cultivars grown in Czech Republic, (ii) to estimate contribution of each volatile to the aroma of the berries by calculating odour activity values (OAVs), (iii) to select compounds best expressing variability among samples and (iv) to compare the selected compounds to describe differences among samples.

MATERIALS AND METHODS

Chemicals

The following chemicals, all of analytical grade purity, were used: benzaldehyde, *cis*-2-octenal, decan-2-one, dodecan-1-ol, heptadecan-1-ol, heptadecan-2-ol, heptanal, hexadecan-2-ol, hexanal, hexen-3-ol, octen-3-ol, pentanal, phenylethanal, propan-2-one, nonan-2-one, undecan-2-one, 2-methylbutan-1-ol (Sigma-Aldrich, St. Louis, Missouri, USA); acetic, benzoic, butanoic, hexanoic, propanoic, 2-hydroxypropanoic, 2-methylpropanoic, 2-methylbutanoic, 3-methylbutanoic acids, 2-methylpropan-1-ol, 3-hydroxybutan-2-one, 3-methylbutan-1-ol, 4-methylpentan-2-one, butan-1-ol, butan-2-ol, butan-2,3-dione, butyl-acetate, benzylalcohol, *cis*-2-hexenal, ethanal, ethanol, ethyl-butanoate, ethyl-ethanoate, ethyl-decanoate, ethyl-dodecanoate, ethyl-heptanoate, ethyl-hexanoate, ethyl-octanoate, decan-1-ol, heptan-2-ol, heptan-2-one, hexan-1-ol, methyl-acetate, nonan-2-ol, nonanal, octan-1-ol, octan-2-ol, octanal, pentan-1-ol, pentan-2-ol, pentan-2-one, phenylethanol, phenylethyl-ethanoate, propan-1-ol, propan-2-ol, propanal, propyl-ethanoate, tridecan-2-one (Merck, Darmstadt, Germany); butan-2-one, methanol (Lachema, Brno, Czech Republic); oct-1-en-3-ol, 3-methylbutan-1-al (Fluka, Seelze, Switzerland).

Plant material

Two cultivars of *Aronia melanocarpa*: Nero (AN) and Viking (AV), and five cultivars of *Amelanchier alnifolia*: Lamarckii Balerina (SLB), Thiessen (ST), Ostravsky (SO), Tisnovsky velkoplody (STV), Tisnovsky skolsky (STS) were analysed. Fruits were obtained from Mendel University in Brno (Czech Republic) during 2011–2013. Fruits were harvested in their full ripeness and immediately stored in the refrigerator at 5 °C. All analyses were performed within seven days. For analysis, 1 g of homogenized berries was placed into a vial for solid-phase microextraction (SPME); three samples of every cultivar were taken, every sample was analysed three times (number of repetitions, $n = 9$).

SPME and GC conditions

SPME was carried out using Carboxen/Poly(dimethylsiloxane) fibre (CAR/PDMS) 85 μm (Supelco, Bellefonte, Pensylvania, USA) under the following conditions: extraction temperature 35 °C; equilibrium time 30 min; extraction time 20 min; desorption temperature 250 °C; desorption time 20 min.

Gas chromatograph TRACE GC (ThermoQuest, Milano, Italy) with capillary column DB-WAX (30 m × 0.32 mm × 0.5 μm, J&W Scientific, Santa Clara, California, USA) was used for gas chromatography with flame ionization detector (GC-FID) analyses under the following conditions: injector temperature 250 °C; splitless desorption 5 min; carrier gas N_2, flow rate 0.9 ml·min^{-1}; flame ionization detector, temperature 220 °C; H_2 inlet 35 ml·min^{-1}; air inlet 350 ml·min^{-1}; make up N_2 30 ml·min^{-1}. The oven temperature was 40 °C for 1 min, then it was increased up to 200 °C at a rate of 5 °C·min^{-1} and maintained at 200 °C for 7 min.

GC-MS analyses were performed on a gas chromatograph HP 6890 with MS detector 5973 N and Mass Spectral Library NIST 98 (Agilent, San-

ta Clara, California, USA). Helium was used as a carrier gas. GC column and conditions of analysis were the same as described above.

The standard addition method was used for quantification of analytes to control the influence of the sample matrix. The mixture of standards was divided into groups consisting of five chemicals that were gradually added (1 ml, each) directly into the sample. These standard mixtures were analysed in the same manner as the samples. Five content levels, in the range of 0.001–200 $mg \cdot kg^{-1}$ (different for various standards), were used to establish the calibration curves. Validation and the validation parameters of the used methods were identical as previously described in details by Vítová et al. [18] The repeatability was verified by repeated extractions ($n = 5$) of the standard mixtures (relative standard deviations < 10 %), detection and quantification limits were in the range of 0.001–0.50 $mg \cdot kg^{-1}$. Linearity was tested within the range of 0.001–200 $mg \cdot kg^{-1}$ (for methanol and ethanol 0.50–2000 $mg \cdot kg^{-1}$); correlation coefficients were all above 0.99 [18].

Odour activity values

OAVs were calculated by dividing contents in the sample by odour threshold acquired from the literature [16, 19–24].

Statistical analysis

The results were evaluated using Microsoft Excel 2010 (Microsoft, Redmond, Washington, USA) and are expressed as mean ± standard deviation ($n = 9$). Principal component analysis (PCA) was used to reveal the differences among samples and to reduce the original data set of experimental characteristics as well as to identify the key volatile compounds. The differences among cultivars and year of production were statistically treated by analysis of variance (ANOVA) using Duncan's test. A probability value of $p \leq 0.05$ was accepted for statistically significant different results. These analyses were performed using Statistica 6 (StatSoft, Tulsa, Oklahoma, USA).

RESULTS AND DISCUSSION

Assessment of volatile compounds in cultivars of chokeberries and saskatoon berries

Two cultivars of *Aronia melanocarpa* and five cultivars of *Amelanchier alnifolia* of 2011–2013 seasons were investigated. Volatile compounds were extracted by SPME, identified by GC-MS and quantified using GC-FID. SPME was used to extract the volatiles, as it is fast, sensitive and does not involve utilization of solvents. It has been previously successfully used for extraction of volatiles from food [25, 26]. Its limitations in quantification ability were obeyed in the recent study by maintaining constant as many experimental conditions as possible.

In total, 39 volatile compounds were identified in chokeberry cultivars, comprising 8 aldehydes: benzaldehyde, ethanal, hexanal, nonanal, octanal, pentanal, propanal, *trans*-2-hexenal; 19 alcohols: 2-methylbutan-1-ol, 2-methylpropan-1-ol, 3-methylbutan-1-ol, butan-1-ol, butan-2-ol, *cis*-3-hexenol, ethanol, heptan-2-ol, hexan-1-ol, methanol, nonan-2-ol, octan-1-ol, octan-2-ol, pentan-1-ol, pentan-2-ol, phenylmethanol, propan-1-ol, propan-2-ol, *trans*-3-hexenol; 6 esters: ethyl-butanoate, ethyl-decanoate, ethyl-ethanoate, ethyl-hexanoate, ethyl-pentanoate, methyl-ethanoate; 3 ketones: heptan-2-one, pentan-2-one, propan-2-one; 2 acids: acetic, hexanoic and 1 terpen: limonene. The example of a chromatogram of compounds identified in chokeberry cultivar Nero (harvested in 2012) is given in Fig. 1.

Alcohols (40–52.6%, w/w) were the most abundant compounds identified in the samples, in good agreement with previously published results of Dolezal et al. [15] but, on the other hand, different from Hirvi and Honkanen [14] and Kraujalytė et al. [16]. Differences in alcohols content could be attributed primarily to differences following from varieties, but also from different climatic and geographical conditions; also sample post-harvest treatment and conditions of storage (fresh vs frozen fruits) could influence the content of compounds. Particularly, the increased content of alcohols (mainly ethanol) could be the result of early-stage fermentation process occurring in fruits [27], although in this case, samples were stored at < 5 °C until analysed. Another group of dominant compounds were aldehydes (13–33.3%, w/w), esters (11.8–22.7%, w/w), ketones (4.3–13.3%, w/w), acids (0–8.7%, w/w) and terpenoids (0–5.9%, w/w).

As regards consistency of the number and character of the identified volatiles, presented results are in good agreement with available studies, as more than 200 compounds were found in chokeberries [16]. Dolezal et al. [15] identified benzaldehyde, hexanal, *trans*-2-hexenal, butan-1-ol, phenylmethanol, hexan-1-ol, pentan-1-ol, pentan-2-on in chokeberry extracts, Hirvi a Honanken [14] identified benzaldehyde, *trans*-2-hexenal, *cis*-3-hexen-1-ol, hexan-1-ol, phenylmethanol and hexanoic acid in chokeberry juice and Kraujalytė et al. [16] identified benzaldehyde, hexanal, nonanal, octanal, *trans*-2-hexenal, 2-methylbutan-1-

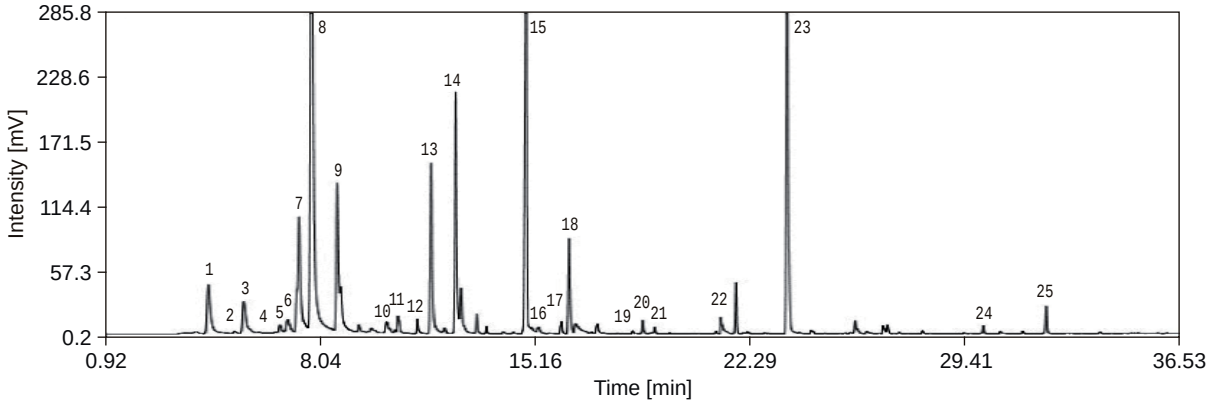


Fig. 1. Chromatogram of compounds identified in chokeberry cultivar Nero harvested in 2012.

Peak assignment: 1 – ethanal , 2 – propanal, 3 – propan-2-one, 4 – methyl-ethanoate, 5 – ethyl-ethanoate, 6 – propan-2-ol, 7 – methanol, 8 – ethanol, 9 – pentan-2-one, 10 – butan-2-ol, 11 – ethyl-butanoate, 12 – hexanal, 13 – 2-methylpropan-1-ol, 14 – ethyl-pentanoate, 15 –3-methylbutan-1-ol, 16 – 2-methylbutan-1-ol, 17 – *trans*-2-hexenal, 18 – pentan-1-ol , 19 – heptan-2-ol, 20 – hexanol, 21 – *cis*-3-hexenol, 22 – nonanal, 23 – benzaldehyde, 24 – hexanoic acid, 25 – benzylalcohol.

ol, butan-1-ol, heptan-2-ol, hexan-1-ol, octan-1-ol, pentan-1-ol, pentan-2-ol, phenylmethanol, ethyl-butanoate, ethyl-ethanoate, ethyl-hexanoate, heptan-2-one, acetic acid and limonene in chokeberries; benzaldehyde was found as the major volatile constituent in chokeberries [16].

It is generally accepted that formation of volatile compounds in fruits is associated mainly with pigment formation during the ripening process; some compounds can be generated from oxidation and degradation of main fruit constituents [28]. The majority of the volatiles identified in the current study are enzymatic degradation products of basic constituents [13, 16]. The degradation products of fatty acids include straight-chain alcohols (2–9C), aldehydes (2–9C), ketones (3–7C) and esters (especially ethyl esters of short-chain acids of 2–10C). Other compounds identified are degradation products of amino acids and cyanogenic compounds, among them aromatic compounds (benzaldehyde, phenylmethanol) and branched-chain alcohols (2-methylbutan-1-ol, 2-methylpropan-1-ol, 3-methylbutan-1-ol). Terpenoid limonene is biosynthesized in plants from two initial isoprenoids by two pathways in the presence of terpene synthases [29]. Ethanol (20.2–322.2 $\mu g \cdot kg^{-1}$), methanol (17.5–139.0 $\mu g \cdot kg^{-1}$), 3-methylbutan-1-ol (0–17.4 $\mu g \cdot kg^{-1}$), 2-methylpropan-1-ol (0–15.6 $\mu g \cdot kg^{-1}$), *trans*-2-hexenal (0–7.3 $\mu g \cdot kg^{-1}$) and benzaldehyde (0.06 $\mu g \cdot kg^{-1}$ – 2.97 $\mu g \cdot kg^{-1}$) were the most abundant volatile compounds of *A. melanocarpa.* The high methanol content could probably be caused by pectin degradation [30].

In contradiction to chokeberry, 31 volatile compounds were identified in saskatoon berries, of which 10 represented aldehydes: benzaldehyde, ethanal, heptanal, hexanal, nonanal, octanal, pentanal, propanal, *trans*-2-hexenal and 3-methylbutan-1-al; 13 alcohols: butan-1-ol, *cis*-3-hexenol, ethanol, heptan-2-ol, hexan-1-ol, methanol, oct-1-en-3-ol, pentan-1-ol, phenylmethanol, propan-1-ol, 2-methylbutan-1-ol, 2-methylpropan-1-ol, 3-methylbutan-1-ol; 4 esters: ethyl-decanoate, ethyl-ethanoate, ethyl-hexanoate, methyl-ethanoate, 3 ketones: heptan-2-one, nonan-2-one, propan-2-one and acetic acid. The typical chromatogram of saskatoon berry cultivar Ostravsky (harvested in 2012) is shown in Fig. 2 illustrating multicomponent composition of the analysed sample.

It is generally known that fruit aroma is based on a mixture of a large number of volatile compounds, whose composition and content is specific to species and often to the variety of fruits [31]. As expected, the composion of volatiles of saskatoon berry cultivars was quite similar to chokeberries (in fact, 25 of the identified volatile compounds were identical), which confirmed the family similarities. On the other hand, also several differences between chokeberries and saskatoon berries were observed, e.g. heptanal, 3-methylbutan-1-al, oct-1-en-3-ol, 2-methylbutan-1-ol, 2-methylpropan-1-ol and 3-methylbutan-1-ol were identified only in saskatoon berries.

In contrast to chokeberry, to the best of our knowledge, no work about saskatoon berry volatiles has been published, with the only exception of Mazza and Hodgins [17] who, however, were interested only in benzaldehyde. As mentioned above, the results obtained indicate similar-

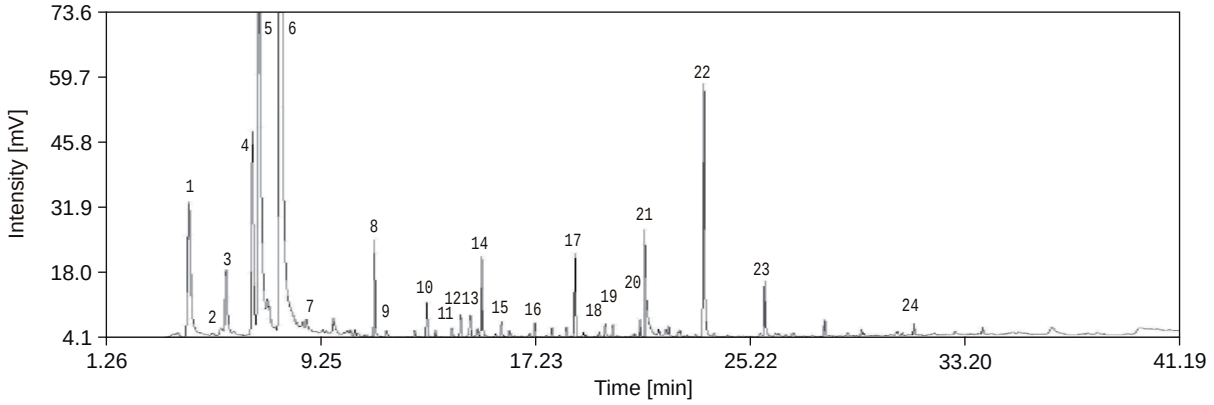


Fig. 2. Chromatogram of compounds identified in saskatoon berry cultivar Ostravsky harvested in 2012.

Peak assignment: 1 – ethanal , 2 – propan-2-one, 3 – methyl-ethanoate, 4 – ethyl-ethanoate, 5 – methanol, 6 – ethanol, 7 – pentanal, 8 – hexanal, 9 – 2-methylpropan-1-ol, 10 – butan-1-ol, 11 – heptanal, 12 – limonene, 13 – 3-methylbutan-1-ol, 14 – *trans*-2-hexenal, 15 – pentan-1-ol, 16 – heptan-2-ol, 17 – hexan-1-ol, 18 – nonan-2-one , 19 – nonanal, 20 – 1-octen-3-ol, 21 – ethanoic acid, 22 – benzaldehyde, 23 – ethyl-decanoate, 24 – benzylalcohol.

ity to chokeberry volatiles. Also in case of saskatoon berry, different alcohols were in the group of most abundant compounds, their content ranged from 26.7% to 46.7%, w/w; followed by the groups of aldehydes (20–34.8%, w/w), esters (12.5–26.7%, w/w), ketones (4.3–15.8%, w/w), acids (0–7.7%, w/w) and terpenoids (0–5.3%, w/w). Composition of individual groups of identified volatiles was also quite similar to chokeberries, confirming thus the above-discussed family similarities. Methanol (883.5–1 426.4 $\mu g \cdot kg^{-1}$), ethanol (149.79–469.0 $\mu g \cdot kg^{-1}$), acetic acid (0–222.2 $\mu g \cdot kg^{-1}$), ethanal (0–84.7 $\mu g \cdot kg^{-1}$) and *trans*-2-hexenal (0–9.44 $\mu g \cdot kg^{-1}$) were the most abundant volatile compounds of *A. alnifolia.* Volatile compounds identified in saskatoon berry cultivars are degradation products of fatty acids, amino acids and cyanogenic glycosides (mainly amygdalin and prunasin) [32].

Odour activity values

The calculated OAVs suggest that the following compounds could be the contributors to aroma of samples in this study: ethanol (OAVs > 100; alcoholic [16]), *trans*-2-hexenal (OAVs > 50; green [16], grassy [19], apple [16, 20]), ethanal (OAVs 3–65; grassy, sweet [19]), hexanal (OAVs 3–91; grassy [16, 20], tallow, fat [16], aldehyde [21]), ethyl-hexanoate (OAVs 19–102; fruity [16, 20], apple peel [16], melon [20]), and then 3-methylbutan-1-ol (OAVs 4–13; green [20], malt [16]), benzaldehyde (OAVs 2–8; candy, sweet [19], bitter almond [16, 21, 22], woody [21], burn sugar [16], roasted pepper [22]), oct-1-en-3-ol (OAVs 3–7; mushroom [21], lavender, rose, hay [21]), acetic acid (OAVs 2–10; acidic [16, 22], sour [16], fruity, plastic [22]), 2-methylbutan-1-ol (OAVs 2–8; fruity [16]), 3-methylbutan-1-ol (OAVs 3–5; alcoholic [19], fruity [19], whisky, malt, burnt [16], whine, ether [21]), methanol (OAVs 1–2; medicinal [23]), heptanal (OAVs 1–2; oily [16], citrus, rancid [16]), and nonanal (OAVs 1–3; floral, citrus [16, 21], fat, green [16], vinegar [21], piney [24]). Most of these compounds were previously recognized as aroma-active, the description of their aroma is in parenthesis. Theoretically, the remaining compounds did not directly contribute (OAVs < 1), they could act only as aroma enhancers because of synergistic effects. As stated above, 12 and 14 aroma active substances were found in chokeberries and saskatoon berries, respectively; they included 6 alcohols, 6 aldehydes, 1 ester and 1 acid. Oct-1-en-3-ol and heptanal were not present in chokeberries.

Several previously published studies dealt with identification of aroma-active components of various fruits [11, 15, 18, 26, 29]. However, the only mention of aroma-active constituents of chokeberry is in the study of Kraujalytė et al. [16]. The authors detected 15 aroma constituents in chokeberries by GC-olfactometry, among them nonanal with pelargonium, green odour, in accordance with the presented results. From the obtained results it is also evident that not all volatile compounds identified are responsible for the typical aroma of these berries. It is also obvious that the compounds with higher content in berries do not need to be aroma-active and, on the other hand, volatile compounds with lower content could be aromatic [12, 33]. That is in accordance with well known facts that aroma depends upon the combi-

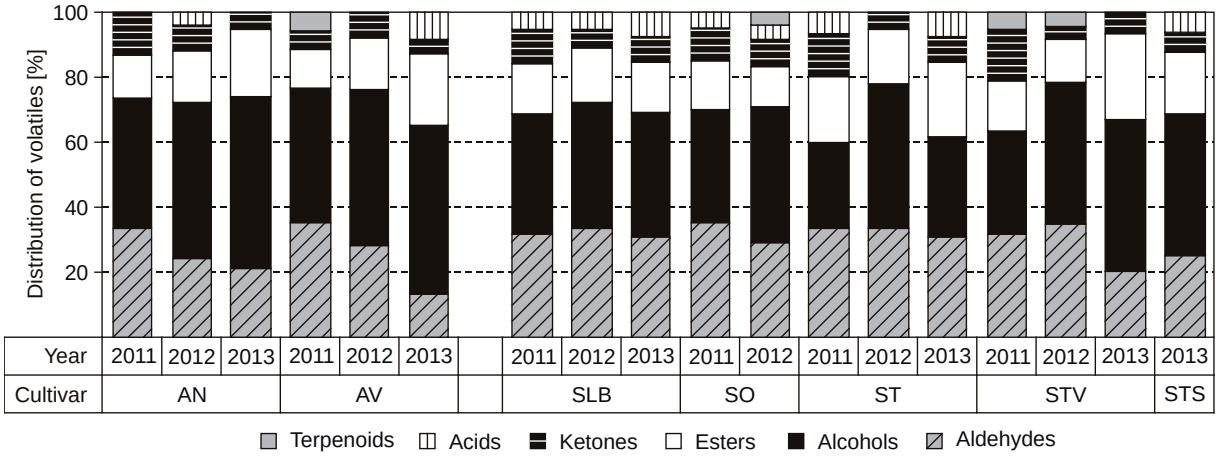


Fig. 3. Distribution of chemical groups of volatile compounds in chokeberry and saskatoon berry cultivars harvested in 2011–2013.

Aronia cultivars: AN – Nero, AV – Viking; Saskatoon berry cultivars: SLB – Lamarckii Balerina, SO – Ostravsky, ST – Thiessen, STV – Tisnovsky velkoplody, STS – Tisnovsky skolsky

nation of volatiles, on the content and the perception threshold of individual volatile compounds [34].

Comparison of selected aroma compounds identified in cultivars of chokeberries and saskatoon berries

It is generally known that volatile profiles of fruits are complex and vary depending on the cultivar, ripeness, pre- and post-harvest conditions and analytical methods employed [31]. As can be seen in Fig. 3, the overall composition of groups of volatiles in chokeberries and saskatoon berries was quite similar. This trend clearly confirmed species similarities mentioned above. There were only small differences among the samples, both between the year of production and individual varieties.

These differences were further investigated using PCA and ANOVA analysis. At first, the PCA analysis was performed taking into consideration results of all 18 samples (berry cultivars) and 39 compounds identified. The cumulative percentage contribution of variance of the first four PCs was 58.4%. PC1 represented 23.7%, PC2 15.3%, PC3 11.1% and PC4 8.3% of the remaining variance.

Samples of aronia and saskatoon berries were well separated from each other (data not presented) along PC1. Samples of saskatoon berries were placed close together, indicating the similarity of cultivars. They were placed in the left part of the plot, while the most of aronia samples were placed in the right part of the plot. The separations along PC2 were rather related to the year of harvest (2011/2012/2013). The aronia samples were placed farther from each other, cultivars produced in 2011 (both AV and AN) being included in a cluster of saskatoon berries, the others being placed individually and so there were larger differences between aronia cultivars as well as between year of production. This fact indicated the importance of both of these factors for their properties, even when comparing, by taxonomic classification, similar products.

On the basis of eigenvalues, the following compounds were found to be the most important parameters for the construction of PC1, i.e. for description of the overall variation: hexan-1-ol (0.88), ethyl-butanoate (0.81), 3-methylbutan-1-ol (0.78), hexanoic acid (0.75), methanol (–0.73), *cis*-3-hexenol (0.74), 2-methylpropan-1-ol (0.70), hexanal (0.70), ethyl-pentanoate (0.68), pentan-1-ol (0.65), octan-1-ol (0.61), nonan-2-ol (0.61), butan-1-ol (0.59), methyl-ethanoate (–0.59) and pentane-2-one (0.57).

Heptan-2-ol (0.72), benzaldehyde (0.71), ethyl-decanoate (–0.70), propan-2-ol (0.65), butan-2-ol (0.65), phenylmethanol (0.65), pentan-2-one (0.63) and *trans*-2-hexenal (0.59) played the key role in PC2, whereas butan-1-ol (0.75), nonan-2-ol (0.73), ethyl-ethanoate (0.56) and 3-methylbutan-1-ol (0.52) played the key role in PC3 and ethanol (0.61) in PC4. These 24 compounds could be considered as the most important to represent and explain the variability of the whole system, so these compounds were selected for expression of differences among the samples.

PCA analysis was also performed individually for chokeberries (data matrix 6 × 24) and

saskatoon berries (data matrix 12 × 24) using the 24 compounds identified above as the most characteristic. Acquired PCA score plots are depicted in Fig. 4 and Fig. 5. In these cases, the cumulative percentage contribution of variance of the first three PCs was 88.9% and 61.4% for chokeberries and saskatoon berries, respectively.

In chokeberry cultivars, differentiation of samples according to the production years was clearly shown, which was mainly related to PC1 (Fig. 4). Cultivars from 2011 were placed close to the left bottom part, while cultivars from 2012 were placed at the right bottom part; cultivars from 2013 were located in the upper part of the plot. Most of the selected compounds correlated positively with PC1, so the cultivars from 2012 contained a high quantity of them, especially of propan-2-ol, butan-2-ol, heptan-2-ol and phenylmethanol. AV cultivar from 2013 was placed individually in the upper part, being rich in butan-1-ol, nonan-2-ol and ethyl-ethanoate. The differences among varieties (AV and AN) were obvious mainly in samples harvested in 2012 and 2013, in contrast to samples of 2011, for which both cultivars showed similar volatile profiles. These results confirmed varietal differences/similarities among samples. Analogous phenomena were previously observed at different varieties of gooseberry [20], raspberry [35] or peaches [36].

Volatile profiles of aronia displayed considerable variation between the different years, suggesting thus important influence of climatic and environmental factors [36, 37]. Nevertheless, it will be necessary to perform a more detailed study of the relationships between the other variables (volatile compounds and standard quality parameters) and the factors considered (harvest dates, shelf life period and storage atmosphere) in order to confirm and quantify the supposed effects of these factors on sample properties in terms of composition of aroma active compounds and also regarding other aspects.

PCA score plot of saskatoon berry cultivars (Fig. 5) is more complicated, with obvious differences mainly among production years related to PC2. All cultivars from 2013 were well separated in the left part of the plot; from the selected compounds, they were rich in ethyl-decanoate and 2-methylpropan-1-ol. Cultivars produced in 2012 were clearly positioned in the centre of the graph, the position being influenced by high contents of butan-1-ol, pentan-1-ol and phenylmethanol. Most of cultivars from 2011 were placed in the right part, being rich in *trans*-2-hexenal, methyl-ethanoate, ethanol, ethyl-ethanoate and heptan-2-ol.

Samples from 2011 and 2012 were not separated clearly, probably due to sharing of some similarities in aroma profiles. The position of samples from 2011 and 2012 was mainly influenced by these volatile compounds: pentan-1-ol, butan-1-ol, *trans*-2-hexenal, heptan-2-ol, hexan-1-ol, methanol, ethyl-decanoate and benzaldehyde. Also analysis of variance, which is discussed in detail below, confirmed these similarities. Anyway, as in the case of aronia, an important influence of climatic and environmental factors [36, 37] on composition of volatiles was confirmed.

If the saskatoon berries from 2011 were compared separately, the varieties ST, SLB and SO were quite similar, but cultivar STV was different from the others. Its specific position in the upper left corner of the plot was probably caused by the highest contents of ethyl-decanoate and hexanal and, on the other hand, the lowest con-

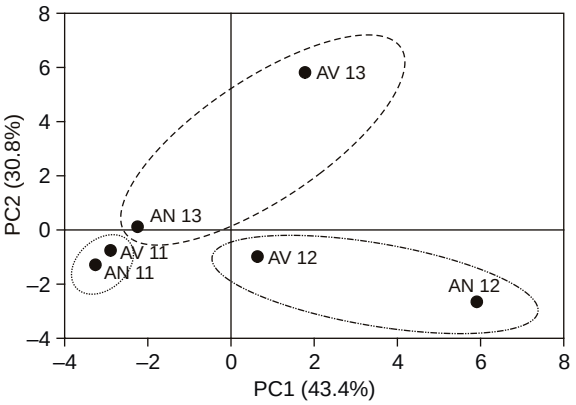
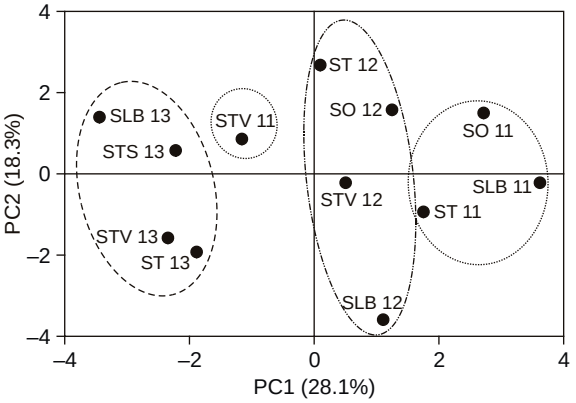


Fig. 4. PCA score plots of *Aronia melanocarpa* cultivars harvested in 2011–2013.

Aronia cultivars: AN – Nero, AV – Viking.



Fig, 5. PCA score plots of *Amelanchier alnifolia* cultivars harvested in 2011–2013.

Saskatoon berry cultivars: SLB – Lamarckii Balerina, SO – Ostravsky, STS – Tisnovsky skolsky, ST – Thiessen, STV – Tisnovsky velkoplody.

tents of ethyl-ethanoate, ethanol and methyl-ethanoate from all of the varieties under study in 2011 season.

Cultivars from the year 2012 revealed different trend, as the varieties ST, SO, STV had quite similar volatile profiles, but cultivar SLB differed from the others. It was placed at the bottom, probably due to the highest contents of benzaldehyde and phenylmethanol.

Fig. 5 also shows that cultivars SLB and STS in 2013 season were quite similar in their profiles, but differed from STV and ST, which were similar to each other. SLB and STS position in PC plot was influenced by the contents of methanol and ethyl-decanoate, while STV and ST position was mainly given by 2-methylpropan-1-ol content. SLB and STS also had similar content of methanol, while ST and STV had similar contents of hexanal, hexan-1-ol and ethyl-decanoate. These differences/similarities were further statistically tested by ANOVA Duncan's test. Results confirmed that they could be directly linked to particular climatic conditions, in accordance with the known fact that the content of fruit constituents could be influenced by climatic conditions, cultivar, habitat and time of collection [38].

ANOVA Duncan's test was applied to the above mentioned 24 most relevant compounds, selected by PCA as the most discriminating parameters, with an aim to better express the differences among the varieties and production years. The comparison is given in Tab. 1 for chokeberries, and in Tab. 2 for saskatoon berries. The significance of differences (at $p < 0.05$) was evaluated for the following criteria: production years (2011 vs 2012 vs 2013) and the fruit variety. Results confirmed the expected differences among the production years (2011–2013), which was also evident from PCA. Significant differences among years of production for variety AN were found in the contents of benzaldehyde, hexanal, *trans*-2-hexenal, *cis*-3-hexenol, ethanol, hexan-1-ol and ethyl-ethanoate; for variety AV in the contents of *cis*-3-hexenol, methanol and ethyl-ethanoate. The highest contents of these volatiles were found in 2012 for both cultivars, which could be influenced by weather

Tab. 1. Comparison of selected volatile aroma compounds in cultivars of *Aronia melanocarpa*.

Cultivar	AN			AV		
Production year	2011	2012	2013	2011	2012	2013
Compound	Content [$\mu g \cdot kg^{-1}$]					
Hexanoic acid	nd	703.1 ± 37.0	nd	nd	nd	615.4 ± 6.2
Benzaldehyde	441.9 ± 7.0 ^{Aa}	2972.9 ± 18.9 ^{Bc}	52.3 ± 0.2 ^{Ce}	146.4 ± 9.1 ^{Db}	2755.3 ± 180.7 ^{Ec}	59.8 ± 1.1 ^{Df}
Hexanal	56.6 ± 0.2 ^{Aa}	203.9 ± 9.6 ^{Bc}	412.8 ± 2.5 ^{Ce}	95.5 ± 1.7 ^{Db}	137.9 ± 4.5 ^{Ed}	137.9 ± 6.5 ^{Ef}
Trans-2-hexenal	5.7 ± 0.1 *^{Aa}	6.9 ± 0.2 *^{Bc}	nd	7.3 ± 0.1 *^{Db}	5.7 ± 0.1 *^{Ed}	nd
2-methylpropan-1-ol	nd	13.4 ± 0.3 *^{c}	nd	nd	7.2 ± 0.1 *^{Dd}	15.6 ± 0.2 *^{E}
3-methylbutan-1-ol	nd	9.7 ± 0.1 *^{Ac}	1.1 ± 0.1 *^{Be}	nd	4.0 ± 0.1 *^{Dd}	17.4 ± 0.5 *^{Ef}
Butan-1-ol	nd	nd	27.4 ± 0.2 ^{e}	18.5 ± 1.0 ^{D}	nd	1445.6 ± 35.3 ^{Ef}
Butan-2-ol	nd	2.1 ± 0.1 *	nd	nd	nd	nd
Cis-3-hexenol	22.5 ± 0.6 ^{Aa}	68.2 ± 5.8 ^{Bc}	166.0 ± 0.9 ^{Ce}	29.8 ± 0.2 ^{Db}	131.8 ± 6.7 ^{Ed}	85.2 ± 0.8 ^{Ff}
Ethanol	20.2 ± 0.3 *^{Aa}	322.2 ± 8.1 *^{Bc}	129.3 ± 0.2 *^{Ce}	33.6 ± 1.1 *^{Db}	192.7 ± 9.9 *^{Ed}	211.1 ± 1.5 *^{Ef}
Phenylmethanol	nd	1114.7 ± 51.4 ^{c}	nd	nd	425.4 ± 15.4 ^{d}	nd
Heptan-2-ol	nd	2.3 ± 0.2 ^{c}	nd	nd	2,0 ± 0.1 ^{c}	nd
Hexan-1-ol	59.1 ± 1.9 ^{Aa}	413.0 ± 25.4 ^{Bc}	193.4 ± 3.5 ^{Ce}	152.9 ± 5.4 ^{Db}	220.4 ± 19.9 ^{Dd}	694.1 ± 59.6 ^{Ef}
Methanol	17.5 ± 0.5 *^{Aa}	139.7 ± 10.9 * ^{Bc}	40.0 ± 0.3 *^{Ae}	28.2 ± 0.3 *^{Db}	125.7 ± 2.2 *^{Ec}	71.2 ± 0.7 *^{Ff}
Nonan-2-ol	nd	nd	nd	nd	nd	14.3 ± 0.1
Octan-1-ol	nd	nd	1.2 ± 0.1 ^{e}	nd	nd	1.0 ± 0.1
Pentan-1-ol	10.9 ± 0.1 ^{Aa}	25.6 ± 3.1 ^{Bc}	14.4 ± 0.1 ^{ABe}	37.2 ± 2.1 ^{DEb}	31.5 ± 0.1 ^{Dc}	48.7 ± 2.9 ^{Ef}
Propan-2-ol	nd	831.5 ± 186.6	nd	nd	nd	nd
Ethyl-butanoate	nd	4.5 ± 0.1 ^{c}	nd	nd	3.7 ± 0.1 ^{Dd}	9.2 ± 0.3 ^{E}
Ethyl-decanoate	7.9 ± 0.2 ^{A}	nd	7.6 ± 0.3 ^{Ae}	nd	11.0 ± 0.1 ^{D}	7.3 ± 0.2 ^{Ee}
Ethyl-ethanoate	10.2 ± 0.1 ^{Aa}	86.2 ± 6.2 ^{Bc}	42.1 ± 0.0 ^{Ce}	16.0 ± 0.6 ^{Db}	124.4 ± 0.4 ^{Ed}	283.0 ± 1.3 ^{Ff}
Ethyl-pentanoate	nd	20.1 ± 0.1 ^{Ac}	15.1 ± 0.3 ^{Be}	nd	3.4 ± 0.2 ^{Dd}	3.2 ± 0.1 ^{Df}
Methyl-ethanoate	nd	73.0 ± 2.6	nd	nd	nd	nd
Pentan-2-one	nd	9.3 ± 0.3 ^{c}	nd	nd	11.0 ± 0.1 ^{d}	nd

Aronia cultivars: AN – Nero, AV – Viking.

* – content in milligrams per kilogram, nd – not detected.

The results are expressed as mean ± standard deviation ($n = 9$). Different capital letters in superscript in the same row indicate significant differences ($p < 0.05$) among the years of production (2011–2013) within the same cultivar. Different small letters in superscript in the same row indicate significant differences ($p < 0.05$) among cultivars in 2011, 2012 and 2013, respectively.

Tab. 2. Comparison of selected volatile aroma compounds in saskatoon berry cultivars.

Cultivar	SLB			SO		STS
Production year	2011	2012	2013	2011	2012	2013
Compound	Content [$\mu g \cdot kg^{-1}$]					
Hexanoic acid	nd	nd	nd	nd	nd	nd
Benzaldehyde	528.8 ± 23.0 ^{Gg}	985.2 ± 76.7 ^{Hk}	45.8 ± 0.7 ^{Io}	72.6 ± 2.4 ^{Jh}	180.4 ± 3.7 ^{Kl}	270.3 ± 2.6 ^{r}
Hexanal	39.9 ± 4.5 ^{Gg}	13.8 ± 1.6 ^{Hk}	73.2 ± 0.2 ^{Io}	40.6 ± 13.5 ^{Jg}	49.9 ± 1.2 ^{Jl}	87.5 ± 1.8 ^{q}
Trans-2-hexenal	8.5 ± 0.2 *^{Gg}	2.1 ± 0.0 *^{Hk}	nd	8.4 ± 0.9 *^{Jg}	4.5 ± 0.1 *^{Kl}	nd
2-methylpropan-1-ol	nd	nd	nd	nd	2515.1 ± 70.0	nd
3-methylbutan-1-ol	nd	nd	5.6 ± 0.1 *	nd	2.3 ± 0.3 *^{k}	nd
Butan-1-ol	nd	nd	nd	nd	85.1 ± 1.4 ^{k}	nd
Butan-2-ol	nd	nd	nd	nd	nd	nd
Cis-3-.hexenol	nd	nd	nd	nd	nd	100.5 ± 1.0
Ethanol	420.6 ± 70.7 *^{Gg}	164.1 ± 11.1 *^{Hk}	302.4 ± 13.1 *^{GHo}	260.7 ± 49.3 *^{Jgh}	224.5 ± 2.7 *^{Jkl}	216.2 ± 7.3 *^{q}
Phenylmethanol	nd	1220.8 ± 31.9 ^{k}	nd	nd	432.6 ± 64.2 ^{l}	nd
Heptan-2-ol	3,2 ± 0.0 ^{Gg}	2.0 ± 0.1 ^{Hk}	nd	3.5 ± 0.1 ^{Jh}	2.1 ± 0.1 ^{Kk}	nd
Hexan-1-ol	46.9 ± 1.2 ^{Gg}	38.4 ± 3.6 ^{Gk}	72.3 ± 1.01 ^{Ho}	88.7 ± 6.2 ^{Jh}	84.1 ± 1.6 ^{Jl}	64.0 ± 1.4 ^{p}
Methanol	1.3 ± 0.4 **^{Gg}	1.1 ± 0.0 **^{Gk}	1.2 ± 0.1 **^{Go}	1.3 ± 0.1 **^{Jg}	0.8 ± 0.1 **^{Kl}	1.3 ± 0.1 **^{o}
Nonan-2-ol	nd	nd	nd	nd	nd	nd
Octan-1-ol	nd	nd	nd	nd	nd	nd
Pentan-1-ol	9.10 ± 2.0 ^{Gg}	9,1 ± 1.5 ^{Gk}	nd	14.8 ± 0.5 ^{Jg}	15.7 ± 1.6 ^{Jl}	16.0 ± 0.1
Propan-2-ol	nd	nd	nd	nd	nd	nd
Ethyl-butanoate	nd	nd	nd	nd	nd	nd
Ethyl-decanoate	6.04 ± 0.3 ^{Gg}	6.6 ± 0.2 ^{Gk}	16.2 ± 0.2 ^{Ho}	6.5 ± 0.4 ^{Jgh}	6.7 ± 0.4 ^{Jk}	7.7 ± 0.1 ^{p}
Ethyl-ethanoate	600.68 ± 37.2 ^{Gg}	73.5 ± 1.5 ^{Hk}	nd	365.9 ± 24.15 ^{Jh}	335.1 ± 7.0 ^{Jl}	nd
Ethyl-pentanoate	nd	nd	nd	nd	nd	nd
Methyl-ethanoate	420.74 ± 48.4 ^{Gg}	152.6 ± 0.9 ^{Hk}	nd	533.2 ± 7.7 ^{Jg}	354.2 ± 0.1 ^{Kl}	69.2 ± 0.3 ^{o}
Pentan-2-one	nd	nd	nd	nd	nd	nd

Cultivar	ST			STV		
Production year	2011	2012	2013	2011	2012	2013
Compound	Content [$\mu g \cdot kg^{-1}$]					
Hexanoic acid	nd	nd	nd	nd	nd	nd
Benzaldehyde	30.7 ± 0.2 ^{Lh}	119.7 ± 10.9 ^{Ml}	96.3 ± 5.9 ^{Mp}	87.1 ± 18.1 ^{Oh}	61.4 ± 2.4 ^{OPl}	18.8 ± 0.3 ^{Pq}
Hexanal	36.9 ± 6.9 ^{Lg}	46.6 ± 2.8 ^{Ll}	29.4 ± 1.2 ^{Lp}	71.6 ± 1.5 ^{Og}	25.1 ± 1.0 ^{Pm}	28.1 ± 1.1 ^{Pp}
Trans-2-hexenal	6.5 ± 0.5 *^{Lg}	3.9 ± 0.0 *^{Mm}	nd	9.5 ± 3.9 *^{Og}	2.1 ± 0.2 *^{Ok}	nd
2-methylpropan-1-ol	nd	nd	0.8 ± 0.1 **^{o}	nd	nd	10.1 ± 0.1 **^{p}
3-methylbutan-1-ol	nd	5.0 ± 0.3 *^{l}	nd	nd	1.9 ± 0.2 *^{k}	nd
Butan-1-ol	nd	48.7 ± 8.9 ^{l}	nd	24.9 ± 1.4 ^{O}	34.5 ± 3.3 ^{Pl}	27.2 ± 0.2 ^{OP}
Butan-2-ol	nd	nd	nd	nd	nd	nd
Cis-3-.hexenol	nd	nd	nd	nd	nd	nd
Ethanol	469.1 ± 40.3 *^{Lg}	297.6 ± 34.8 *^{Ml}	284.8 ± 1.1 *^{Mo}	128.0 ± 54.5 *^{Oh}	336.1 ± 41.4 *^{Pl}	149.8 ± 14.2 *^{Op}
Phenylmethanol	nd	nd	nd	nd	344.0 ± 41.4 ^{l}	nd
Heptan-2-ol	2.5 ± 0.0 ^{Li}	2.4 ± 0.1 ^{Ll}	nd	nd	2.1 ± 0.0 ^{kl}	nd
Hexan-1-ol	37.7 ± 12.0 ^{Lg}	103.5 ± 3.4 ^{Mm}	65.4 ± 0.8 ^{Lop}	72.8 ± 8.9 ^{Ogh}	53.6 ± 5.9 ^{Ok}	65.1 ± 1.7 ^{Oop}
Methanol	1.2 ± 0.1 **^{Lg}	1.1 ± 0.1 **^{Lk}	0.9 ± 0.1 **^{Mop}	1.3 ± 0.1 **^{Og}	1.1 ± 0.0 **^{Ok}	1.4 ± 0.1 **^{Ooq}
Nonan-2-ol	nd	nd	nd	nd	nd	nd
Octan-1-ol	nd	nd	nd	nd	nd	nd
Pentan-1-ol	nd	17.5 ± 1.1 ^{l}	nd	8.8 ± 1.4 ^{Og}	12.1 ± 1.0 ^{Okl}	nd
Propan-2-ol	nd	nd	nd	nd	nd	nd
Ethyl-butanoate	nd	nd	nd	nd	nd	nd
Ethyl-decanoate	4.8 ± 0.3 ^{Lg}	7.8 ± 0.6 ^{Mk}	6.4 ± 0.4 ^{LMp}	7.9 ± 0.6 ^{Oh}	7.2 ± 0.4 ^{Ok}	7.4 ± 0.1 ^{Op}
Ethyl-ethanoate	300.3 ± 18.7 ^{Lh}	145.7 ± 0.3 ^{Mmn}	17.2 ± 0.0 ^{No}	34.1 ± 7.4 ^{Oi}	160.4 ± 9.9 ^{Pn}	15.2 ± 0.1 ^{Op}
Ethyl-pentanoate	nd	nd	nd	nd	nd	nd
Methyl-ethanoate	212.3 ± 15.2 ^{Lh}	144.3 ± 7.4 ^{Mk}	nd	32.3 ± 4.8 ^{Oi}	112.4 ± 17.4 ^{Pk}	66.9 ± 1.2 ^{Po}
Pentan-2-one	nd	nd	nd	nd	nd	nd

Saskatoon berry cultivars: SLB – Lamarckii Balerina, SO – Ostravsky, STS – Tisnovsky skolsky, ST – Thiessen, STV – Tisnovsky velkoplody.

* – content in milligrams per kilogram, **– content in grams per kilogram, nd – not detected

The results are expressed as mean ± standard deviation (n = 9). Different capital letters in the same row indicate significant differences ($p < 0.05$) among the years of productions (2011–2013) within the same cultivar. Different superscript letters in the same row indicate significant differences ($p < 0.05$) among cultivars in 2011, 2012 and 2013, respectively.

conditions [36, 37]. ANOVA analysis also confirmed the differences among the chokeberry varieties; between AN and AV cultivars in 2011 (in all the identified volatiles), in 2012 (in 12 from 15 volatiles) and also in 2013 (in 11 from 13 volatiles). AN exhibited, in most cases, higher contents of volatiles than AV cultivars, which probably resulted from species-varietal composition, ontogenesis and age of shrub [36, 39]. In case of saskatoon berry cultivars, the significant differences following from different production seasons were found in benzaldehyde, hexanal, *trans*-2-hexenal, ethyl-ethanoate and methyl-ethanoate. ANOVA also proved significant differences among saskatoon berry cultivars in 2011, 2012 and 2013 (Tab. 2).

The results of the performed statistical analysis (PCA and ANOVA) suggest that volatile profiles of chokeberry and saskatoon berry are strongly influenced by environmental factors (year of production) and species-varietal composition. It was confirmed that PCA analysis, combined with analysis of variance, are good tools for differentiation of samples of chokeberry and saskatoon berry on the basis of the profiles of aroma components.

CONCLUSION

Characterization of profiles of volatile compounds in selected chokeberry and saskatoon berry cultivars grown in Czech Republic was performed. It was evident that the applied separation and identification method, based on the combination of SPME and GC, was sufficiently sensitive, precise and repeatable for identification and quantification of a large number of volatile compounds present in fruits. As expected, the overall profile of volatiles was similar to other berry fruits, being characterized by the dominant presence of alcohols, aldehydes, esters and acids. In total, 39 and 31 volatile compounds were identified in chokeberries and saskatoon berries, respectively, several of them being reported for the first time. Fourteen of all the identified compounds were assumed to be aroma-active and could contribute to the overall aroma of samples. Significant differences among varieties were found for both chokeberry and saskatoon berries, being influenced not only by varietal diversity but also by the growing/climatic conditions and the season of production. However, further studies focused on monitoring of the volatile and aroma active compounds profiles in berries during their maturation, processing and storage under various conditions are still necessary to understand the biological processes in these fruits. From the industrial point of view, further optimization of production, post-harvest treatment, conservation and processing conditions oriented to quality improvement is needed to support the wide utilization of chokeberry and saskatoon berry in food industry.

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Original Research Paper

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Validation of SPME–GC–FID Method for Determination of Fragrance Allergens in Selected Cosmetic Products

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Summary. An analytical method for simultaneous determination of 24 legislatively restricted fragrance allergens in various cosmetic products was developed and validated using a combination of solid-phase microextraction and gas chromatography with flame ionization detector. The divinylbenzene–polydimethylsiloxane (DVB–PDMS) fiber was evaluated as the best for extraction of all studied fragrance allergens at 40 °C and 20 min extraction time. All calibration curves showed good linearity in the range of 10^{-1} to 10^{3} μg mL^{-1}. Good repeatability and inter-day precision was determined for the proposed method. Detection limits for individual allergens ranged from 0.007 to 2.7 μg mL^{-1}. The validated method was used to analyze real cosmetic samples. The obtained results indicated that not all of the analyzed cosmetic samples were labeled in accordance with European Cosmetic Directive.

Key Words: SPME, GC, fragrances, cosmetics

Introduction

Fragrances have been used since ancient times. Fragrance substances are defined as organic compounds naturally derived from plants or synthesized in laboratories with characteristic, usually pleasant odors [1]. Fragrances cover wide range of different consumer products. They can be found not only in cosmetics, but also in detergents, fabric softeners, and other household products where fragrance may be used to mask unpleasant odors from raw materials [2–4].

Besides pleasant odor, fragrances can trigger several negative reactions. It has been proved that some fragrances may have a negative effect on sensitive individuals even at very low concentrations. Fragrance components are frequent causes of cosmetic allergies. The danger of irritation or allergic reaction is increased when a surface of skin is broken down by, e.g., shaving or peeling and subsequently when cosmetic product is applied on eroded skin. Fragrances are not only responsible for contact allergies but also for various systemic effects (bioaccumulation in human adipose tissue), respiratory effects (asthma or migraine headaches), neurological effects (neurotoxicity), or carcinogenicity [5–7].

Due to increasing frequency of allergic contact dermatitis associated with the use of perfumed products, the Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP) has selected 26 compounds as likely causing contact allergies. According to European Cosmetic Directive, these suspected allergens have to be labeled on cosmetic and detergent products packaging when the concentrations of these allergens exceed certain values. Labeling is required if such a component is present at ≥ 10 ppm in leave-on cosmetic products or ≥ 100 ppm in rinse-off cosmetic products, irrespective of their function or sources [8, 9]. This mandatory labeling on cosmetics products helps the dermatologists to diagnose fragrance allergy and the patients to avoid the fragrance ingredients [10].

The matter of fragrance allergens is currently very topical theme, and various scientists are concerned with determination of fragrance allergens in different products using the diverse analytical methods. Gas chromatography coupled with mass spectrometry (GC–MS) is the most widely used technique, because 24 from 26 allergens are volatile compounds [11–13]. Besides the GC–MS technique, high-performance liquid chromatography coupled with mass spectrometry (HPLC–MS) or diode array detector (DAD) was used by several authors [14, 15]. The isolation from the matrix is an important step in the assessment of these substances. Methods that can be used for isolation include pressurized liquid extraction (PLE) [16], size exclusion chromatography (SEC) [17], or ultrasound-assisted emulsification microextraction (USAEME) [18]. The main drawbacks of these methods and techniques are their high acquisition and operating costs.

The aim of this study was to optimize and validate an analytical method for simultaneous determination of fragrance allergens present in cosmetic products using solid-phase microextraction method (SPME) and gas chromatography coupled with flame ionization detector (GC–FID). The SPME method became very popular in recent years because it is simple, low-cost, solvent-free, and sensitive [19]. In comparison with GC–MS, GC–FID is widely available in many laboratories and it is used for routine analysis of organic compounds [20–22]. Only a little work has been done before in the field of simultaneous determination of all fragrance allergens using SPME–GC–FID, and this method is often used only as complementary technique [23, 24]. Moreover, most of the authors are focused only on determination of several selected allergens or on determination of fragrance profiles comprising the tens of the various fragrance compounds including few fragrance allergens. Thus, the new validated technique can be very useful for many laboratories.

Experimental

Chemicals and Reagents

The following chemicals were used as standards: 2-(phenylmethylene)-heptanal, 97% (amyl cinnamaldehyde); benzene methanol, 99% (benzyl alcohol); 3-phenyl-2-propen-1-ol, 98% (cinnamyl alcohol); 3,7-dimethylocta-2,6-dienal, 95% (citral, cis/trans); 2-methoxy-4-prop-2-enyl phenol, 99% (eugenol); 7-hydroxy-3,7-dimethyloctanal, ≥ 95% (hydroxycitronellal); 2-methoxy-4-(1-propenyl) phenol, 98% (isoeugenol, cis/trans); 2-(phenylmethylene)-1-heptanol, ≥ 85% (amyl cinnamyl alcohol); 2-hydroxy-phenylmethyl ester benzoic acid, 99% (benzyl salicylate); 3-phenyl-2-propenal, ≥ 93% (cinnamaldehyde); 2H-1-benzopyran-2-one, 98% (coumarin); 3,7-dimethyl-2,6-octadien-1-ol, 97% (geraniol); 4-(4-hydroxy-4-methylpentyl)-cyclohex-3-ene-1-carbaldehyde, 97% (lyral); 4-methoxybenzyl alcohol, 98% (anis alcohol); 3-phenyl-phenylmethyl ester-2-propenoic acid, 99% (benzyl cinnamate); 3,7,11-Trimethyl-2,6,10-dodecatrien-1-ol, 95% (farnesol); 2-(4-tert-butylphenyl)-2-methylpropanal, 95% (lilial); 3,7-dimethyl-1,6-octadien-3-ol, 97% (linalool); phenylmethyl benzoate, 99% (benzyl benzoate); 3,7-dimethyl-1,6-octadien-3-ol, 96% (citronellol); 2-(phenylmethylene) octanal, 95% (hexylcinnamaldehyde); 1-methyl-4-prop-1-en-2-yl-cyclohexene, 97% ((R)-(+)-limonene); methyl ester 2-octynoic acid, 99% (methyl 2-octynoate); 3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one, ≥ 85% (ionone); these were purchased from Sigma-Aldrich (Germany). All the chemicals were of chemically pure grade.

Standard Solutions

Individual stock solutions of each fragrance compound, mixtures of standards, and the further dilutions were prepared in methanol (Sigma-Aldrich, Germany). The working solutions were prepared daily as individual standard solutions or in the mixture. Each allergen standard solution was diluted by methanol in suitable ratio. Total volume of standard solution was 1 mL.

SPME–GC–FID Conditions

The various SPME fibers were used in this study: polydimethylsiloxane–divinylbenzene, 65 μm (PDMS–DVB); divinylbenzene–carboxen–polydimethylsiloxane, 50/30 μm (DVB–CAR–PDMS); carboxen–polydimethylsiloxane, 85 μm (CAR–PDMS); polyacrylate, 85 μm (PA); polydimethylsiloxane, 100 μm (PDMS) (Supelco, Bellefonte, USA). All fibers were conditioned according to the manufacturer's instruction prior to their use.

Fragrance extraction was performed in the headspace above 1 mL of the sample introduced in 4 mL vials. The vial was heated at a specified temperature using a water bath. After equilibrium, the fragrance compounds were adsorbed onto an SPME fiber that was introduced into headspace vial through septum and persisted for a specified time. After a suitable extraction time, the fiber was withdrawn into the needle, removed from the septum, and then inserted directly into the injection port of the GC system for thermal desorption.

All analyses were carried out using a Trace GC chromatograph (ThermoQuest Italia S. p. A.) coupled to flame ionization detector. Analytes were separated on DB-WAX capillary column (30 m × 0.32 mm I.D.; film thickness, 0.5 μm) and identified by comparing their retention times with those of standards analyzed under the same conditions. Nitrogen (purity, 99.999%) was employed as the carrier gas at a constant column flow of 0.9 mL min^{-1}. The oven temperature was programmed from 40 °C (held for 1 min) to 220 °C at 5 °C min^{-1} (held for 28 min). Total analysis time was 65 min. The splitless mode (maintained for 5 min) was used for injection, and injector temperature was kept at 250 °C. The retention times for target compounds as well as their main properties are shown in *Table I*. Chromatograms of the standard mixtures are presented on *Fig. 1a–1c*.

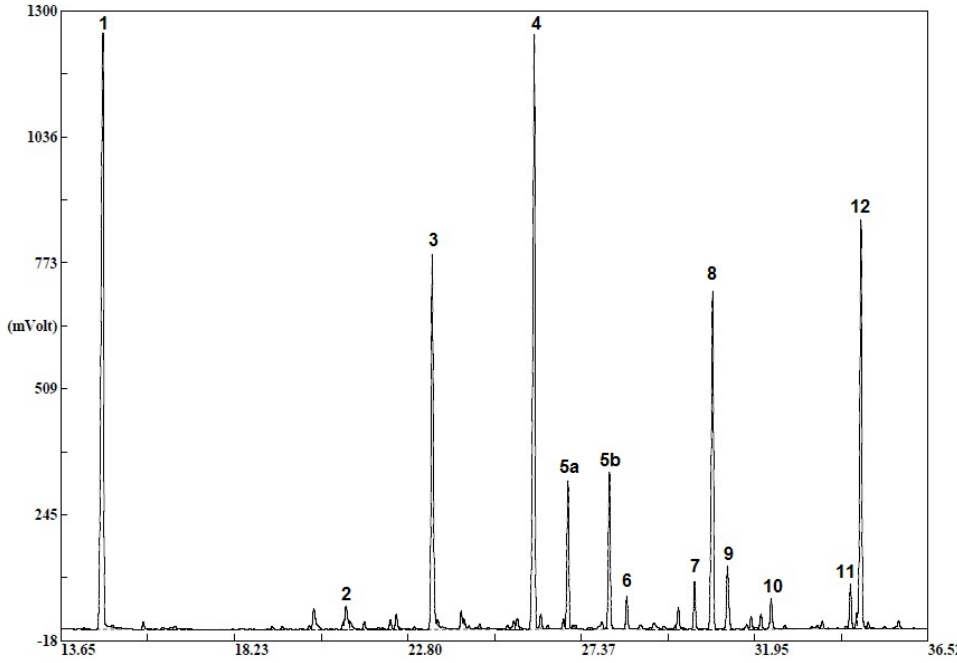

Fig. 1a. GC–FID chromatogram of a standard mixture of the fragrance allergens in methanol showing 1–12 peak (see number code equivalence in *Table I*)

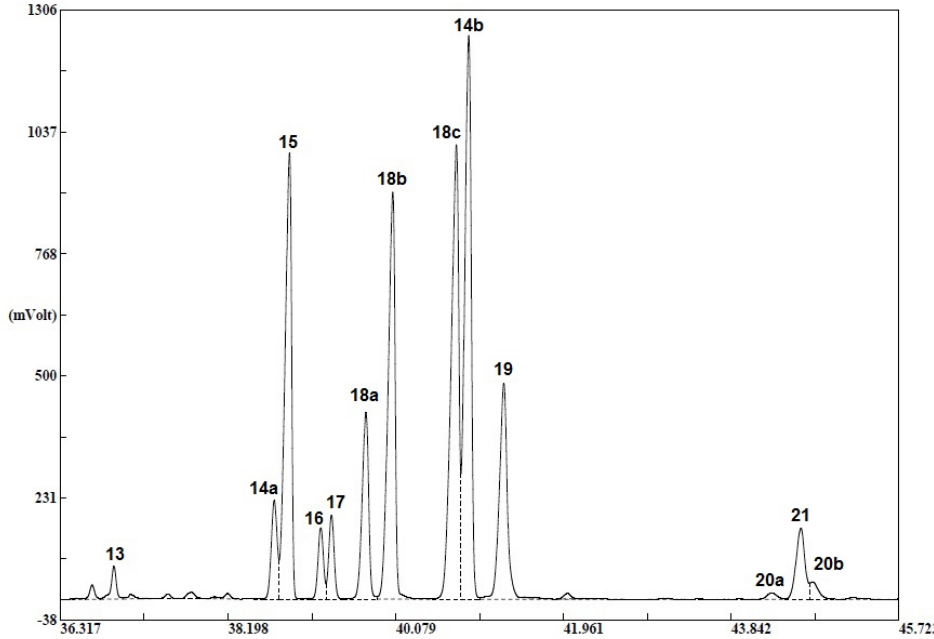

Fig. 1b. GC–FID chromatogram of standard mixture of the fragrance allergens in methanol showing 13–21 peak (see number code equivalence in *Table I*)

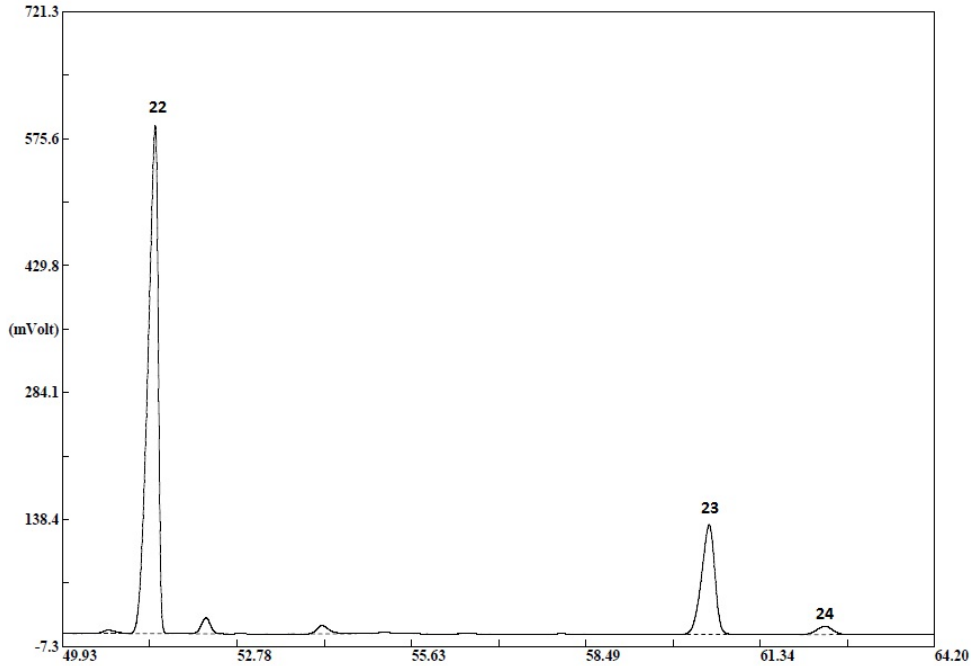

Fig. 1c. GC–FID chromatogram of standard mixture of the fragrance allergens in methanol showing 22–24 peak (see number code equivalence in *Table I*)

Table I. List of 24 fragrance allergen compounds

Key	Retention time (min)	Fragrance substance	CAS number	Molecular weight	Boiling point (°C)
1	14.71	Limonene	5989-27-5	136	176
2	20.91	α-Amylcinnamyl alcohol	101-85-9	204	> 200
3	23.50	Linalool	78-70-6	154	198
4	26.14	Methyl 2-octynoate	111-12-6	154	219
		Citral isomers			
5a	27.05	Citral 1	5392-40-5	152	229
5b	28.16	Citral 2			
6	28.59	Citronellol	106-22-9	156	225
7	30.45	Geraniol	106-24-1	154	229
8	30.83	α-Isomethyl ionone	127-51-5	206	266
9	31.27	Benzyl alcohol	100-51-6	108	205
10	32.48	7-Hydroxycitronellal	107-75-5	172	240
11	34.69	Lilial	80-54-6	204	279
12	34.78	Cinnamaldehyde	104-55-2	132	252
13	36.92	Eugenol	97-53-0	164	256
		Isoeugenol isomers			
14a	38.70	Isoeugenol 1	97-54-1	164	267
14b	40.87	Isoeugenol 2			
15	38.82	α-Amylcinnamaldehyde	122-40-7	202	289
16	39.24	Anisalcohol	105-13-5	138	259
17	39.37	Cinnamyl alcohol	104-54-1	134	250
		Farnesol isomers			
18a	39.69	Farnesol 1	4602-84-0	222	283
18b	39.95	Farnesol 2			
18c	40.65	Farnesol 3			
19	40.86	α-Hexylcinnamaldehyde	101-86-0	216	174
		Lyral isomers			
20a	44.32	Lyral 1	31906-04-4	210	319
20b	44.80	Lyral 2			
21	44.59	Coumarin	91-64-5	146	301
22	51.17	Benzyl benzoate	120-51-4	212	324
23	60.22	Benzyl salicylate	118-58-1	228	320
24	63.52	Benzyl cinnamate	103-41-3	238	371

Validation Parameters

The calibration plots were constructed using standard solutions containing different concentration of analytes. Peak area of each analyte was plotted against the corresponding analyte concentration. The linearity was tested in the concentration range 10^{-1}–10^{3} μg mL^{-1}. The calibration curves were evaluated by correlation coefficients.

Repeatability and intermediate precision were expressed as relative standard deviation of standard solution analysis. The repeatability was determined from five independent analyses of standard solution carried out during 1 day. Intermediate precision was determined by single analysis of standard solution during 10 different days. The concentration of each fragrance allergen in standard solution was near the middle of linearity range. For the recovery test, real sample of face lotion was spiked by each fragrance standard. Recoveries were calculated as the ratio of the measured concentration, after subtracting the initial concentration in the non-spiked sample, to the spiked concentration.

Limits of detection (LOD) and limits of quantification (LOQ) were calculated as the analyte concentration that generates a signal which is 3 times (LOD) and 10 times (LOQ) higher than the noise generated from the analysis of unexposed fiber.

Samples

Ten various representative commercially available cosmetics (baby oil, lip balm, olive cream, deodorant stick for men, antiperspirant for women, face mask, face lotion, shampoo, and toothpastes) were employed in this study to examine the applicability of the proposed method. All the samples were purchased on the local market and stored at 5 °C until analysis. The real samples were processed the same way as the standards; every sample was analyzed in triplicate under the optimized conditions.

Statistical Analysis

The statistical analysis was performed using Microsoft Office Excel 2003 and statistical software Statgraphics Centurion XVI (Statpoint Technologies, Inc., USA).

Results and Discussion

Selection of SPME Fiber

In the frame of the optimization process, initially, five types of fibers were tested for the preconcentration of fragrance compounds. The extraction temperature during the test was 40 °C, and equilibrium and extraction times, 15 min. The test solution contained all 28 fragrances dissolved in methanol. After analysis, total peak area of all fragrances was calculated from the chromatograms. The obtained results are shown in *Fig. 2*. As can be seen, the highest yields were observed by CAR–PDMS fiber; however, DVB–CAR–PDMS and DVB–PDMS fibers can be also used for the fragrances extraction. The obtained chromatograms showed that DVB–PDMS fiber has a higher affinity for amylcinnamal, farnesol, hexylcinnamal, coumarin, benzyl benzoate, benzyl salicylate, and benzyl cinnamate in comparison to other compounds, while CAR–PDMS fiber for highly volatile compounds specifically for limonene, linalool, citral, methyl 2-octynoate, citronellol, geraniol, benzyl alcohol, hydroxycitronellal, eugenol, ionone, and cinnamaldehyde. The lowest yields were observed with PDMS and PA fibers and so these fibers were found to be unsatisfactory for our purposes. Even though that CAR–PDMS fiber achieved the best results in terms of total peak area, DVB–PDMS fiber was selected as the most suitable and universal fiber for simultaneous determination of all aimed fragrance allergens.

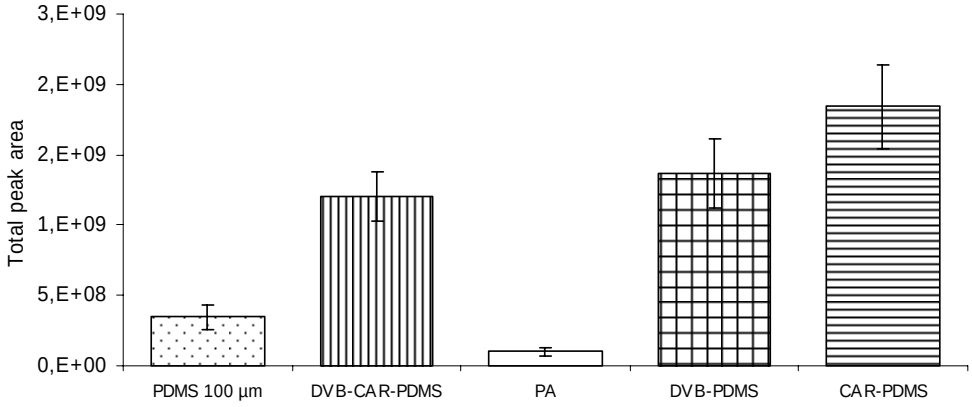

Fig. 2. Influence of the fiber coating on the absorption of the regulated fragrance allergens

Robustness of the Method

The optimization of the headspace (HS)-SPME conditions was performed by the use of a statistically designed experiment by means of the statistical software Statgraphics Centurion XVI.

The selected design allows studying the results using various statistical tests and graphic tools to determine which factors and interactions have a statistically significant effect.

The selected factors theoretically influencing final result were equilibrium time (A), extraction time (B), extraction temperature (C), and desorption time (D). All these factors were included in screening design appropriate for four factors by means of a 2^{n-1} ($n = 4$) half fraction process, involving 10 runs in randomized order inclusive two central points.

The factors that were changed during the course of the experiment and their levels considered are listed in *Table II*. The response evaluated for all experiments was the total sum of peak areas, obtained in the GC analysis.

Table II. Factors and levels in the screening experimental design

Factor	Key	Low level (−)	High level (+)
Equilibrium time	A	10	25
Extraction time	B	5	25
Extraction temperature	C	25	40
Desorption time	D	3	10

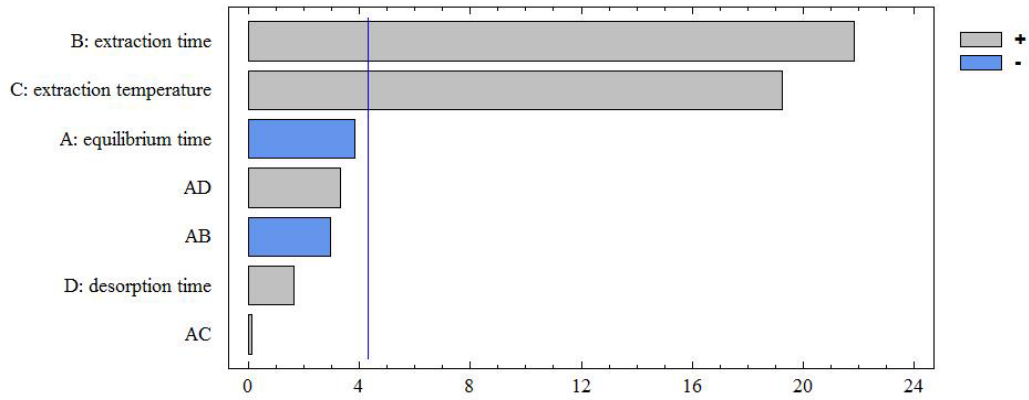

Fig. 3. Pareto chart of standardized effects of 2^{4-1} half fraction process for total chromatographic peak

Standardized Pareto chart for the main factors and two-factor interactions is presented in *Fig. 3* displaying the effects in decreasing order of significance. The vertical line in the graph shows the statistically significant

level. As can be seen in the figure, only the extraction time and the extraction temperature effects were significant at 95% confidence level, and therefore, they were further optimized. However, the effects of equilibrium time and desorption time were not statistically significant bound at the 95% confidence level; the effect of this insignificant parameters was examined experimentally on the basis of one variable at a time. The final values of equilibrium time and desorption time were set to 15 min and 10 min, respectively. These times correspond to the maximum measured response of studied compounds (data not shown).

Optimization of Extraction Time

The application of six extraction times (5, 10, 15, 20, 30, and 40 min) was tested to find the optimum for extraction and preconcentration of aimed fragrances by DVB–PDMS fiber. As illustrated in *Fig. 4*, the best value of extraction time was 20 min. At this time, all active sites on the fiber were saturated, and consequently, the highest peak area of all analytes was obtained. Application of longer extraction time led to gradual desorption of analytes until the active sites on the fiber surface were not saturated again.

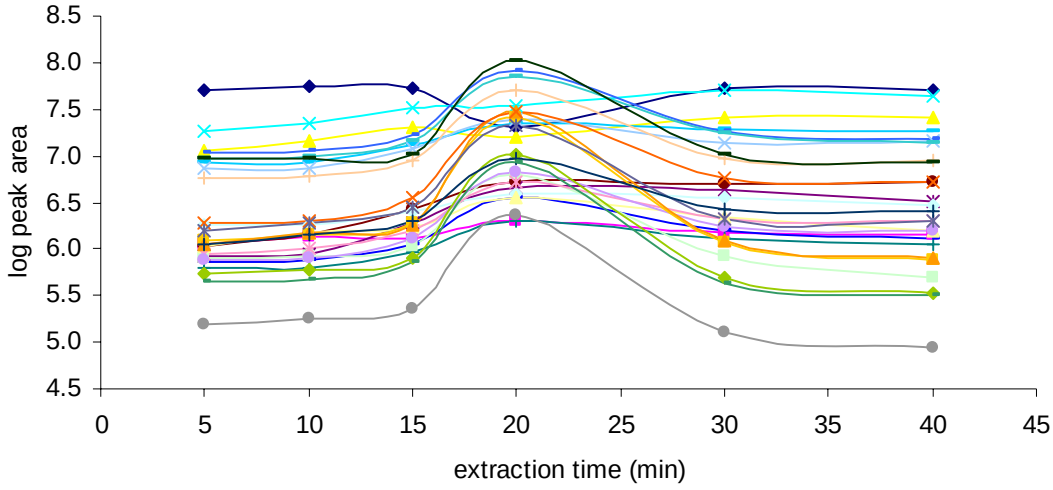

Fig. 4. The peak area of studied compounds collected on the SPME fiber at different extraction time

Optimization of Extraction Temperature

The influence of six different temperatures (23, 30, 35, 40, 45, and 50 °C) on the extraction yields of studied analytes was investigated. As shown in *Fig. 5*, slightly larger extraction yields were achieved at 30 °C; however, at 40 °C,

satisfactory results were obtained too. This temperature (40 °C) is closer to the human body temperature, which plays an important role in cosmetology.

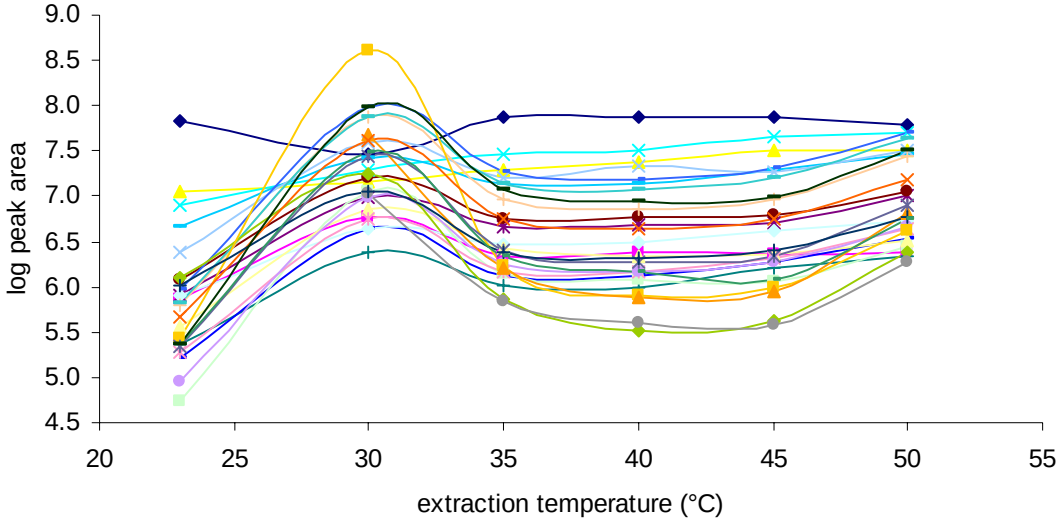

Fig. 5. The peak area of studied compounds collected on the SPME fiber at different extraction temperature

Validation of Method

The validation parameters of the methods are summarized in *Table III*.

All calibration curves were linear in the tested concentration range. The R^2 values were above 0.999 for all compounds. The repeatability and inter-day precision of proposed method were very good. In general, the relative standard deviations of measured concentrations during these tests were about 5% *(Table III)*. The limits of detection and quantification were determined in the range of the units of μg mL^{-1} *(Table III)*. Because of the lack of reference material, the recovery test has been performed to verify the accuracy of proposed method. The results were satisfactory, and the recovery values were in general over 80%.

During the study of possible carry over effect of all fragrance allergens, it was observed that some compounds are not completely desorbed after the end of analytical run. This effect was eliminated by insertion of fiber after each analytical run to the injector for 10 min and by running the fast mode analysis.

Table III. Validation parameters of the method

Fragrance compound	Linearity range (μg mL^{-1})	LOD (μg mL^{-1})	LOQ (μg mL^{-1})	Repeatability (RSD %)	Inter-day precision (RSD %)
α-Amylcinnamaldehyde	2.4–970	0.7	2.4	0.5	7.2
Amylcinnamyl alcohol	3–1000	0.8	2.5	1.4	6.3
Anisalcohol	4.18–1113	1.25	4.17	2.8	5.3
Benzyl alcohol	0.05–104	0.015	0.05	3.5	6.6
Benzyl benzoate	1.12–560	0.3	1.09	5.3	7.6
Benzyl cinnamate	4.9–949	1.5	4.9	5.8	7.1
Benzyl salicylate	1.18–590	0.4	1.16	1.1	4.3
Cinnamaldehyde	0.12–1153	0.04	0.13	1.2	4.1
Cinnamyl alcohol	5–1000	1.4	4.1	2.6	6.4
Citral 1	8.9–1340	2.7	8.9	1.7	6.1
Citral 2	8.9–1340	2.7	8.8	1.2	6.7
Citronellol	8.6–1659	2.6	8.5	1.5	6.5
Coumarin	1–1000	0.28	0.95	5.8	7.4
Eugenol	6.6–1166	1.9	6.4	2.1	6.6
Farnesol 1	8.9–1331	2.6	8.7	1.8	5.7
Farnesol 2	8.9–1331	2.6	8.6	1.3	5.1
Farnesol 3	8.9–1331	2.6	8.7	2.6	4.3
Geraniol	2.22–1334	0.7	2.2	2.1	6.9
Hexylcinnamaldehyde	2.14–1048	0.6	2.09	1.7	6.1
Hydroxycitronellal	2.5–1104	0.7	2.18	1.4	5.3
Isoeugenol 1	6.8–1080	2	6.7	0.9	3.9
Isoeugenol 2	6.8–1080	2	6.7	1.3	4.5
Isomethyl ionone	5.8 –1116	1.7	5.8	2.6	4.7
Lilial	0.23–930	0.06	0.21	1.4	4.4
Limonene	4.2–1680	1.26	4.2	0.9	5.7
Linalool	8.5–1275	2.5	8.4	1.8	5.3
Lyral 1	1–1000	0.3	0.97	3.4	5.2
Lyral 2	0.25–1000	0.23	0.96	3.1	5.2
Methyl 2-octynoate	2.9–1201	0.007	0.023	4.4	4.6

Application of Validated Method to Real Samples

Finally, the validated method was applied to the 10 different commercial products of cosmetics comprising rinse-off and leave-on products. The contents of particular fragrance ingredients in samples were determined by external standard procedure. The results indicated that several of the cosmetic products analyzed were not properly labeled in accordance with European

Cosmetic Directive. Several cosmetic products contained more than 10 or 100 μg mL^{-1} of fragrance allergens, and these allergens were not listed on the packaging. *Table IV* summarizes all measured samples and fragrance compounds found in these samples. As can be seen, only in case of the olive cream information about the composition of the product on the packaging agreed with results obtained by SPME–GC–FID analysis. In some cases, the limit at which it is necessary to indicate the presence of a substance on the packaging was only slightly exceeded; however, in baby oil sample, very high concentration of citral which was not mentioned on the packaging was found. Similarly, high concentrations of isoeugenol in deodorant stick for men or citral, isoeugenol, and linalool in toothpaste were found.

Table IV. Concentrations (μg mL^{-1}) of the suspected fragrance allergens in cosmetic products

Fragrance compound	Leave-on products						Rinse-off products			
	Baby oil	Lip balm	Olive cream	Deodorant stick for men	Antiperspirant for women	Face lotion	Face mask	Shampoo	Toothpaste 1	Toothpaste 2
Amylcinnamaldehyde	5	5	143[a]	4						
Amylcinnamyl alcohol		6		11[b]	15[b]				10	
Anisalcohol										
Benzyl alcohol	10	1	19[a]	36[a]	40[a]	18[a]				
Benzyl benzoate	38[a]		44[b]	11[a]						
Benzyl cinnamate										
Benzyl salicylate			10[a]	19[a]	44[a]	17[a]				
Cinnamaldehyde			9			6		8	111[a]	15
Cinnamyl alcohol	32[b]		153[a]	19[b]						
Citral 1	35[b]		134[a]	89[a]	77[a]	64[b]	1195[a]	991[a]	156[b]	82
Citral 2	765[b]		1151[a]	106[a]	191[a]	105[b]	1047[a]	151[a]	346[b]	61
Citronellol	107[a]		706[a]	215[a]		107[a]	23			
Coumarin	12[a]		5		8[a]	10				
Eugenol	52[b]		3	25[a]		52[a]		82	380[a]	44
Farnesol 1										
Farnesol 2										
Farnesol 3										
Geraniol	100[a]		264[a]	119[a]	8	6	1197[a]	91		
Hexylcinnamaldehyde	4		14[a]	8[a]	10[a]		5	974[a]		
Hydroxycitronellal	77[a]		18[a]	11[b]			8	48		
Isoeugenol 1				403[b]					95	
Isoeugenol 2				42[b]					72	
α-Isomethyl ionone	109[a]	29[b]	6	136[a]	381[a]	4	36	18	31	17
Lilial	18[a]		3	77[a]		152[a]				
Limonene	209[a]	945[a]	144[a]	1274[a]	1132[a]	209[a]	160[a]	164[a]	921[a]	770[b]
Linalool	475[a]	70[b]	715[a]	1033[a]	927[a]	475[a]	18	1109[a]	204[b]	48
Lyral 1				23[a]						
Lyral 2				19[a]						
Methyl 2-octynoate	2	24[b]	2			8		5		

[a]Analytes listed on the cosmetic label.

[b]Analytes exceeding the concentration limits (> 10 or 100 ppm [9]) and not listed on the packaging.

Relative standard deviations were less than 10% in for all samples.

Conclusion

In the present work, a simple and effective method based on HS–SPME coupled to GC–FID has been developed and validated for the assessment of 24 regulated fragrance allergens in cosmetic products. The main advantages of this method are low cost and less laborious sample preparation than other conventional techniques. Selection of SPME fiber with suitable coating has been shown as a crucial factor for the extraction of studied compounds. Extraction time and temperature were identified as other factors that may significantly affect the extraction. It is necessary to take into consideration that the choice of optimal extraction conditions is a compromise because the substances differ in their physical and chemical characteristics. After considering all the results, the following final extraction conditions were selected: equilibrium time, 15 min; extraction time, 20 min; extraction temperature, 40 °C; and desorption time, 15 min. The validated method was used for analysis of real cosmetic products. Only 3 of 10 analyzed samples were in compliance with the European Cosmetic Directive which establishes the obligation to indicate the presence of an allergen in the product packaging if the concentration of allergen in the product is higher than 10 ppm (leave-on cosmetic products) or 100 ppm (rinse-off cosmetic products). In view of the fact that the fragrance allergies are still increasing worldwide, all the fragrance allergens controlled are recommended to be listed namely on the product labels instead of inadequate overall terms "perfume" or "fragrance" often used in practice.

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