EXTRACTION OF GRAPPA AROMA BY SUPERCRITICAL CARBON DIOXIDE

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Grappa is an Italian spirit obtained by distillation of fermented grape marc. The use of the supercritical carbon dioxide extraction of grappa in order to obtain high quality grappa aroma extracts was investigated and preliminary results are presented.

Supprimé : with low alcoholic content

INTRODUCTION

Grappa is the spirituous beverage made in Italy from marcs distillation, which are the residual from wine-making. The EC regulation 1576/89 established the general production procedure of grappa and fixed common analytical composition limits [1]. Traditionally, grappa is a spirit ready to drink. Consequently, ageing in wood, absolutely necessary for other kinds of alcoholic beverages such as brandy, cognac, whisky, is for grappa more a demand of the market than a real need [2]. Grappa when purchased by the consumer is by analysis essentially a mixture of water, ethyl alcohol and volatile compounds in the approximate proportions 59 per cent, 40 per cent and 1 per cent by volume respectively. The content of volatile compounds in grappa is higher than in whisky (0.1%) [3] or wine−spirits such as brandy, Cognac and Armagnac (0.15-0.2%) [4]. The flavor of grappa originates from a number of sources, including the varietal origin of grape, the storage and fermentation times and methods of marcs, the distillation equipments and methods, and the substances released from the wooden barrel when ageing is performed. The bulk of aroma substances is mainly composed of higher alcohols, acetaldeyde and ethyl acetate, and, at a smaller level, of fatty acids and relevant ethyl esters, of higher alcohols esters of some others ethyl esters, and aryl and long-chain aliphatic alchols.

Supercritical fluid extraction (SFE) has been employed in dealcoholisation of alcoholic beverages with several patent application and references in bibliography [5, 6, 7]. However, the extraction of the aroma of spirits, with supercritical CO_2 has been scarcely studied [8, 9]. Carbon dioxide due to the low critical temperature (31°C) allows working at mild conditions and its gaseous standard state provides a solvent-free product. These properties lead to choose supercritical CO_2 in the extraction of fragrance compounds. Up to now it seems that SFE has never been applied to grappa.

The aim of this work was to establish a preliminary set of supercritical fluid extraction conditions through experimental design to obtain grappa aroma extracts to use as flavoring in food products (i.e. ice-cream, confectionery products, jams, etc.).

EXPERIMENTAL

The alcoholic beverage used was a commercial grappa at 40 % ethanol content. Carbon dioxide was supplied by Siad, Italy.

A laboratory scale plant was used. It consists of a solvent cooler Biorad Criotherm C-30 Control Unit, a solvent pump Isco-Model 260 D-Syringe Pump, a water bath , an extraction cell with an internal volume of 100 mL, and two separators filled with distilled water and maintained at a temperature of 0°C, in which the extract was collected and the solvent depressurized. For each experiment the extraction cell was filled with 50 mL of grappa and after sealing, the temperature was set at the desired value.

The ethanol content of grappa extracts were determined by electronic densimetry [10]
A Carlo Erba 8000 Top series gas chromatograph (CE Instruments, Milan, Italy) equipped with a flame ionization detector and split-splitless injector was used for the analyses of the following volatile compounds: 2-methyl-1-propanol, 1-hexanol, 1-heptanol, 1-nonanol, 2-phenylethanol, linalool, α-terpineol, nerol, ethyl lactate, ethyl butyrate, ethyl caproate, ethyl caprylate, ethyl pelargonate, ethyl laurate, benzaldehyde, diethylsuccinate, isoamyl acetate. The column used was a Supelcowax 10 column (Supelco, Milan, Italy) (30 m x 0.32mm i.d., film thickness 0.3 μm). The injection and detector temperature was 250°C. The GC oven temperature was programmed as follows: 60°C held for 8 min - then 8°C min^{-1} to 170°C, from 170°C to 240°C with rate 13°C min^{-1} (20 min isothermal). The carrier gas was helium with a flow rate of 1.2 mL/min, with a split ratio of 1:30. An aliquot of 5.0 mL of each sample was added with 100μL of n-dodecanol (3.94 g/L solution in ethanol) as internal standard. Identifications were made using a reference mixture of volatile compounds. Quantification was expressed both as mg/L. The repeatability of the HRGC analysis was tested using a sample analysed six times. The coefficients of variation were less than 8 % .

RESULTS AND DISCUSSION

Given the objectives of the study, the experimental runs were carried out by following the arrangement of a classical full factorial design for two-level factors (a 2^3 design) [11, 12] applied in the experimental domain reported in Table 1. The process parameters (the independent variables or factors) here considered were: *i*) the temperature (expressed in °C), *ii*) the pressure (expressed in bar), and *iii*) the CO_2 flow rate(expressed in kg/h).

Variable	Factor	Unit	Center	Step of variation
X_T	Temperature	°C	50	10
X_P	Pressure	bar	140	60
X_F	CO_2 Flow	kg/h	0.2	0.1

Table 1. Experimental domain for quantitative factor influence study.

Two experimental responses were considered: namely, the extraction yield of aroma compounds, defined by Perrut and Nunes da Ponte [7] as the ratio R between the amount of aroma contained in the extract and in the feed obtained by HRGC analysis of volatiles (η_H) and the extraction yield of ethanol expressed by the ratio between the quantity of ethanol in the extract and that contained in the feed (obtained by electronic densimetry (η_E)). Each response can be described by a linear model with two-factor interaction terms, such as:

$$\eta_i = \beta_0 + \beta_T X_T + \beta_P X_P + \beta_F X_F + \beta_{TP} X_T X_P + \beta_{TF} X_T X_F + \beta_{PF} X_P X_F \qquad (1)$$

Where: η_i is the theoretical response function; X_j are the coded variables; β_o, β_i and β_{ij} are the true model coefficients.
The observed response y_i for the i_{th} experimental run, indicating with e_i the error, is:

$$y_i = \eta_{i} + e_i \qquad (2)$$

The model coefficients β_o, β_i and β_{ij} are estimated by a least squares fitting of the model to the experimental results obtained in the design points. For the estimated values of these coefficients, the symbols b_o, b_i and b_{ij} will be used. The response values estimated by the model are designated as:

$$\hat{y}_i = b_0 + b_T X_T + b_P X_P + b_F X_F + b_{TP} X_T X_P + b_{TF} X_T X_F + b_{PF} X_P X_F \qquad (3)$$

In order to estimate the model here considered the full factorial design involving 8 experiments was chosen. For the estimation of the pure error variance three replicates at the centre of the experimental domain were included for the experimentation. The additional experimental points at the centre of the

domain enable the experimenter to compare the variance due to the lack of fit of the model to the pure error variance by performing a statistical test based on the *F*-test. The fitted model is considered adequate if the variance due to the lack of fit is not significantly different from the pure error variance [12].

The generation and the data treatment of the experimental design were performed by NEMRODW, Released 2000 [13].

Only the volatile compounds often present in those aroma fractions (significant in the odour of grappa) have been determined in the CO_2 - extract samples. The extracts were analyzed in order to evaluate the amount of volatile compounds extracted by means of the sum of their concentration, expressed as mg for mL of extract. Based on the two experimental responses measured for the eleven experiments performed by following the experimental plan it has been possible to assess the statistical significance of the mathematical model proposed in Equation 1. The coefficients of the model estimated by multilinear regression are graphically shown in Figure 1 (*a*) and (*b*). Here, the estimated values of the model coefficients are displayed using the Pareto Chart where the absolute value of each effect – ordered in increasing values - is shown. As it can be seen, for both the experimental responses under study it would seem that there is not an interaction effect to be considered important. Only determined main effects would be clearly active: the one associated to the factor "pressure" for response y_H and the ones related to factors "pressure" and "CO_2 flow" for response y_E. The Bayesian analysis of the model coefficients, that calculates *a posteriori* the probability that each of the effects is active, also confirms this interpretation as displayed in Figure 2 (*a*) and (*b*). Given the maximum probability of no effect being active (here, 42.3 % for response y_H and 20.78% for y_E), the solid boxes show the maximum and minimum probabilities to be active for each single effect [12].
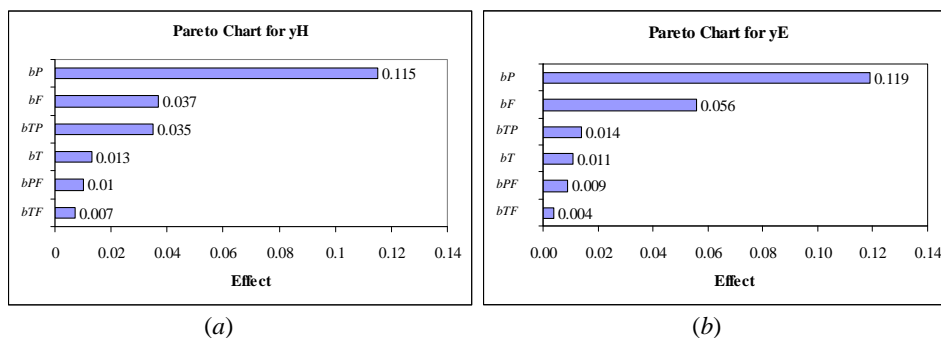

(*a*)   (*b*)

Figure 1. Pareto Charts of the main and interaction effects for y_H (*a*) and y_E (*b*).

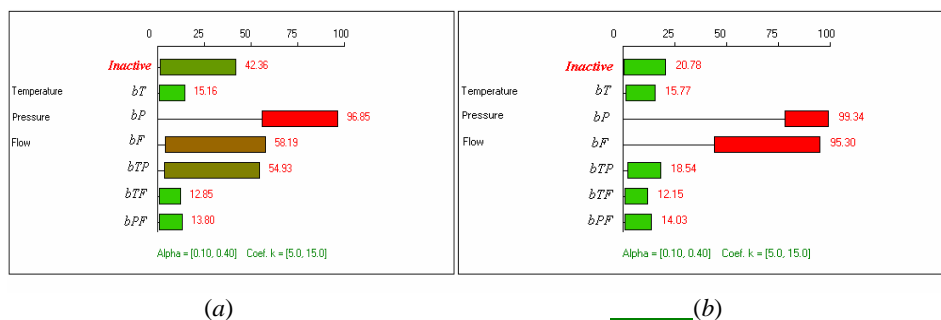(*a*)   (*b*)

Figure 2. Bayesian analysis of model coefficients for y_H (*a*) and y_E (*b*).

Also the assessment of the results based on the analysis of variance (ANOVA) of the regression pointed out that the proposed model in Eq.1 was inadequate for both experimental responses under study. In both cases, in fact, not only the lack of fit was statistically significant, but also the regression itself was not significant.

According to these results a simplified model for both experimental responses was therefore proposed:

$$\eta_i = \beta_0 + \beta_T X_T + \beta_P X_P + \beta_F X_F \tag{4}$$

corresponding to the following equations expressed in coded variables for y_H and for y_E, respectively:

$$\hat{y}_1 = 0.181 + 0.013 X_T + 0.115 X_P + 0.037 X_F \tag{5}$$

$$\hat{y}_2 = 0.225 + 0.011 X_T + 0.119 X_P + 0.056 X_F. \tag{6}$$

The analysis of variance for each of the fitted equations is reported in Table 2 Thanks to the replications of the experimental point corresponding to the centre of the experimental domain, it was possible to validate the postulated linear models given in Eqs. 5 and 6 by comparing the pure error sum of squares to the lack-o-fit sum of squares. In this case, the regression was significant for both responses, whereas the lack of fit was not statistically significant [12]. In this way, it was possible to accept the assumption made about the existence of a local planarity for y_H and y_E in the explored experimental region.

Source of variation	Sum of squares	d.f.	Mean square	F	Significance
(a)					
Regression	0.1183	3	0.0394	10.5393	0.547 **
Residuals	0.0262	7	0.0037		
Lack of fit	0.0249	5	0.0050	7.8708	11.7
Pure error	0.0013	2	0.0006		
Total	0.1445	10			
(b)					
Regression	0.1391	3	0.0464	21.7375	0.0635 ***
Residuals	0.0149	7	0.0021		
Lack of fit	0.0121	5	0.0024	1.6840	41.3
Pure error	0.0029	2	0.0014		
Total	0.1541	10			

Table 2. ANOVA of the regression for the experimental responses y_H (a) and y_E (b)

The contours diagrams and response surfaces of the fitted planar (or linear) models of Eqs. 5 and 6 are shown graphically in Figure 3 (a) and (b), respectively. Here, in order to assess graphically the joint effect of pressure and flow on the behaviour of the two responses under study, the temperature results fixed at T = 50 °C. From these graphs it is possible to see the increasing of both the experimental responses with the augmentation of pressure and CO_2 flow: in both cases the values of the contours lines increase from the lower left to the top right of the diagrams. Moving along each of the straight lines the same values for the calculated responses $\hat{y}$ by the fitted model here considered are obtained.
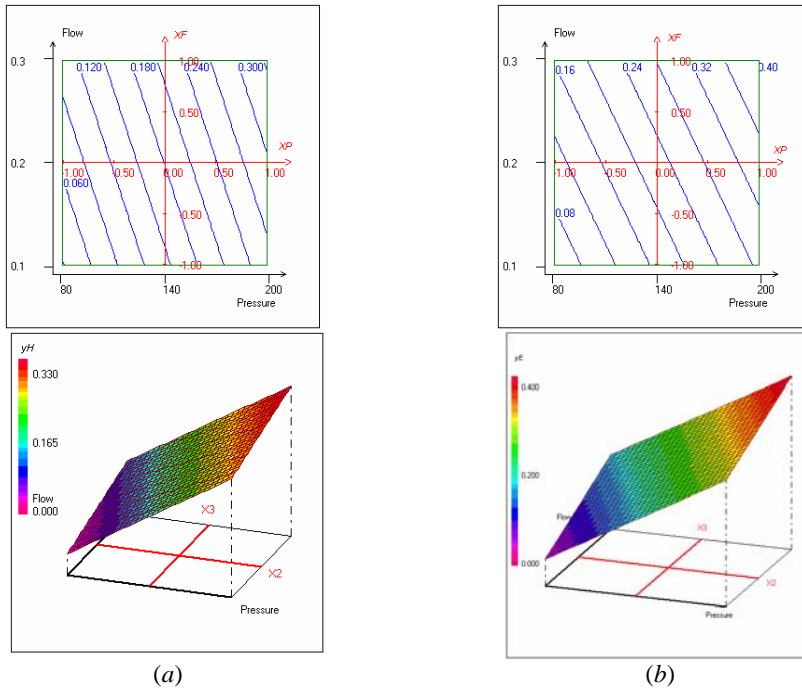
Figure 3. Contour plots and response surfaces representing y_H (*a*) and y_E (*b*) versus pressure and flow with the factor temperature fixed at 50° C.
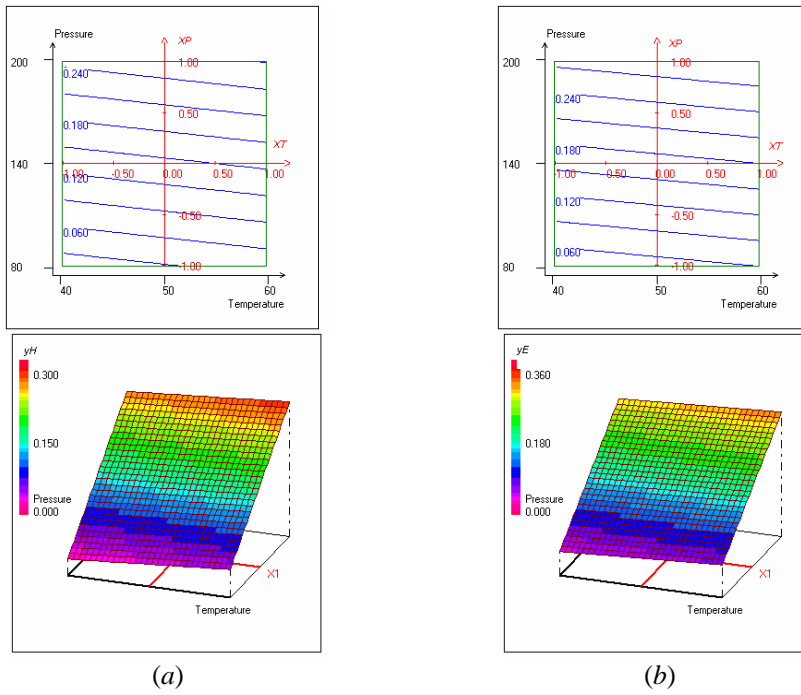

Figure 4. Contour plots and response surfaces representing y_H (*a*) and y_E (*b*) versus pressure and temperature with the factor flow fixed at 0.1 kg/h.

The contours diagrams and response surfaces representing y_H and y_E as a function of the pressure and temperature maintaining as a fixed factor the flow rate are reported in Figure 4 (*a*) and (*b*).

CONCLUSIONS

Due to the many variables affecting the performance of a process based on the use of supercritical fluids a first statistic study has been made and experimental data are collected on real matrices.
A preliminary set of supercritical fluid extraction data has been established in order to reduce the tests necessary to design the process related to extraction of aroma compounds from grappa.

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