Characterization of *craft beer* through flavour component analysis by GC-MS and multivariate statistical tools

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ABSTRACT

Beer is a rather popular drink and represents the most widely consumed alcoholic beverage in the world.The present research aims at characterizing the flavour profile of lager pilsner, the category of low fermentation beers most common in Europe. The largest portion of the global market is dominated by a few multinational companies, but in the last years the number of independent craft breweries has increased very rapidly also in countries where there weren't an established craft brewing tradition. According to the Italian Brewers Association, in Italy there are eight brewing companies which operate 14 industrial breweries, which in the years have standardized the product to increase their slice of market. The craft-beer sector represents a niche market, about 3% of total production (1% in 2011) [EU Report, 2016]. Italy has a relatively young craft brewing tradition, but the data together with the new ways of consumption, can be considered promising for the sector development. In 2016, the "craft beer" has been defined for the first time in Italy with DDL 1328-B (art.35). Legislation does not consider the quality of the raw materials, but only the manufacturing processes: the artisanal beer-making is a beer obtained without microfiltration and pasteurization steps, unlike industrial products. In this contest, the aim of the study was to characterize the beers (all lager style) purchased on the market through the analysis of the aromatic profile of samples produced under different processes (*craft methods or industrial processes*). In fact, in addition to smaller production scale and independent, the main characteristic of craft beer is to put the emphasis on flavour and brewing techniques. Flavour, consisting of a large number of volatile compounds, has a great influence on consumer acceptability and, when safety and nutritional value are guaranteed, sensory parameters become the discriminating factor in the product quality assessment which determines the differentiation on the market. The identification of specific compounds, which confer a particular aroma, suitable to be used as potential quality/process markers in order to discriminate beer samples according to their production method. A headspace solid-phase microextraction coupled to gas chromatography-mass spectrometry (HS-SPME-GC-MS) was performed to evaluate the beer samples fingerprint. Multivariate statistical methods were then applied to the collected profiles to built model which could allow differentiating craft beers from all the others. Hence, the proposed method may represent an interesting tool to authenticate craft beer by verification of the compliance with their label description which, at the same time, can entail brand protection.

INTRODUCTION

Brewing industry is a huge global business and one of the most important sectors in the food and beverage industry. After water and tea, beer is considered the most popular drink being consumed all over the world, accounting for the 78.2% of the alcoholic beverages share [Gómez-Corona, C., 2016]. During the years, the global expansion of beer market has necessarily led to the industrialization of production methods and the standardization of the products, and so the industrial beers gradually replaced the craft beers. Only in the 1970's, the standardization of beer consumption, typical of industrial automation, was no longer considered a successful model and the brass sector has returned to craft beerproduction, considered a higher quality product. According to the Italian law n. 1354 of 1962 [Law 1354/1962], beer is "*a product obtained from alcoholic fermentation with Saccharomyces cerevisiae or Saccharomyces carlsbergensis strains of a must prepared with barley malt (also roasted), or wheat or their mixtures, and water, with the addition of hops, or its derivatives, or both*". The denomination is attributedto the products with Plato grade higher than 10.5 and alcoholic strength ($\%_{v/v}$) more than 3.5%. Legislation regardingraw materials and sugar content of the must, but does not consider any restriction concerning the production process, which includes different steps. However, end product characteristics are related also to manufacturing conditions; indeed, throughout the transformation process several factors can affect the nutritional and organoleptic properties of the final product.In recent years, Italy has been focusing on beer production made fromselected raw materials,following the artisan production methods, that positively contribute to the end product quality (*craft beer*). In 2014, Italy was the third European country for handicraft microbreweries, after the UK and Germany and, from 2009 to 2014, Italian microbreweries rose by 141.74% [The Brewers of Europe, 2016]. However, the absence of a definition to identify a craft beer has generated numerous discontents in the years among both producers and consumers. On July 2016 [DDL 1328-B/2016], for the first time in Italy, the craft beer was defined as: "*beer produced by small independent breweries, and not subjected, during the production phase, to pasteurization and microfiltration processes …… small brewery is legally and economically independent of any other brewery that uses plants physically distinct from those of any other brewery that does not operate under a license for the use of immaterial property and whose annual production does not exceed 200,000 hectoliters … ….*". Pasteurization and microfiltration conditions could influence several characteristics of the end product; therefore, the production technologies play an essential role on the chemical composition and aroma profile of beer. As a matter of fact, the new Italian legislation aims to restoring a qualitative approach to production and consumption of beer product. Apparently beer is a simple product, made exclusively with malt, water, yeast and hops [Hindy, S., 2014]. Malt are germinated cereal grains obtained with a process aimed at reducing the grains' starch into sugars readily available for brewing. Beer is brewed principally from malted barley, but in some countries other cereals, such as maize, rice, sorghum and unmalted wheat, are often used as additions. Water represents the main ingredient of beer, accounting 85-93% of its weight, and its level of dissolved minerals does influence beer's finished taste. The presence of yeast allows the alcoholic fermentation process with the production of ethanol and carbon dioxide. In addition, it is responsible of character and flavor of beer with the production of secondary metabolites such as esters, higher alcohols, organic acids, carbonyl compounds (aldehydes and ketones) and sulfur compounds. These substances may be classified according to their positive or negative sensory properties (*olfactory descriptors*) [Manzano, M., 2007]. However, the post-fermentation aroma evolves during maturation and aging by enriching the beer of new positive or negative fragrances. Moreover, the presence of off-flavor may depend on the action of light and the presence of oxygen generating degradation and/or oxidation reactions. Hop is used as a flavouring which add bitterness and other aroma in beer and act as a natural preservative and stabilizing agent. So, each ingredient plays a significant role in product realization and the different peculiarities of raw materials are the base of the intrinsic characteristics of the beer. In terms of production process, craft beer made following the artisan production methods shows significant differences from beer produced in industrial plants. Pasteurization process is undoubtedly a critical step for the final beer characteristics since modification of its main components can take place. This treatment is widely adopted in the industrial production and its involve high temperatures: 60-80°C up to 30 minutes. The application of heating-process ensures reduction of microbial contamination, extending the biological stability and increasing shelf life of final product; but the development of uncontrolled cross-reactions, such as the Maillard Reaction (MR), could be induced too. The MR may affect the nutritional value of cereal grains proteins reducing nutritionally-available. In addition, the sugars transformation mechanism in alcohol is stopped, reducing the innumerable flavours normally found into an unpasteurized beer. Furthermore, the heating step tends to standardize the organoleptic characteristic of beer, with the consequence that in most case it will be difficult to distinguish a brewery's production from that of another. In industrial beers, the pasteurization step is followed by a microfiltration phase, a physical-mechanical separation used to remove yeast residual after secondary fermentation. In absence of this treatment, the beer appears cloudy and the yeasts can develop specific chemical reactions generating undesirable aroma; therefore, the unfiltered beers are adapted for limited distribution, with shorter shelf life (typically, from 90 days to one year) and storage under more restrictive conditions (low temperatures). For these reasons, pasteurization and microfiltration treatments are essential to multinational companies in order to extend the storage period and to standardize the taste of their products in order to level expectations of the widest possible range of consumers. In addition, commercial beers are often produced with chemical additives, preservatives and surrogates of barley malt, which allow to lower production costs but negatively affect the taste experience. Instead, craft beers are no-pasteurized and no-filtered beverages andare produced with high quality raw materials, without the addition of chemicals and substitutes ingredients, and in small volume. The "*craft beer revolution*" is today motivated by cultural change i.e. a "Slow Food" approach, the consumer's learning in tasting, "the pleasure of tasting different and new flavours, sharing passion and experiencing cultural enrichment"[Donadini, G., 2017]. Scientific studies shows that, in a quest for taste authenticity, craft beer is chosen by consumers for its particulars flavours which increase the probability of perceiving craft beers to be of superior quality to industrial beers [Aquilani, B., 2015]. For these reasons, the study of the aromatic profile may represent an interesting tool to characterize and valorize the single product in relation to the production processes. In this work the volatile fraction of different beer samples were determined optimizing a HS-SPME/GC-MS method and the results were elaborated by multivariate statistical method in order to correlate the chemical information with the manufacturing conditions. The bibliographic studies carried out so far with this technique do not focus on the different production methods of beer, but rather on the characterization of a different hops varieties [Kovacevic, M., 2001], different styles of beer [Cajka, T., 2010], on a precise geographical origin [Alvin, R.P.R., 2017], or on a specific analytes determination [Moreira, N., 2013]. According to the new Italian law, it could be necessary to assess the authenticity of beers obtained by handcraft method in order to protect the products of local craft breweries and to protect consumers from possible commercial frauds.

MATERIALS AND METHODS

The study was performed analyzing beer samples made by artisanal and industrial processes. All samples were *Pilsner style Lager*. Lager is a name used to indicate the low fermentation beers and Pilsner is a brand name registered at the Chamber of Commerce and Trade in Pilsen (Czech Republic). This category represents the type of beer themost widely consumed in Europe. Forty-one different samples of craft beer and thirty-six of industrial beer were purchased from the supermarket (*large-scale retail trade*) and specialized beershop. Craft beer samples were selected based on the information claimed on the label (eg "*unfiltered product*, *unpasteurized*" or "*craft beer*"). All samples were stored in their unopened glass bottle at 4°C until their use and analyzed before the expiration date. 5.00g of the cooled beer was directly weighted into a 20 mL autosampler vial to minimize the loss of volatile compounds, and 2.00 g of NaCl were added into the vial to preserve samples and to improve extraction efficiency of volatile fraction in the headspace (*salting out effect*). The vials were finally sealed with a PTFE/silicon septum and aluminum crimp cap, and sonicated for 5 minutes at 20°C to establish equilibrium between headspace and sample prior of the exposure fiber. Determination of aromatic profile was performed by headspace solid-phase microextraction (HS-SPME) procedure following by GC-MS analysis (Trace GC-UItragas chromatograph) equipped with a Programmed Temperature Vaporized (PTV) injector interfaced with a single quadrupole ISQ LT mass spectrometry (ThermoFisher Scientific system). A DVB/CAR/PDMS (divinylbenzene/carboxen/polydimethylsiloxane) fiber (StableFlex, 50/30 µm, 2 cm) by Supelco through TriPlusRSH autosampler (ThermoFischer Scientific) was used for flavour analysis. The selected fiber combines the characteristics of three different polymer coatings and is thus able to extract volatile compounds in a wide range of polarities compared to other simpler types of fibers [Da Silva, G.C., 2015]. Separation was performed with HP-INNOWAX capillary column (30 µm, 0.25 mm i.d., 0.15 µm) by Agilent Technologies, with helium as carrier gas at flow rate of 1 mL/min.Before chromatographic analysis, samples were incubated at 20°C for 5 minutes, then the fiber was exposed for 10 min in the headspaceat the same temperature. After the extraction time, volatile compounds were thermally desorbed in splitless mode for3 minutes in PTV injector of the GC with a programmed temperature from 60to 230°Cat 14.5°C/s held for 5 minutes.The GC oven temperature was programmed at 40°C for 3 min, ramped at 5°C/min to 150°C, then at 15°C/min to 200°C held for 2 minutes (analysis time: 30.3 minutes). The mass spectrometry operated in electron impact mode at 70 eV and in scan range (m/z) from 40 to 350 a.m.u., with both ion source and transfer line at 250°C. The identification of analyteswas carried out by comparison of theirmass spectra with those of the US National Institute of Standards and Technology (NIST) mass spectra data bank. Analysis were performed in duplicate. Multivariate statistical analysis of the data set was performed using the Principal Component Analysis (PCA) obtained with "The Unscrambler" software by CAMO.

RESULTS AND IMPLICATIONS

The optimized method, based on HS-SPME/GC-MS, was used to study the volatile fraction composition of beer samples in order to identify potential markers which discriminate craft products from industrials ones. In the analyzed samples it was possible to identify 116 volatile compounds: esters, alcohols, ethers, carboxylic acids, terpenes, aldehydes, ketones, phenols, heterocyclic and aromatic compounds. In Figure 1 the chromatograms comparison between two commercial Italian beer samples. The top chromatogram shows the aromatic profile of a sample selected between the most common products consumed by Italian people and produced on industrial scale; the bottom chromatogram refers to a more expensive product which reports the wording "*unpasteurized*, *unfiltered*, *craft*" on the label, belonging to a niche production.

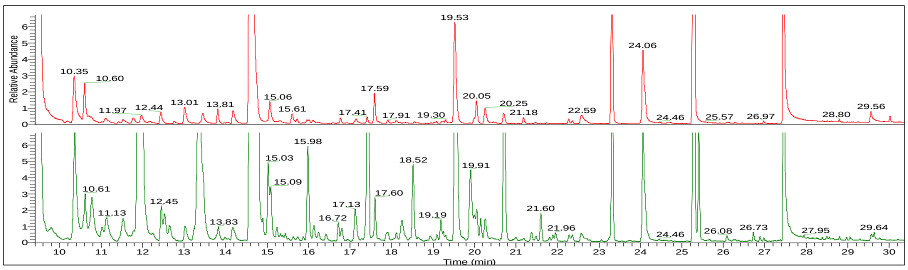

Figure 1. Chromatograms comparison of industrial (top) and craft (bottom) beer. TIC (total ion current) = $2.87x10^9$.

The fingerprints comparison highlights significant differences both in qualitative and quantitative composition of peaks among the two considered categories of beer (*industrial* vs. *craft*). The aromatic profile of all industrial beers analyzed was poorer in volatile compounds and the intensity of some characteristic substances of the craft beers was lower. Pasteurization and microfiltration processes could justify the classic flattening of the aroma of industrial productions. For the chemometric analysis, several potential markers (volatiles) were selected after the overlay of GC profiles of the analyzed beer samples. The key selection criterionwas a distinct difference in intensity of a particular peakamong examined samples and known relation with beer aroma,considering for the Reverse Search Indices (Rmatch) a threshold of 920 for each compound detected in the sample. In this way, 30 selected markers were the main investigated substances (Table 1). According to other scientific approaches, in the first analysis, is possible to suppose that most of the compounds identified are derived from the fermentation process, Maillard aging/degradation reactions (dependent on the glucose and protein fraction), and by oxidation of the terpenes and/or lipids component.

Table 1. Flavour compounds detected in the beer samples.

Marker compounds		Olfactory descriptors	Marker compounds		Olfactory descriptors
Esters			**Alcohols**		
1	Isobutyl acetate	Fruity, floral	17	Isobutanol	Ethanol
2	Ethyl butyrate	Fruity, pineapple	18	Isoamyl alcohol	Ethanol
3	Isobutyl isobutyrate	Fruity	19	2-Furanmethanol	Burnt
4	Isopentyl acetate	Sweet “banana oil”	20	Phenylethylalcohol	Fruity, floral
5	2-Methylbutyl propionate	Fruity, sweet	**Carboxilic Acids**		
6	2-Metylbutyl isobutyrate	Fruity	21	Acetic acid	Acrid, pungent
7	Ethyl hexanoate	Apple	22	Hexanoic acid	Pungent
8	Hexyl acetate	Apples and plums	23	Octanoic acid	Smoked
9	Methyl 4-methylenehexanoate	Fruity	**Carbonyl compounds**		
10	Ethyl heptanoate	Brandy “cognac oil”	24	Furfural	Caramel
11	Ethyl (L)-(-)-lactate	Fruity, coconut	25	2-Undecanone	Fruity
12	Heptyl acetate	Fruity	**Terpenes**		
13	Ethyl octanoate	Apple	26	β-Myrcene	Sweet, balmy
14	Ethyl decanoate	Fruity	27	β-Linalool	Rose and lily wood
15	Ethyl 9-decenoate	Floral, fruity	28	β-Caryophyllene	Woody, spicy
16	Phenethyl acetate	Fruity	29	Humulene	Woody
			30	(R)-(+)-β-Citronellol	Rose and geranium

In Figure 2, the comparison of the mean values of the marker areas (n=2), shows that the volatiles production is higher in products which have not undergone thermal stress treatments; therefore, it could be assumed that the high temperature used in pasteurization step could reduce flavour pattern. Accordingly, the adoption of handicraft process in beer manufacturing could be important to preserve the organoleptic and nutritional peculiarity of final product.

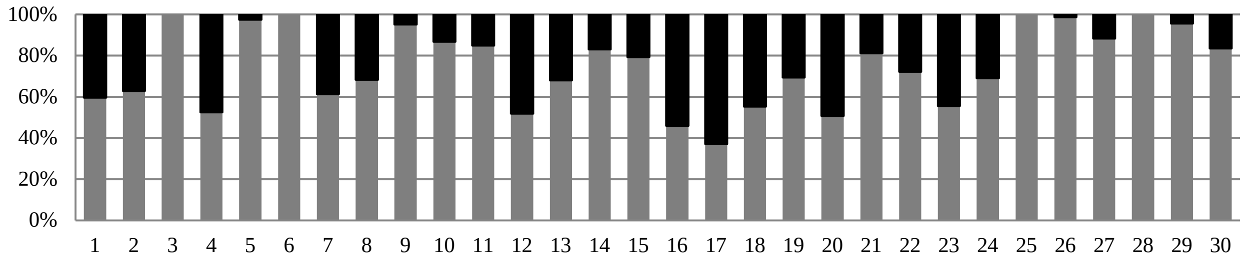

Figure 2. Relationships of the mean value of the areas, expressed in arbitrary units, of the 30 markers selected (the numbering is based on the Table 1): industrial (black) vs. craft (grey)beers.

Volatile esters constitute the most abundant class in samples analyzed, theyareresponsible for the fruity character of fermented beverages. Esters are only trace compounds in beer, but they play an important role in overall flavour balance. A number of factors could influence flavor-active ester production, including wort composition and production process; in fact, a thoughtful control allows brewers to steer ester concentration and thus to control the fruity character of their beers. During storage, esters concentration can decrease with consequent reduce in fruity flavour of beer, therefore these substances are also considered a characteristic of young products. In samples analyzed, esters were observed at different concentration levels: intensity was greater in all handcraft beers; while, isobutyl isobutyrate and 2-metylbutyl isobutyrate were not detected in any of the industrial beers studied. Also higher alcohols are important precursors of more active esters, so it is necessary to control the formation ofthese substances to ensure optimal production of esters and to balance the beer flavour. High concentrations of alcohols cancontribute to beer strong and pungent smell and taste, while

optimal levels give fruity character [Gonçalves, João L., 2014]. Isobutanol was identified in all samples analyzed, it is produced by yeast in its normal metabolism, but too high concentration of this compound (as well as propanol and isoamyl alcohol) was not desirable in beer, as it has a solvent taste. However, in industrial beer samples large amount of isobutanol was identified compared to the craft ones; so it could be considered as one of the specific markers to discriminate the two classes of beer considered. As well as the 2-furanmethanol,considered an off-flavor of beer due to its burning flavour, probably produced in the thermal step of pasteurization process, could be a process marker. Acetic acid, octanoic acid and hexanoic acid were the main acids in beers analyzed; industrial products showed a greater acid content. According to literature the presence of these substances is acceptableat low levels, since their flavourcharacteristics may be quite aggressive (smoked, pungent flavour)in end product [Jeff Sparrow, 2015].Carbonyl compounds are responsible for the stale flavour in beer: aldehydes are the major deterioration agents which negatively affect beer aroma and flavour [Moreira, N., 2013]. They may have originated from a wide range of chemical reactions, such as lipid oxidation, Maillard reactions, Strecker degradation and aldol condensation. For example, 5-HMF (5-hydroxymethyl-furfural) is the most important heterocyclic compound formed during beer aging by Maillard reaction between the carbonyl group of a hexose and the amino group of an amine; while furfural is produced when starting form a pentose instead of a hexose [Rakete, S., 2014]. Furfural is a dehydration product of pentoses and an important marker of beer flavour deterioration, although it is not an off-flavor product at the concentration at which it is found in beer [Li, M., 2009]. Furfural was identified at higher concentrations in the industrial beers compared to craftproducts and a significant correlation (P value < 0.05) with isobutanol was found, so these two analytescould be considered as qualityprocess markers for industrial beers. 2-Undecanone, anaromatic ketoneresponsible for a positive fruity aroma,was not found in industrial products but low amounts was found in craft samples: so could be a discriminating compound of the two categories investigated.In addition, several terpenes were identified mainly in the handicraft products, or a higher quantity in craft beers than industrial. Terpenes derived directly from the hops used in the every products analyzed, and so were found in amount significantly different in the beer samples considered. Craft beers showed greater presence of these compounds and probably it is the consequence of the absence of pasteurization and microfiltrationstepsthat flattening the flavour pattern. In particular, β-caryophyllene and humulene were not found in industrial beer samples and, together with β-myrcene and β-linalool could be used to distinguish the two categories of beers.
In this preliminary study, PCA was used to classify beer samples based on the chemical information provided from 30 target flavour compoundsreported in Table 1and selected among those with high Rmatch. The data set consisted of a 77x30 matrix: rows represented the samples analyzed (77 scores) belonging to two categories (craft and industrial beers), while columns represented the peak area of 30 selected analytes (30 loading) obtained from the chromatographic plots. Figure 3 shows samples (*score*) corresponding to the three principal components (PCs) with eigenvalues > 1 which explain 84% of the total variance.

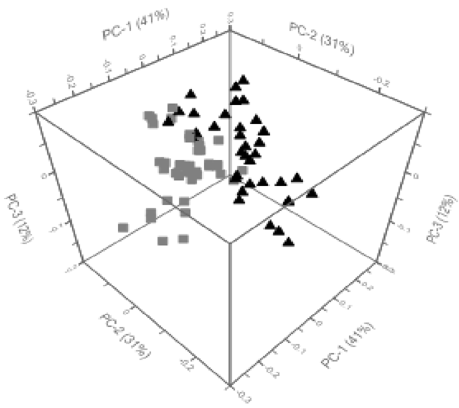

Figure 3. Result of PCA 3D scatter scores plot: craft (box) and industrial (triangle) beers.

The analysis of score plot emphasized separation between the craft samples and industrial ones on the first principal component (PC1), while the second principal component (PC2) discriminates beer samples within each group. At the same time, multivariate analysis suggests that there are several analytes significantly contributing to discriminate the two beer categories. So, the preliminary results suggest that the flavour footprint of beers could be significantly affected by the method of production, both in terms of qualitative and quantitative composition of volatiles.

CONCLUSIONS

This study is timely considering the increasing trend of beer consumption worldwide as well as the continuous growth of handcraft microbreweries and the importance of volatile compounds in beer flavour. In this work,the HS-SPME/GC-MS method was used to identify flavour compounds able to discriminate beer samples produced under different process steps (craft vs. industrial process). In order to establish the relationship between some of the identified compounds and the heat treatments applied during pasteurization step, a multivariate statistical analysis was performed. PCA showed a well-defined separation among the two groups of beer samples considered could potentially be used as quality markers of the end product. In first analysis, several compounds, such as terpenic compounds (β-caryophyllene, humulene, β-myrcene, β-linalool), higher alcohols (isobutanol, isoamyl alcohol), esters (isobutyl isobutyrate, 2-metylbutyl isobutyrate) and acids (acetic acid, octanoic acid, hexanoic acid), seem to discriminate the two categories of beer examined. So, these results could suggest that flavor of beer may significantly differ depending on conditions applied during its production (process marker). In addition, some of the detected compounds have a low odour thresholds and thus they may contribute to beer aroma (quality markers). This research could be have a potential interest, from the market point of view, to characterize different commercial products made with industrial or handicraft processes (high added value), that reflects on different final selling prices. In conclusion, even though Italian law does not attribute any quality to the artisanal title, this study it could confirm that a different productive process could effectively affect the sensorial and nutritional features of the final product. Further studies aimed at a more thorough examination of the data obtained will be needed to identify additional volatile compounds that could potentially be used as product quality markers, also in order to protect the consumer from possible fraud, and that these compounds can therefore be considered as process markers for the purpose of discriminating between craft and industrial processes, especially on the basis of the use of high temperature steps.

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