Article

Optimal Design Approach Applied to Headspace GC for the Monitoring of Diacetyl Concentration, Spectrophotometric Assessment of Phenolic Compounds and Antioxidant Potential in Different Fermentation Processes of Barley

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Abstract: The present study aimed to validate a control method on the gas chromatography system (GC) based on the experimental design strategy, to examine the changes and correlation between the fermentation process and the quality of alcoholic and non-alcoholic beer product, especially the formation of diacetyl. On the other hand, spectrophotometric methods were applied to the determination of polyphenols content and the potential antioxidant activity of beer during different fermentation processes. with this aim, three modes of barley fermentation were used, specifically classical fermentation, stopped fermentation and thermal process. The results showed that the different fermentation modes had a major impact on diacetyl production. The highest concentration was obtained using stopped fermentation 0.36 mg/L, the lowest concentration value 0.07 mg/L was detected using the thermal process. Monitoring the increase of oxygen concentration between fermentation, filtration, and filling of the final product (32, 107, 130 ppm, respectively) has a significant impact on the concentration of diacetyl. The obtained results of spectrophotometric analysis showed that the total antioxidant activity changed during beer fermentation process and demonstrate that the extend of the antioxidant activity was very much dependent on the total polyphenolic content with a higher value in Hopped wort (13.41%, 65 mg GAE 100 mL^{-1}, 28 mg CE 100 mL^{-1}) for antioxidant potential, total phenolic content, and total flavonoids content, respectively, whereas the lowest values was detected in Non-alcoholic beer using thermal process (7.24%, 35 mg GAE 100 mL^{-1}, 10 mg CE 100 mL^{-1}) for antioxidant potential, total phenolic contents, and total flavonoids contents, respectively. Based on the results achieved, we reveal the impact of the fermentation process on the nutritional value of the final product.

Keywords: experimental design; headspace GC; diacetyl; fermentation; barley; spectrophotometric; antioxidant activity; TPC; TFC

1. Introduction

Beer is one of the first-born and popular fermented drinks in mankind's history, The process by which beer is brewed has not changed significantly since its discovery thousands of years ago and the production of this beverage can be considered as very traditional and remain still the same, without important variations ever since its earliest origin. Generally, the process is based on natural enzymatic activity happening throughout the malting of grain, mashing of grist and fermentation of wort [1]. Beer is a complicated combination of ingredients, fermented from raw materials including water, yeast, malt and hops, and comprises a wide range of several chemical components that may react and interact at all stages of the brewing process [2]. Various groups of compounds have been shown to play an important role in the flavor characteristics [3], e.g., esters, higher alcohols, aldehydes, ketones, organic acids), which contribute to the last sensorial character of beer [4]. Some of these by-products are unsuitable because they impart beer off-flavors/aromas [5]. Particularly interesting topics are the reduction of diacetyl, dimethyl sulfide (DMS) or hydrogen sulfide and an enhanced production of sulfur dioxide or esters [6]. They are originated from two amino acids namely valine and leucine, and are responsible for toffee, butterscotch, honey and vanilla-like off-flavors when they are present at concentrations higher than their threshold values (>0.1–0.4 mg/L) [7,8]. In addition, they may play the most important role in the final flavor [9].

The Diacetyl (2,3 butanedione), is a volatile α-diketone, produced naturally in fermented products and as a yeast metabolism product during fermentation by yeast and bacteria. Remaining to its characteristic butter such as aroma, diacetyl has been widely used as a synthetic butter flavoring agent in food products [10] such as microwave popcorn [11], confectionery, baked goods [12] and in flavored electronic cigarette liquids [13,14]. On the other hand, Dimethyl sulfide (DMS), as the majority of unpleasant sulfury flavors, originates mainly from wort and hops, however, another possible origin is dimethyl sulfoxide (DMSO) by yeast during fermentation or wort-spoilage bacteria [15], DMS may significantly affect the aroma of beer and may lead to undesirable flavor impressions such as cooked cabbage or sweet-corn [7,15]. Despite the increasing recognition of diacetyl toxicity, various studies have shown shedding of epidermal growth factor receptor (EGFR) ligand amphiregulin following the exposure to diacetyl [16,17] and increased secretion of inflammatory cytokines [17,18] and the augmentation of reactive oxygen species (ROS) production, reduced antioxidant defenses [19,20], epithelial injury [21] and protein damage [22], development of bronchiolitis obliterans (BO), a severe and irreversible lung disease [12,23–25]. Furthermore, DMS is recognized to cause toxic central nervous system (CNS) effects, including diaphragmatic paralysis and coma, accompanied by asphyxia because of oxygen depletion at very high concentrations of DMS. Coroners from Japan and Russia [26,27] report lung edema and visceral hyperemia in victims that have succumbed to paper-mill by product exposures rich in DMS (up to 80%).

During the first four days of fermentation the conversion into the vicinal diketones, such as diacetyl and 2,3-pentanedione, is the highest, after which the formation of other products from these ketones are superior. So, there is a reduction of the concentrations of diacetyl and 2,3-pentanedione during fermentation and lagering. It is important to achieve low concentrations of these compounds in the final product because they cause an off flavor. Several reasons (e.g., fermentation, temperature, aeration) can be the cause of high concentrations. Therefore, during the production of beer it is important to maintain the optimum adjustments to prevent off- flavors caused by diacetyl and DMS. Since the determination described here concerns relatively volatile compounds it is possible to apply headspace injection, meaning that part of the vapor above the beer sample is injected after equilibration at a fixed temperature.

The overall objective of this study was designed to develop a simple and rapid method of control using GC based on strategy of the experimental design to check out the effect of oxygen on the chemical breakdown of Diacetyl during fermentation processes, so as to achieve the improvement of the technological control of the flavor profile, in the production

of the flavor active compounds. On the other hand, the application of a spectrophotometric methods to evaluate the total phenolic, flavonoids content and the antioxidant capacity of each beer type to evaluate the impact of fermentation processing on the nutritional value on the final product.

2. Materials and Methods

2.1. Materials

Barley malt was subjected to three fermentation methods, namely normal fermentation, arrested fermentation, and fermentation coupled with a thermal process. Beer samples from different fermentation methods were analyzed during the production of alcoholic and alcohol-free beer, *Saccharomyces cerevisiae* is the yeast utilized during the different process of fermentation. The yeast was propagated from a freezer stock maintained at −150 °C.

2.2. Stopped Fermentation Methods

Fermentation can be stopped by removing the yeast cells or by rapidly cooling the fermenting must. This technique requires musts with low concentrations of fermentable carbohydrates. The fermentation stage is conducted at low temperature, about 2–3 °C, with a contact time of about 200 h. During fermentation, the must is not aerated to prevent yeast reproduction and to prolong the lag phase during which the yeast consumes and metabolizes but does not propagate or produce ethanol.

2.3. Thermal Process

The beer is degassed and subsequently preheated in a plate heat exchanger. The beer is fed into the stripping section of a rectification column. The product flows through the column at a temperature between 43 °C and 48 °C. In countercurrent, the product meets rising vapors that cause selective separation of the alcohol from the product. The alcohol-free beer is then introduced into an evaporator from the bottom of the column. In the evaporator, the vapors necessary for the rectification process are produced and redirected to the column. The completely dealcoholized product is pumped out of the plant after passing through a cooler [28].

2.4. Chemicals

GC parameter Chromatographic condition: The GC esters separation was performed using a DBWAX (Agilent Technologies, Stevens Creek Blvd., Santa Clara, CA, USA) Column with a capillarity of (60 m × 530 μm × 1 μm) nominal with a temperature max of 240 °C for the reference 50 m × 530 μm × 1 μm nominal temperature max of 320 °C. Column used for diacetyl is the DB5 (Agilent Technologies, Stevens Creek Blvd., Santa Clara, CA, USA) with a capillarity of (60 m × 530 μm ×1 μm) with a max temperature of 320 °C for the reference the capillarity is 50 m ×530 μm × 1 μm with a max temperature of 320 °C. This difference in column type and the design of the apparatus may have led to variations in temperature and pressure, especially in the furnace, injectors, columns, and detectors, because of the method used. To create a reliable method, we used an experiment plan based on a matric that allowed us to determine the different settings and subsequently the injections.

2.5. Concepts on Experimental Designs

In the present study we conducted to develop a method for determining the parameters that affect the quality of beer taste, we took a reference sample with known concentrations and each time we change the data according to an experimental design to find the same concentrations as the reference.

The experimental designs make it possible to organize the trials that accompany scientific research or industrial studies in the best strategy, With the plans of experiments the maximum information are obtained with the minimum of experiments. In this context, we must follow mathematical rules and adopt a rigorous approach [29]. The factors considered in an experimental design are the input factors.

The factorial experimental designs all use the following mathematical model that links the response y to the factors x_1, x_2, ... xi. This theoretical model is postulated a priori. This is a polynomial model (1).

$$Y = a_0 + a_1 \cdot x_1 + a_2 \cdot x_2 + \ldots\ldots\ldots\ldots + a_n \cdot x_n \quad \sum_{i.j=1 \ i \neq j \neq k}^{n} aijk \cdot xi \cdot xj \cdot xk + \cdots \quad (1)$$

A response surface plan is a set of advanced techniques (experiment plan) that allow us to better understand and optimize the response. The response surface design methodology is often used to develop models following the determination of important factors using factorial designs, particularly if you suspect a curvature in the response surface [30].

Regarding the characteristics of the studied factors, the choice was based on a surface plan with three factors: Where a_0, a_1. ... Are the coefficients of the polynomial (2).

$$Y = a_0 + a_1 \cdot x_1 + a_2 \cdot x_2 + a_3 \cdot x_3 + a_{12} \cdot x_1 \cdot x_2 + a_{13} \cdot x_1 \cdot x_3 + a_{23} \cdot x_2 \cdot x_3 + a_{123} \cdot x_1 \cdot x_2 \cdot x_3 \quad (2)$$

The product terms of type for example aij.xi.xj correspond to interactions.

A final mandatory step before using the model in production will be to test by an experiment in the center of the experimental domain if the value predicted by the model is close to the experimental value [30].

2.5.1. Methods
Nemrodw

This software used for experimental designs allows the construction of classic experimental matrices, specific to the type of objective pursued: in this case it is a study in an experimental field (response surfaces).

Factors and Response

Three factors influence principally on the retention time of diacetyl during GC analysis.

- Oven temperature. (Measured by x_1 in °C).
- Intel Back Temperature. (Measured by x_2 in °C).
- Column pressure. (Measured by x_3 in ATM).

The response (y) quantified by the retention time of diacetyl corresponding to its concentration. The objective is to validate the values of the factors on the machine that allows us to have a better response.

2.6. Qualitative and Quantitative Methods
2.6.1. The Determination of Diacetyl and 2,3-Pentanedione in Packaged Beer Using Headspace Gas Chromatography

A- Internal standard stock solution

150 μL 2,3-hexanedione in 100 mL ethanol

Preparation: Pour about 90 mL cold (0 °C) ethanol in a 100 mL volumetric flask. Place the flask in a tray with melting ice. Add 150 μL 2,3-hexanedione using a 250 μL syringe. Make up to 100 mL at 0 °C with cold (0 °C) ethanol. Stability: In refrigerator 1 week.

B- Internal standard working solution

2.0 mL stock solution (A) in 200 mL 5% (*v/v*) ethanol

Preparation: Pour about 50 mL cold (0 °C) 5% (*v/v*) ethanol/water in a 200 mL volumetric flask. Pipette 2.0 mL of the cold (0 °C) stock solution (A) into the flask. Make up to 200 mL with cold (0 °C) 5% (*v/v*) ethanol/water and mix. Transfer the solution to the dispenser when used. Stability: In refrigerator 1 week.

2.6.2. The Determination of Acetaldehyde, Dimethyl Sulfide, Esters, and Higher Alcohols by Gas Chromatography

X-Internal Standard Stock Solution

Preparation: Pour 50 mL ethanol in a 100 mL volumetric flask. Pipette 3.0 mL 4-heptanon and 30.0 mL n-butanol into the flask. Make up to 100 mL with distilled-water and mix. Stability: In refrigerator (0–10 °C) 1 month.

Y-Internal Standard Working Solution

Preparation: Pour 50.0 mL ethanol and about 400 mL distilled water in a 1000 mL volumetric flask. Pipette 20.0 mL of the stock solution (A) into the flask. Make up to 1000 mL with distilled water and mix. Transfer the solution to the bottle with dispenser when used. Stability: In refrigerator (0–10 °C) 1 month.

The normal procedure for sample preparation is as follows: Slowly fill a 250 mL graduated measuring cylinder with the cold (0–10 °C) beer sample. Remove the excess of beer and/or foam by using a waterjet suction pump to obtain 250 mL. Pipette 2.0 mL (volumetric pipette or dispenser) of the internal standard working solution B and D into an infusion bottle. Immediately add the beer sample to the infusion bottle. Close with a screwcap and rubber septum and carefully mix. In this way the samples are prepared one by one. After a series of samples has been prepared, each infusion bottle should be kept closed for at least 5 min to prevent foaming over. Prepared samples can be stored in the refrigerator (0–10 °C) for 2 days.

Preparation: Pour about 50 mL of the 250 mL prepared sample (with internal standard) into another clean infusion bottle, close and place in a water bath of 40 °C, place a (vinyl-covered) lead weight around the neck of the bottle to prevent floating, (Lead donut 50 mm, Tamson 486K32 or order via GSC), pierce a disposable needle through the septum to prevent overpressure, after 30 min of conditioning inject about 0.5 mL of the headspace.

2.6.3. Spectroscopic Analysis of Polyphenols

The spectrophotometric analyses were performed using a UV-1601 spectrophotometer from Shimadzu (Duisburg, Germany) and were replicated three times for each extract or calibration point ($n = 3$).

2.6.4. Determination of Total Phenolic Content (TPC)

The total phenolic content was determined based on a spectrophotometric method using the Folin–Ciocalteu reagent according to the method of Signleton et al. (1999) [31]. This reagent leads to oxidizes the phenolic compounds, which turns the solution to blue. The TPC was quantified from a calibration curve prepared with gallic acid standard and expressed as mg of gallic acid equivalents (GAE) per 100 mL of sample (mg GAE 100 mL^{-1}). The calibration curve was drawn, and the equation of linear regression was obtained:

$$y = 1.552x + 0.208, R^2 = 0.962$$

2.6.5. Determination of Flavonoid Content (TFC)

Total flavonoid content was performed using the aluminum chloride ($AlCl_3$) based on the protocol described by Kim et al. (2003) [32]. The TFC in samples was quantified from a calibration curve prepared with Catechin standard and expressed as mg of catechin equivalents (CE) per 100 mL of sample (mg CE 100 mL^{-1}). The calibration curve was drawn, and the equation of linear regression was obtained: $y = 2.857x + 0.080, R^2 = 0.999$.

2.6.6. Determination of Free Radical Scavenging Activity in Beer by DPPH

Regarding the analyses and evaluation of the described methods, the results of the ruggedness test for determination of the conditions of the method, coefficients of variation and recommendations of some authors was elaborated a simple modification of the protocol

for determination of antioxidant capacity using DPPH (2,2-diphenyl-1-picrylhydrazyl), a stable free radical in beer and beverages [33].

Preparation of Diluted sample. Dilute 13.3 mL of degassed beer with water to 100 mL at temperate to 20 °C, attemperate to 20 °C in volumetric ask and the solution was vortexed thoroughly, 2.5 mL of diluted beer was added to 20 mL of ethanol and kept the mixture to stand 20 min at 20 °C, then fill up the volume to 25 mL with attemperated ethanol, the solution was homogenized rigorously, finally the mixture was transferred to a fluted filter, filtered, and stored at 20 °C before using.

Diluted blank. Dilute 2.5 mL of water with ethanol in volumetric ask 25 mL. Attemperate 20 min to 20 °C. The mixture could be filtered If is necessary through fluted filter.

Preparation of DPPH stock solution. 0.06 mM of DPPH methanolic solution (0.0024 g DPPH/100 mL ethanol) Attemperate 20 min to 20 °C. The stock solution should be freshly used.

Determination

Sample. In a test tube, 1.5 mL of diluted sample was added to 1.5 mL of DPPH solution, the mixture was homogenized and incubated for 30 min in darkness at room temperature, the absorbance was measured at 517 nm, against diluted blank.

Control. 1.5 mL of diluted blank was added to 1.5 mL of DPPH solution, vortexed thoroughly. In addition, kept in the dark for 30 min, the absorbance was measured at 517 nm against diluted blank.

Calculation of the Results

DPPH scavenging activity was expressed as a percentage of free radical scavenging activity (FRSA) with DPPH or as % inhibition of the free radical with DPPH according to the following formula:

$$\%I = \frac{A\ control - A\ sample}{A\ control} \times 100$$

3. Results and Discussion

3.1. Experimental Area

The classical factors studied on the experimental design.

The obtained results (Tables 1–4) showed that after the application of the experimental design the optimal adjustment for determination of volatile components was based on three parameter values:

- Oven temperature = 70 °C
- 70 °C $\leq$ Intel Back Temperature $\leq$ 130 °C
- 39 atm $\leq$ Column Pressure $\leq$ 40 atm

Table 1. The factors influenced on the retention time of diacetyl analyzed by experimental design.

Experience	Factors	Centre	Variation Degree
X1	Oven temperature.	100 °C	30
X2	Intel Back Temperature.	100 °C	30
X3	Pressure Column.	39.5 atm	0.5

Table 2. Application of factorial design (Three factors).

Experience	Factor 1	Factor 2	Factor 3
1	−1	−1	−1
2	+1	−1	−1
3	−1	+1	−1
4	+1	+1	−1
5	−1	−1	+1
6	+1	−1	+1
7	−1	+1	+1
8	+1	+1	+1

Table 3. The values of the factors studied with no coding unit.

N° Test	Factor 1	Factor 2	Factor 3
1	70	70	39
2	130	70	39
3	70	130	39
4	130	130	39
5	70	70	40
6	130	70	40
7	70	130	40
8	130	130	40

Table 4. The results obtained (response) after the application of the experimental design.

ECH	Oven Temperature (°C)	Intel Back Temperature (°C)	Pressure Column (atm)	RT	[Diacetyl] µg/L
Reference Sample	70	150	39	5.6	16.5
1	70	70	39	5.602	17.06133
2	13	70	39	ND (5.58)	0
3	70	130	39	5.605	16.346
4	130	130	39	ND	ND
5	70	70	40	5.459	16.708
6	130	70	40	ND (5.462)	0
7	70	130	40	5.457	16.70835
8	130	130	40	ND	ND

ND: Not detected.

3.2. Interpretation of Analysis in Various Fermentation Conditions

During classical fermentation the first peak of the diacetyl concentration normally appears early. The concentration of diacetyl is then reduced when the yeast assimilates diacetyl and converts it enzymatically into acetone, which in turn is then metabolized. Diacetyl is thought to be a by-product of amino acid biosynthesis involving a relationship between amino acid biosynthesis and the formation of acetolactate, the diacetyl precursor. The second peak of diacetyl is explained by the decrease in fermentable sugars and a maximum activity of the yeast, hence a concentration of diacetyl proportional to the multiplication of the yeast and the degradation of fermentable sugars (production associated with growth). However, problems arise when continuous metabolic fluctuations lead to late production of diacetyl in amounts that create a 3rd peak which may be due to the presence of a high concentration of diacetyl in the beer wort. The higher levels of diacetyl resulting from late fermentation cannot be reduced below its gustatory threshold in the allotted time.

The total concentration of diacetyl in the beer at a given time during and after fermentation results from the combination of the rate of formation of α-acetolactate, its rate of conversion, and the rate of reduction of diacetyl to 2,3-butanediol and acetone. Any latency of high diacetyl concentration resulting from late fermentation cannot be reduced until the beer undergoes further treatment, which may require prolonged maturation or other treatments or remedial measures to bring the final diacetyl concentration within the target specification of the desired final product (0–40) microgram per liter (Figure 1).
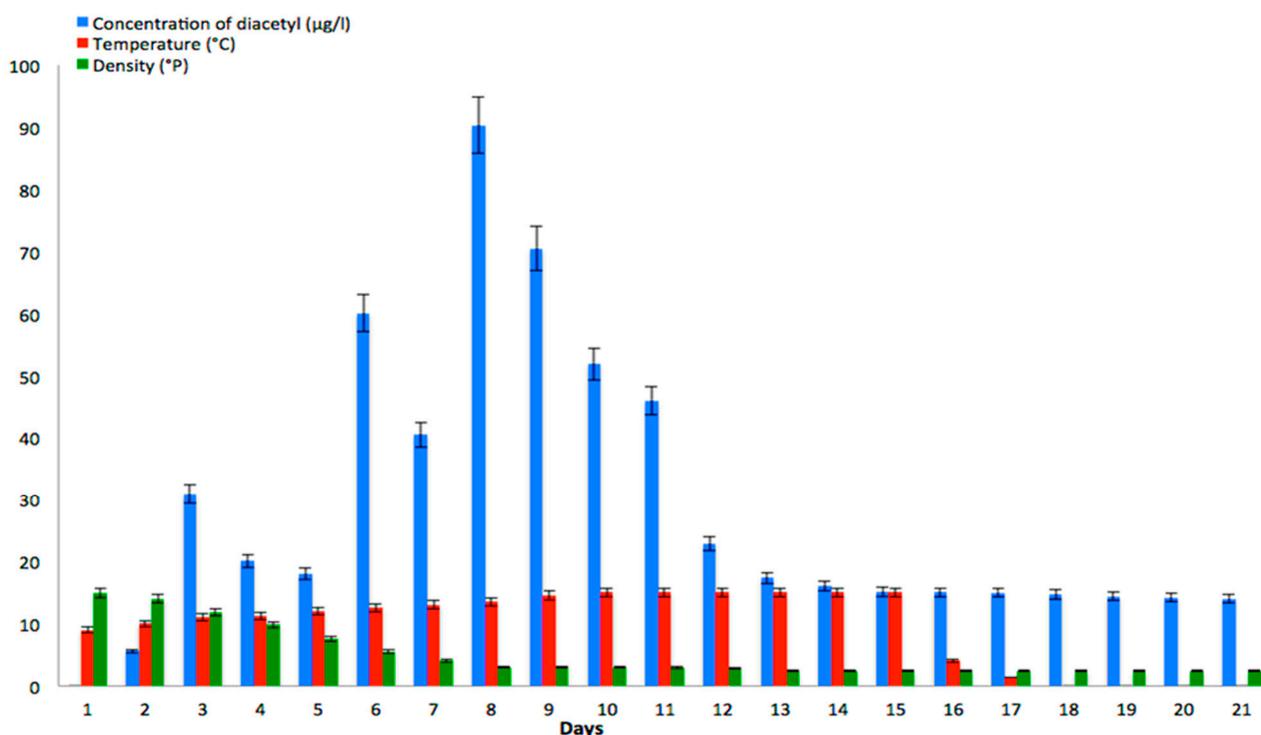

Figure 1. Monitoring of diacetyl concentration during classical fermentation.

In the process of stopping fermentation the wort has been cooled to about 20 °C, is stripped under pressure to avoid foam production. This method produces a good quality beer with less than 0.2% alcohol by volume, but it requires precise analytical control. The yeast mass and alcohol content must be monitored every 8 h. In Super high gravity production, starting at 17 °P, 6.5 °P is obtained after centrifugation at about 0.4 °C and dilution, before or after final filtration, with desalted water to reach the desired alcohol and saccharometric level. When producing non-alcoholic beers by stopping fermentation, the main objective is to reduce the impression of bad taste or to limit it from the beginning and for diacetyl concentrations between 11 and 12 µg/L (Figure 2).

Fermentation coupled with the thermal process represents the easiest method to have an alcohol-free beer, the application of heating between 35 °C and 60 °C until boiling results in the elimination of alcohol and other volatile substances such as esters and higher alcohols consequently increasing the turbidity of the 0.3 to 2.5 IBC (Table 5) [34].

The final beer product under different conditions was analyzed by GC to identify its aroma compounds. The results show that the concentration of diacetyl also increases between the stop of fermentation and the release of the final product, with the concentration of diacetyl at the last day of fermentation is equal to 12 µg/L this concentration increases up to 160 µg/L in the final product. This difference requires control of factors such as oxygen level and temperature between the fermentation stage and the release of the final product (Table 6, Figure 3).
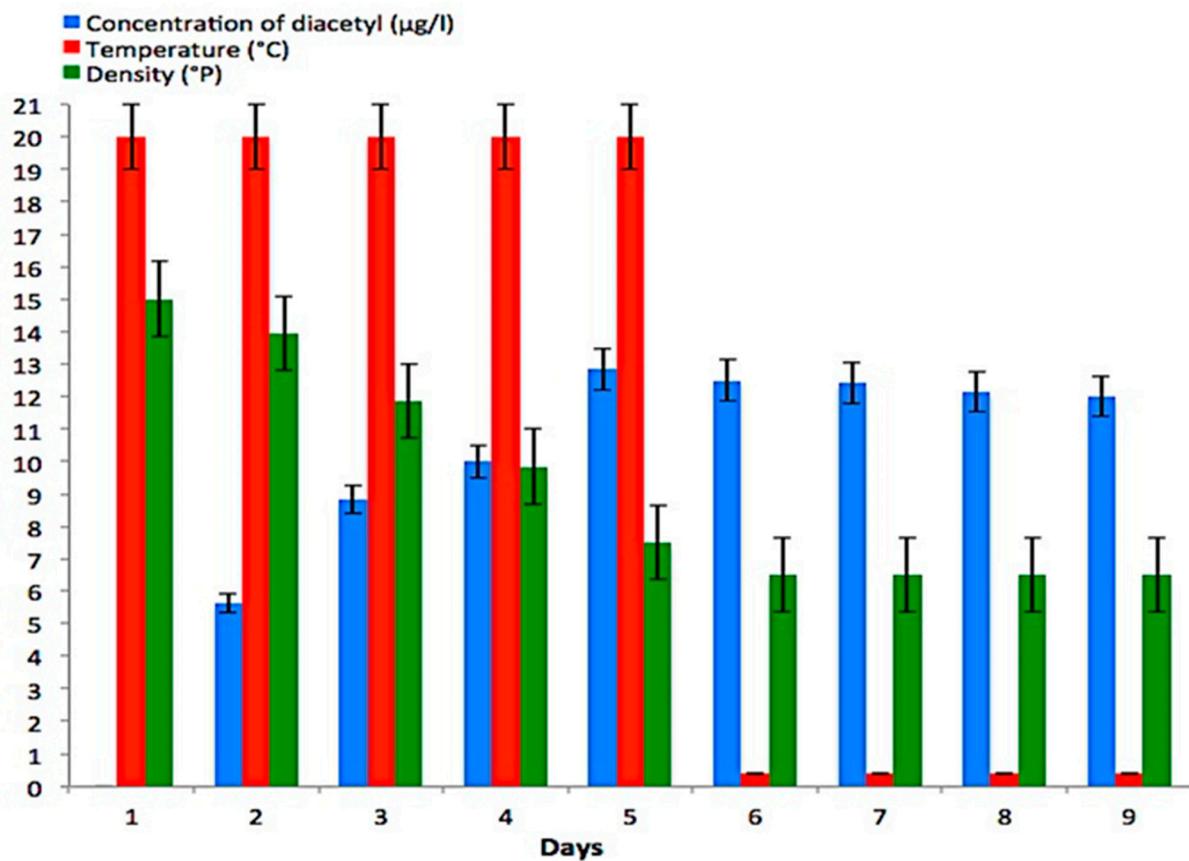
Figure 2. Monitoring of diacetyl concentration during stopping fermentation.

Table 5. Aromatic compounds and turbidity of the final product of alcoholic and de-alcoholized beer in various fermentation conditions determined by GC.

Detected Compounds	Final Product of Beer by Using Classical Fermentation	Final Product of Non-Alcoholic Beer by Using Stopping Fermentation	Final Product of Non-Alcoholic Beer by Using Thermal Process
Ethanol (vol%)	5.2	0.4	0.02
Acetaldehyde (mg/L)	7.2	8.5	4.0
Propanol (mg/L)	22	9	Not detected
Ethyl acetate (mg/L)	20.2	3.4	Not detected
Isobutanol (mg/L)	21.4	3.2	Not detected
Isoamyl acetate (mg/L)	2.35	0.12	Not detected
3-methylbutanol (mg/L)	60.1	1.25	0.25
2-methylbutanol (mg/L)	20.2	Traces	Traces
Phenylethanol (mg/L)	33.11	35.1	38.5
Furfuryl alcohol (mg/L)	3.11	2.6	2.411
Diacetyl (mg/L)	0.16	0.36	0.07
DMS (μg/L)	22	45	Not detected
Turbidity (IBC)	0.35	1.5	2.5

At the end of fermentation, oxygen is eliminated from the milieu because it is consumed during the respiration phase of the yeast. The remaining quantity is used during fermentation because it is essential in the biosynthesis of sterols, which are essential molecules for the maintenance of cell walls and give the yeast a high rate of viability.

After filtration process, the oxygen concentration is higher than measured in the holding tank (after the end of fermentation). This concentration decreases over time. This occurs due to removing the air bubbles formed at the beginning of the filtration process.

The water used in the preparation of the filtration layers contains a significant amount of oxygen.

Table 6. Aromatic compounds of a final product of beer under classical fermentation.

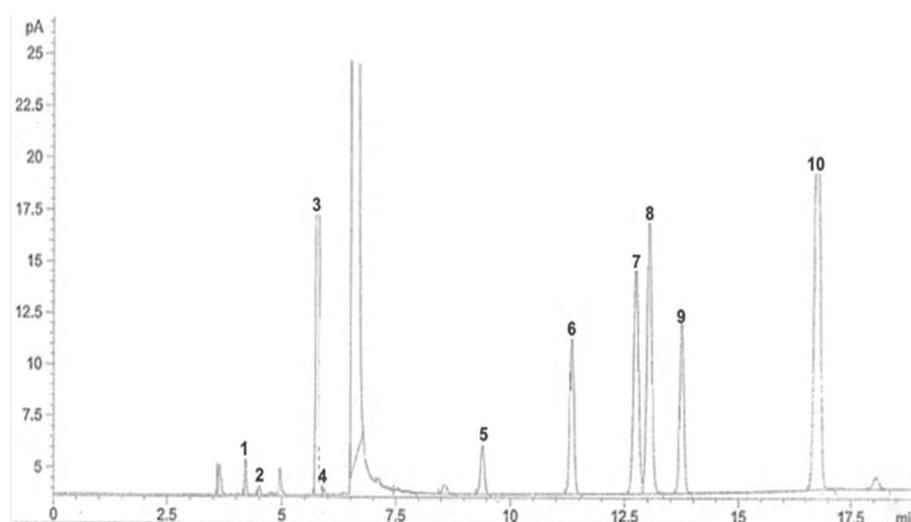| Peak	Compound	Retention Time (min)	Amount
1	Acetaldehyde (mg/L)	4.211	7.29
2	DMS (μg/L)	4.508	21.989
3	Ethyl acetate (mg/L)	5.777	20.838
4	Methanol (mg/L)	5.928	2.210
5	n-Propanol (mg/L)	9.412	13.546
6	Iso-Butanol (mg/L)	11.349	21.439
7	Isoamyl acetate (mg/L)	12.748	2.456
8	Heptanone(mg/L)	13.035	1.000
9	Butanol (mg/L)	13.750	1.000
10	Amyl alcohols (mg/L)	16.760	73.130

Figure 3. Chromatogram of a beer sample on a DBWAX column.

At the filler inlet, the oxygen concentration decreases proportionally with increasing temperature, this is due to the fact that oxygen is less soluble at higher temperatures. (HENRY's law), (Figure 4).

In general, the dissolution of oxygen in beer is high if the temperature is low and the pressure is high. The length of the pipes increases the risk of oxygen saturation in the beer. Aeration at the beginning of fermentation with less than 10 g/L is not a potential risk of oxidation since the yeast consumes the remaining oxygen during secondary fermentation. Controlling the pressure stability of clear beer tanks should be carried out to optimize the rate of oxygen removal by carbon dioxide.

The FTIR spectra analysis of the beer, shows the presence of -NH bonds of amines between 3000–3400 cm^{-1} as well as primary and secondary amine bonds between 2800–3000 cm^{-1}, C=O bonds of amides and aromatic ketones, C-O bonds of esters between 1300–1450 cm^{-1}, C-O bonds of primary and secondary alcohols between 1040–1090 cm^{-1} and finally Ar-C bonds of aromatics between 850–890 cm^{-1}.

The total concentration of diacetyl in the beer must during and after fermentation results from a combination of the rate of formation of α-acetolactate, its rate of conversion, and the rate of reduction of diacetyl to 2,3-butanediol and acetone. Any latency of removed diacetyl concentration resulting from late fermentation production cannot be reduced until the beer undergoes further processing, which may require prolonged maturation or other processing or corrective measures to bring the final diacetyl concentration within

the desired product target specification (0–40) micrograms per liter. This increase due to bacterial contamination reactions and the presence of oxygen which oxidizes ketones and alcohols is confirmed by Figure 5 which indicates that the oxygen level decreases as a function of time in the beer in parallel with other parameters such as temperature.
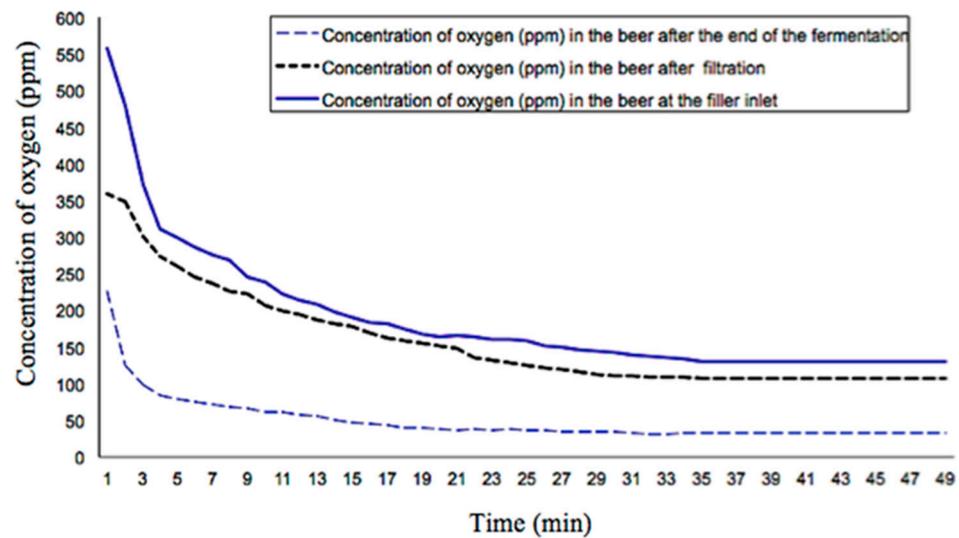

Figure 4. Monitoring of oxygen concentration during the process of beer production.
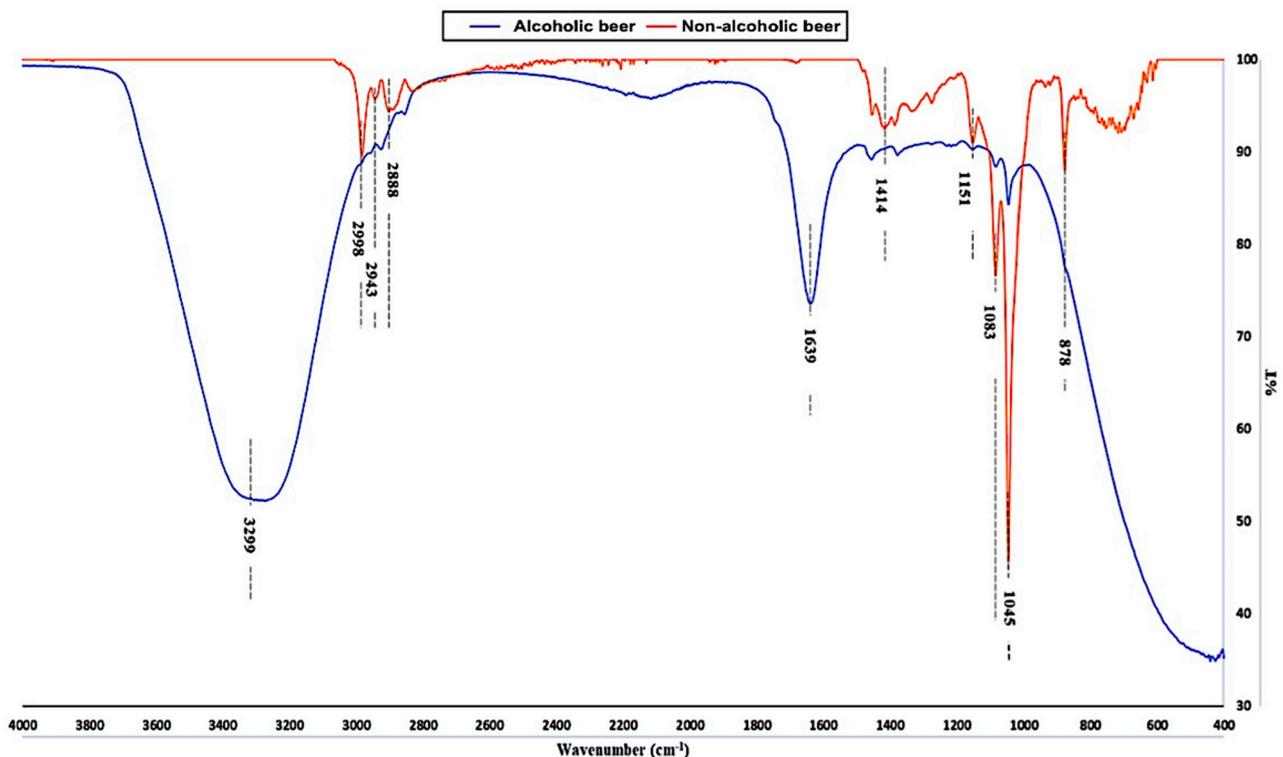

Figure 5. FTIR (Fourier transform infrared spectroscopy) spectra of alcoholic and non-alcoholic beer.

3.3. Interpretation of Spectroscopic Analysis

According to the mean values of the analyzed beer types presented in Figure 6, hopped wort (HW) sample contained flavonoids and total phenolic in higher concentrations (28 mg CE 100 mL^{-1}, 65 mg GAE 100 mL^{-1}, respectively) than sweet wort (SW), which is reflected in their very high antioxidant activity with a free radical inhibition capacity of 13.41%, Figure 6a.
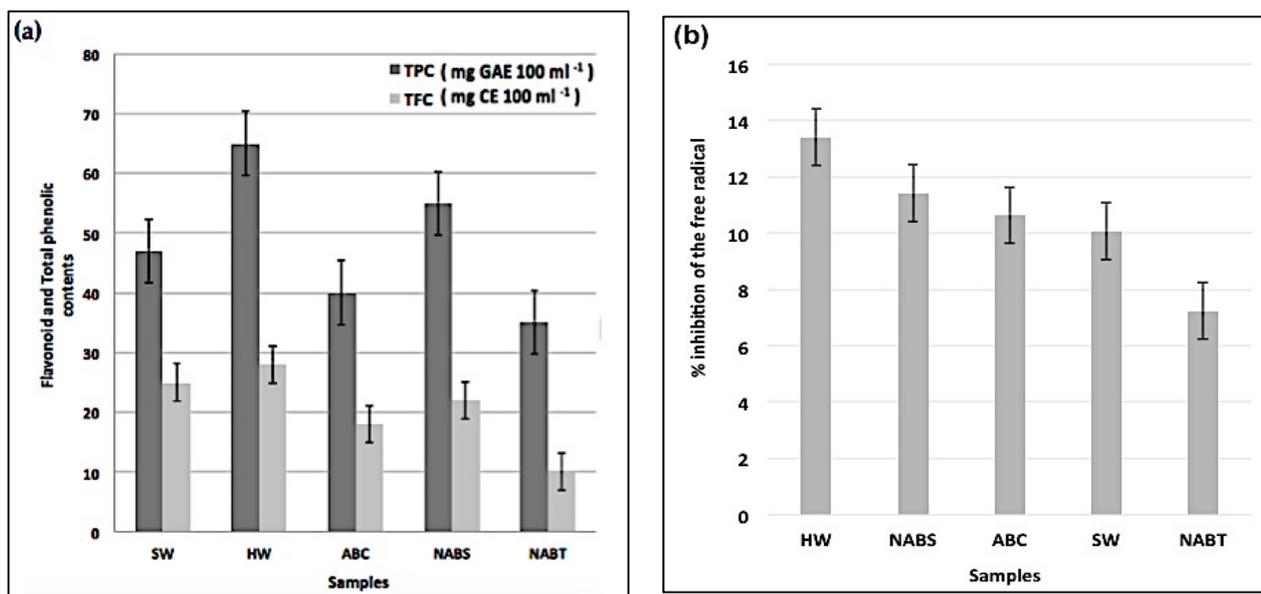
Figure 6. Flavonoid, total Phenolic Contents (**a**) and percentage inhibition of free radical scavenging activity (**b**) of the analyzed samples.

After different fermentation processes, the highest concentration of flavonoids and total phenolic contents (22 mg CE 100 mL^{-1}, 55.12 mg GAE 100 mL^{-1}, respectively) was observed in the non-alcoholic stopping fermentation beer (NABS), with a higher free radical scavenging capacity than alcoholic beer by using classical fermentation (ABC) and non-alcoholic beer by using thermal process (NABT). The lowest concentration of flavonoids and total phenolic contents (10.01 mg CE 100 mL^{-1}, 35.1 mg GAE 100 mL^{-1}, respectively) could be observed in non-alcoholic beer by using thermal process (NABT) Figure 6b.

The beer filtration process reduces the content of phenolic compounds, as well as the antioxidant activities of the wort. With the higher consumption of diatomite, a high concentration of iron ions is introduced, which decreases the scavenging rate of DPPH, During the cooling phase, the spontaneous adsorption of phenolic compounds and melanoidins on the wort lees and polymerization lead to the decrease of TPC in beer [35–37].

4. Conclusions

Analytical chemistry is playing an increasingly important role in beer flavor analysis, chemical analysis of beer is essential and is widely used in various research areas related to beer production. Furthermore, beer is a complex alcoholic beverage containing both volatile and non-volatile components. The validation of a method to control and monitor the concentration of diacetyl in the different stages of alcoholic and non-alcoholic beer production to understand how to maintain the balance between the different types of flavors.

The application of spectrophotometric analysis is essential in the evaluation of the antioxidant quality in the final product. The antioxidant potential of beer depends not only on the quality of the raw materials, but also on the technological process.

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