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Characterization and antioxidant activity of nocino liqueur

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Abstract

Nocino is an after-dinner liqueur made with green, unripe walnuts, appreciated both for its appealing flavour and aroma, and for its properties as a tonic and digestive aid. There is a large number of recipes for the production of nocino and the industrial production coexists with preparations at home. The liqueur has never been scientifically investigated; thus the aim of this work was to examine various kinds of it. Three sets of samples were considered: Commercial brands, home-made nocino and, among the latter, samples made for years with the same recipe and aged for different times, up to 25 years. In the commercial products, the phenolic profiles and the antioxidant activities were widely variable. The antioxidant activity proved to be directly correlated with the content of total phenols, total tannins and non-tannin phenolics. Study of samples aged for different lengths of time showed that the characteristics considered remained substantially unchanged during the aging process.

Keywords: antioxidant; DPPH; Nocino liqueur; Phenols; Walnut

1. Introduction

Nocino is an after-dinner liqueur typical of Modena, but it is also produced in other regions of Italy. It was first made by the Celts and its use was spread in the areas under their control. In Italy it was apparently introduced by the Druids, coming from the area around Brittany.

The production of nocino is connected with St. John the Baptist's day, on June 24, the day of the end of celebrations for the summer solstice, on which the green, unripe walnuts used to prepare it are traditionally collected. Indeed, it was held in ancient times that the night of the summer solstice had purifying benefits on the earth and up on mankind: Herbs and nuts gathered on that night were strongly affected by this feature.

Nocino is appreciated for its bitter, sophisticated and appealing flavour, for its very special aroma, its dark brown colour and its properties as a tonic and digestive aid (Bergonzini, 1978). The latter properties, which are linked to its ability to increase the secretion of gastric juices, could derive from the high content of phenol-related substances found in the liqueur and thus from their antioxidant power.

There is a large number of recipes for the production of nocino. Since it is relatively easy to make, industrial production coexists with preparations at home. The production phases essentially consist in nut-harvesting, maceration/infusion in ethanol together with selected herbs, filtration, addition of a sugary syrup and aging.

The walnuts are picked unripe, when the husk is green and the endocarp is developed but not completely lignified. The nuts are carefully collected during the second half of June, because it is feared that any delay might have a negative effect on the quality of the liqueur. The whole nuts are washed, cut into quarters and left to steep in food-grade ethanol for one to three months, together with various herbs and spices, such as cloves, cinnamon, coriander, coffee beans and lemon zest, which create diversity in the formulations.

When the steeping process is over, the liquid is separated from the solid matter, which is sometimes soft-pressed. After filtration the infusion is diluted by adding a syrup made up of sugar and water, so that the alcohol

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content reaches around 40% v/v. In order to obtain the right balance for the aromatic substances contained in the nocino, it is left to age for a period ranging from three months to more than one year.

Although this liqueur is widely known and appreciated – and not only in Italy – it has never been scientifically investigated, and thus practically nothing can be found in the literature.

The aim of this work was to analytically examine samples of nocino found in the shops or prepared at home, and home-made samples produced using the same recipe between 1977 and 2000. The research was focussed on the phenolic fractions, which are the major feature of this liqueur, and on its antioxidant properties. Indeed, while many studies have been carried out on the antioxidant properties and the phenol content of wines (Burns et al., 2001; Burns et al., 2000; De Beer, Joubert, Gelderblom, & Manley, 2002; Kanner, Frankel, Granit, German, & Kinsella, 1994; Lopez Velez, Martinez Martinez, & Del Valle Ribes, 2003; Manzocco, Mastrocola, & Nicoli, 1999; Minussi et al., 2003; Teissedre et al., 1996; Vinson & Hontz, 1995), little research has been done on spirits (Da Porto, Calligaris, Celotti, & Nicoli, 2000; Duthie et al., 1998; Goldberg, Hoffman, Yang, & Soleas, 1999; Heinonen, Lehtonen, & Hopia, 1998; Trevithick, Chartrand, Wahlman, Hirst, & Trevithick, 1999).

2. Materials and methods

2.1. Materials

A total of 30 nocino samples were analysed during 2001. Ten were commercial samples, bought at supermarkets or wine shops; three were undated home-made samples; 17 were aged home-made samples, produced by one of the authors (Scaramuzzi) from walnuts picked up in the same geographical area within the period running from 1977 to 2000. In the case of the latter samples, the walnuts were always harvested around 24th of June and the liqueur was made using the same recipe: 40 walnuts, cut into quarters, were put in a glass container, together with 2 l of food-grade ethanol (95% v/v), a cinnamon (*Cinnamomum zeylanicum*) stick, ten cloves and the peel of a large lemon. The hermetically sealed uncoloured glass container was exposed to the sun for 40 days, periodically shaken, and finally filtered. A sugar syrup, prepared with 0.4 l of water and 1 kg of sucrose, was added to the filtered infusion. The liqueur was aged in one litre glass bottles kept in the dark at 15–18 °C.

Commercial samples were identified by a letter, home-made samples by HM followed by a number (from 1 to 3) and dated home-made samples were indicated by means of the production year.

2.2. Phenolic composition

Total phenols were determined using the Folin–Ciocalteau reagent according to Singleton and Rossi (1965). Non-tannin phenolics were analysed by precipitation with cinchonine sulphate (Peri & Pompei, 1971). Total tannins were calculated by the difference between total phenols and non-tannin phenolics. All results are expressed as milligrammes of catechin/l.

2.3. pH

pH was measured using a pH meter mod. pH M62 (Radiometer, Copenhagen, Denmark).

2.4. Sugar and ethanol contents

Sugar and ethanol contents of nocino samples were determined by HPLC, following the method reported by Yuan and Chen (1999). The method was modified as follows: The mobile phase consisted of 0.01 N sulphuric acid aqueous solution, and samples diluted 1:500 in the mobile phase were injected with a 50 μl loop. The equipment for the analysis consisted of an Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA), coupled with a pre-column Micro Guard Cation-H (Bio-Rad), and a 1037A Refractive Index detector (Hewlett–Packard, Milano, Italy). Chromatographic peaks were quantified by comparison with calibration curves of sucrose, glucose, fructose and ethanol standard solutions (all purchased from Aldrich Chemical Company, Inc., Milwaukee, WI, USA).

2.5. Antioxidant activity

Antioxidant activity was measured by two methods: The free radical 2,2-diphenyl-1-picryl-hydrazyl (DPPH·) method (Brand-Williams, Cuvelier, & Berset, 1994) and the electrochemical method suggested by Mannino, Brenna, Buratti, and Cosio (1998).

In the DPPH· method, a volume of 3.0 ml of 6.1×10^{-5} M DPPH· (2,2-diphenyl-1-picrylhydrazyl hydrate, free radical, 95%, Sigma–Aldrich Chemie GmbH, Steinheim, Germany) in methanol solution was used. The reaction was started by the addition of 30 μl of five different sample dilutions in 40% v/v ethanol. The reaction was carried out for each sample at 40°C and the bleaching of DPPH· was monitored at 515 nm (Uvidec-610, Jasco, Tokyo, Japan) for 30 min. Results are reported as $1/I_{50}$. I_{50} was defined as the amount of original nocino sample (in μl) required to lower the initial DPPH· concentration by 50% and was extrapolated from a dose–response curve.

In the electrochemical method, the mobile phase was modified to make it suitable for nocino samples: A solution of methanol and pH 4 acetate buffer (70:30 v/v)

with the addition of 2% w/v sodium perchlorate was used. The flow injection analysis system consisted of a Waters 510 pump (Waters Co., Milford, MA, USA), with a Rheodyne 7725 Valve (Rohnert Park, CA, USA), having a loop of 20 μl, and an EG&GPrinceton Applied Research (Princeton, NJ, USA) Model 400 thin-layer electrochemical detector equipped with a glassy carbon electrode, operating at a potential of +0.5 V, a reference (Ag/AgCl saturated) electrode and a platinum counter electrode. Data were recorded using an integrator D-7500 (Merck Hitachi, Ltd., Tokyo, Japan). A flow rate of 0.8 ml/min was employed. Prior to analysis samples were suitably diluted with mobile phase and filtered through a GS 0.22 μm membrane (Millipore Co., Milford, MA, USA). Results are expressed as trolox equivalent (TE), referred to as micromoles of trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, 97%, Sigma–Aldrich) in 100 ml, calculated with respect to a calibration curve of trolox standard solutions in a concentration range of 2–7 mg/l.

All the above analyses were carried out at least in duplicate, with the exception of the antioxidant power by the DPPH· method, which was performed only once.

2.6. Statistical analyses

The data obtained were statistically processed (Pearson correlation matrix, principal component analysis, and clustering analysis) by using Systat 5.03 for Windows (Systat, Inc., Evanston, IL, USA) and The Unscrambler 7.5 (CAMO ASA, Trondheim, Norway) software.

3. Results and discussion

Tables 1 and 2 show pH, sugar and ethanol contents of the commercial, undated home-made, and aged home-made samples of nocino.

Apart from one commercial sample (L) with a pH of 5.4, the pH values of all other samples fell between 4.0 and 4.9. The pH was not affected by the aging process, as can be seen from Table 2.

The average total sugar content was slightly over 30 g/100 ml. The commercial and home-made aged samples had a lower intersample variability (approximately 11–13%), whereas the variation was considerably higher for the three undated home-made samples (approximately 30%), due to the different recipes used.

HPLC analysis clearly showed that, in addition to sucrose, glucose and fructose were present in all the samples in very similar concentrations. This was an expected finding since, in view of the fact that this is an acid product, these sugars can derive from a partial hydrolysis of the sucrose. Indeed, observing the samples from the different years (Table 2), in the production of which exclusively sucrose was used, it can be noted that there is a low content of monosaccharides in the younger samples and that it gradually builds up in the older ones. In the latter, glucose and fructose together total about one third of total sugars, with a maximum of almost 50% in the 1980 sample, which has the lowest pH.

The profile of the commercial samples (Table 1) is more difficult to interpret, as the amount of aging is not always indicated on the bottle and one finds the generic

Table 1
pH, sugar content and ethanol concentration (average ± SD) of commercial and undated home-made nocino samples

Sample	pH	Sucrose (g/100 ml)	Glucose (g/100 ml)	Fructose (g/100 ml)	Total sugars (g/100 ml)	Ethanol (%v/v)
A	4.8	18.3 ± 0.1	11.3 ± 0.1	11.2 ± 0.1	40.8	51.3 ± 0.1
B	4.6	31.3 ± 3.1	1.4 ± 0.1	1.7 ± 0.1	37.4	50.7 ± 0.4
C	4.8	27.0 ± 0.1	1.1 ± 0.1	1.3 ± 0.1	29.4	46.5 ± 0.1
D	4.3	22.3 ± 0.1	6.9 ± 0.1	6.4 ± 0.1	35.6	47.7 ± 0.3
E	4.3	19.1 ± 0.1	4.6 ± 0.1	4.2 ± 0.1	27.9	48.4 ± 0.1
F	4.3	24.5 ± 0.1	3.2 ± 0.1	2.8 ± 0.1	30.5	47.9 ± 0.1
G	4.4	24.2 ± 0.1	3.3 ± 0.1	2.7 ± 0.1	30.2	49.1 ± 0.6
H	4.0	23.7 ± 0.2	4.0 ± 0.1	3.4 ± 0.1	31.1	38.3 ± 0.2
I	4.8	26.7 ± 0.1	0.5 ± 0.1	0.7 ± 0.1	27.9	50.3 ± 0.1
L	5.4	31.6 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	32.3	50.1 ± 0.2
Mean	4.6	24.9	3.7	3.5	32.3	48.0
CV%	–	18.0	92.5	93.3	13.3	7.8
HM1	4.8	14.4 ± 0.1	7.5 ± 0.1	7.6 ± 0.1	29.5	37.9 ± 0.1
HM2	4.1	13.7 ± 0.1	3.8 ± 0.1	3.8 ± 0.1	21.3	36.7 ± 0.1
HM3	4.6	36.1 ± 0.1	1.3 ± 0.1	1.6 ± 0.1	39.0	53.3 ± 0.1
Mean	4.5	21.4	4.2	4.3	29.9	42.6
CV%	–	59.5	74.3	70.0	29.6	21.7

CV = coefficient of variation.

Table 2
pH, sugar content and ethanol concentration (average ± SD) of aged home-made nocino samples

Sample	pH	Sucrose (g/100 ml)	Glucose (g/100 ml)	Fructose (g/100 ml)	Total sugars (g/100 ml)	Ethanol (%v/v)
1977	4.6	23.5 ± 0.1	7.1 ± 0.1	7.0 ± 0.1	37.6	50.2 ± 0.1
1978	4.5	24.7 ± 0.1	6.9 ± 0.1	6.4 ± 0.1	38.0	52.1 ± 0.1
1980	4.3	15.8 ± 0.1	7.7 ± 0.1	7.3 ± 0.1	30.8	40.5 ± 0.1
1982	4.5	20.0 ± 0.4	5.6 ± 0.2	5.4 ± 0.3	31.0	39.0 ± 0.1
1983	4.5	23.8 ± 0.1	5.9 ± 0.1	5.3 ± 0.1	35.0	34.5 ± 0.1
1985	4.8	22.8 ± 0.1	3.7 ± 0.1	3.4 ± 0.1	29.9	38.4 ± 0.1
1986	4.4	23.8 ± 0.1	4.6 ± 0.1	4.1 ± 0.1	32.5	32.0 ± 0.5
1988	4.4	22.4 ± 0.1	3.6 ± 0.1	3.1 ± 0.1	29.1	42.1 ± 0.1
1990	4.5	20.3 ± 0.1	3.8 ± 0.1	3.5 ± 0.1	27.6	37.9 ± 1.0
1991	4.7	20.8 ± 0.2	4.5 ± 0.1	4.1 ± 0.2	29.4	40.8 ± 0.1
1993	4.9	27.7 ± 0.3	1.4 ± 0.1	1.6 ± 0.1	30.7	36.9 ± 1.8
1995	4.4	24.0 ± 0.3	3.4 ± 0.1	3.0 ± 0.1	30.4	36.3 ± 0.7
1996	4.8	27.6 ± 0.5	1.5 ± 0.1	2.0 ± 0.1	31.1	35.8 ± 0.3
1997	4.6	22.9 ± 0.1	2.6 ± 0.1	2.3 ± 0.1	27.8	37.0 ± 0.2
1998	4.7	25.8 ± 0.1	1.2 ± 0.1	1.4 ± 0.1	28.4	29.3 ± 0.9
1999	4.8	24.2 ± 0.4	0.7 ± 0.1	0.9 ± 0.1	25.8	37.8 ± 0.3
2000	4.9	30.5 ± 0.2	0.8 ± 0.1	1.1 ± 0.1	32.4	32.3 ± 0.2
Mean	4.6	23.6	3.8	3.6	31.0	38.4
CV%	4.1	14.3	59.4	56.2	10.7	15.2

CV = coefficient of variation.

term "sugar" in the list of ingredients. For example, in commercial sample A, over 50% of total sugars were glucose and fructose. However, this sample had a relatively high pH (4.8) and underwent limited aging, as it was produced in 2000. It is thus quite clear that not only sucrose was added to that sample. In samples C and I, which had also been aged for a short time and had a pH of 4.8, the two monosaccharides accounted for, respectively, 8% and 4% of the total sugars; it can therefore be supposed that only sucrose was added to these products. The same goes for sample L, which had the lowest glucose and fructose contents, equal to approximately 2% of the total sugars, and the highest pH value.

Although sample HM1 had been produced using only sucrose and had a pH of 4.8, it proved to have a concentration of monosaccharides reaching approximately 50% of total sugars. Since we have no precise information regarding the year of production, it can be hypothesized that it was not a recent sample and that the liqueur had been kept at a relatively high temperature, promoting the hydrolysis of sucrose.

As regards the average ethanol content (Tables 1 and 2), the three groups of liqueurs showed only slight differences. The average content of the commercial samples was 48.0% v/v, with a low coefficient of variation (7.8%). The home-made samples tested had a similar alcohol content (42.6% v/v), but a relatively high coefficient of intersample variation (21.7%), due to sample HM3 which has a high alcohol content compared with the other two. The dated nocino had an average alcohol content of 38.4% v/v, with an intersample variability falling between that of the other two groups (15.2%). For these samples the reason for the relatively high coefficient of variation was the presence of the 1977 and 1978 samples, which had the highest concentrations of alcohol (approx. 50% v/v), and sample 1998, which had an alcohol content lower than 30% v/v.

In Tables 3 and 4, the phenolic profiles of the samples of nocino analyzed are reported.

The total phenol content of the thirty samples varied from 239 to 3884 mg/l. These values are comparable to those reported for red wines (507–3205 mg/l) by Mannino et al. (1998). Two commercial samples (L and

Table 3
Contents (average ± SD) of total phenols, non-tannin phenolics, and total tannins in commercial and undated home-made nocino samples

Sample	Total phenols (mg catechin/l)	Non-tannin phenolics (mg catechin/l)	Total tannins (mg catechin/l)
A	3884 ± 34	2043 ± 42	1841
B	3248 ± 34	1604 ± 20	1644
C	3130 ± 11	1296 ± 26	1834
D	1964 ± 25	1111 ± 36	853
E	1894 ± 12	895 ± 27	999
F	1727 ± 25	780 ± 9	947
G	1634 ± 24	823 ± 27	811
H	1490 ± 2	642 ± 23	848
I	474 ± 9	274 ± 17	200
L	239 ± 11	141 ± 35	98
Mean	1968	961	1008
CV%	59	60	61
HM1	3017 ± 75	1473 ± 36	1544
HM2	950 ± 4	541 ± 58	409
HM3	678 ± 1	509 ± 10	169
Mean	1548	841	707
CV%	83	65	104

CV = coefficient of variation.

Table 4
Contents (average ± SD) of total phenols, non-tannin phenolics and total tannins in aged home-made nocino samples

Sample	Total phenols (mg catechin/l)	Non-tannin phenolics (mg catechin/l)	Total tannins (mg catechin/l)
1977	2701 ± 6	1166 ± 18	1535
1978	2207 ± 3	973 ± 8	1234
1980	2181 ± 22	1122 ± 35	1059
1982	2510 ± 1	1135 ± 15	1375
1983	2728 ± 6	1389 ± 51	1339
1985	3017 ± 42	1446 ± 8	1571
1986	2001 ± 26	1600 ± 21	401
1988	2934 ± 62	1411 ± 10	1523
1990	2387 ± 45	1149 ± 1	1238
1991	2592 ± 6	2073 ± 5	519
1993	3330 ± 10	1664 ± 18	1666
1995	2401 ± 20	1062 ± 21	1339
1996	3522 ± 5	1755 ± 3	1767
1997	3021 ± 19	1322 ± 66	1699
1998	3451 ± 32	1508 ± 22	1943
1999	2746 ± 28	1312 ± 5	1434
2000	3166 ± 150	1410 ± 43	1756
Mean	2759	1382	1376
CV%	16	20	30

CV = coefficient of variation.

I) proved to have extremely low values, totalling 239 and 474 mg/l, respectively. From the information given on the label, there is no reason to suppose that these two samples had a composition differing from the others: In the case of sample L, "tincture of walnut husks" is declared to be amongst the ingredients, while for sample I we find "infusion of walnut husks". Since there was no reason for excluding these two samples, in Table 3 we find an average phenolic content of 1968 mg/l, with a high coefficient of variation (59%).

Two of the home-made samples analyzed (HM2 and HM3) contain rather low levels of total phenols (950 mg/l and 678 mg/l, respectively), probably owing to the small quantity of walnuts used to make the liqueur. Actually sample HM2 was made with only 10 walnuts in one litre of food-grade ethanol; whereas sample HM3 was produced with 20 walnuts in one litre ethanol, but they were gathered riper than they traditionally are and, as the endocarp had hardened, only the husks were used instead of the whole nut.

The dated samples had an average phenol content of 2758 mg/l, with a limited intersample variability (16%), as they had always been prepared following the same recipe and, starting from 1996, with walnuts gathered from the same tree. A general observation relating to samples of dated nocino is that total levels of total phenols do not decrease (for instance by insolubilization) upon aging the liqueur.

The content of tannins accounted for slightly over 50% of total phenols in the commercial samples and in the home-made dated ones (respectively, 51% and 53%). Amongst the undated home-made samples analyzed, the nocino HM1 gave the same results as the other two groups, whereas HM2 and HM3 had a lower percentage of tannins with respect to non-tannin phenolics. In particular, in sample HM3, the tannins amounted to only 23% of total phenols. As explained above, for the production of this sample only the husks were used instead of the whole nut. This suggests that the phenolic profile of the husks is different from that of the whole unripe nut.

Total tannin content proved to be strongly correlated ($P > 99.9\%$) with that of total phenols (Fig. 1).

Tables 5 and 6 show the results relating to the analysis of antioxidant power in vitro of the samples of nocino, performed using the DPPH· ($1/I_{50}$) and electrochemical (TE) methods. The data obtained by the DPPH· method were substantially confirmed by the electrochemical method. Indeed, even if the results are expressed in a different manner, the relationships

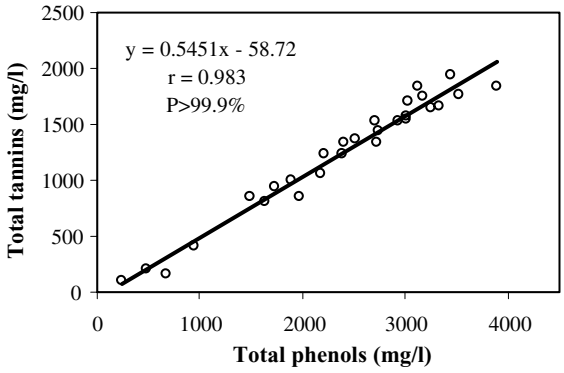

Fig. 1. Correlation between total phenols and total tannins.

Table 5
Antioxidant activity (average ± SD) of commercial and undated home-made nocino samples

Sample	$1/I_{50}$ (μl^{-1})	TE (μmol trolox/100 ml)
A	0.50	206 ± 66.0
B	0.50	238 ± 29.1
C	0.40	169 ± 40.3
D	0.26	117 ± 3.7
E	0.25	117 ± 1.8
F	0.21	85.7 ± 16.4
G	0.21	95.9 ± 8.7
H	0.14	73.8 ± 17.4
I	0.05	17.8 ± 1.9
L	0.01	1.9 ± 0.7
Mean	0.25	112.1
CV%	67.23	67.3
HM1	0.50	155 ± 40.2
HM2	0.12	53.4 ± 17.1
HM3	0.09	33.9 ± 5.3
Mean	0.24	80.9
CV%	96.57	80.7

CV = coefficient of variation.

Table 6
Antioxidant activity (average ± SD) of aged home-made nocino samples

Sample	$1/I_{50}$ (μL^{-1})	TE (μmol trolox/100 ml)
1977	0.38	201 ± 56.6
1978	0.32	n.d.
1980	0.31	153 ± 1.2
1982	0.37	204 ± 42.7
1983	0.42	218 ± 27.9
1985	0.53	n.d.
1986	0.30	168 ± 53.7
1988	0.45	202 ± 7.4
1990	0.34	182 ± 18.6
1991	0.48	n.d.
1993	0.59	n.d.
1995	0.31	n.d.
1996	0.59	246 ± 73.8
1997	0.48	n.d.
1998	0.53	n.d.
1999	0.45	221 ± 51.1
2000	0.50	n.d.
Mean	0.43	199.5
CV%	22.68	14.2

CV, coefficient of variation; n.d., not determined.

between the different samples are confirmed and the two methods prove to be highly correlated ($P > 99.9\%$), as shown by Fig. 2. Both methods therefore proved suitable for analysis of the antioxidant powers of nocino.

From Table 5, three classes of commercial products can be identified: One with a high antioxidant power (samples A, B and C), one with a low antioxidant power (samples I and L) and one with intermediate values (samples D, E, F, G and H). The same kind of classification is also possible on the basis of total phenol content, which indeed proved to be highly correlated with antioxidant activity (Fig. 3). This correlation has already been observed for red, white, and rosé wines (Brenna & Pagliarini, 2001; Sanchez-Moreno, Larrauri, & Saura-Calixto, 1999; Simonetti, Pietta, & Testolin, 1997) and for cognacs (Da Porto et al., 2000). The antioxidant power measured by the DPPH· method also proved to have a strong correlation ($P > 99.9\%$) with the other classes of phenolic compounds.

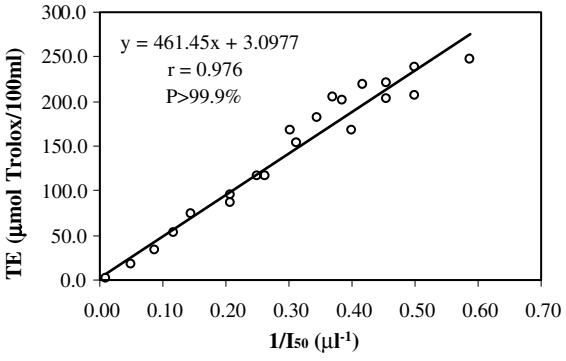

Fig. 2. Correlation between DPPH· ($1/I_{50}$) and electrochemical (TE) methods for the analysis of antioxidant power.

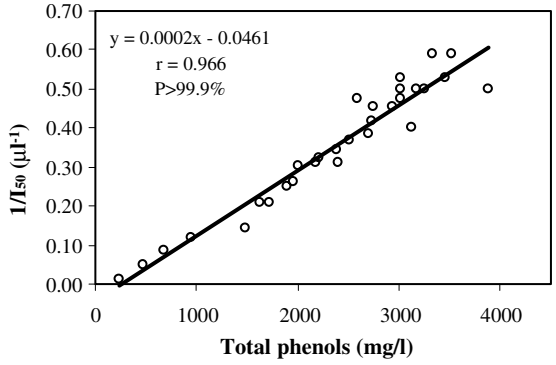

Fig. 3. Correlation between total phenols and antioxidant activity.

The importance of antioxidant activity in the prevention of illnesses typical of old age led us to assign a level of "quality" to the three classes of product identified. In particular, a recent work by Anderson et al. (2001) showed that the polyphenols contained in walnuts effectively inhibit oxidation of lipoprotein LDL in blood plasma.

The three home-made samples can be positioned within the three classes already indicated for commercial products. In this case, the differences in antioxidant activity surely derive from the different recipes followed.

As regards the samples produced in different years from the dated home-made group, it can be seen from Table 6 that there is no definite decrease in antioxidant power in the samples undergoing the longest aging process, even if the samples produced between 1996 and 2000 have a higher antioxidant activity than the samples produced between 1977 and 1983. However, as in the case of total phenol content, this might depend on the differing degrees of ripeness of the walnuts when they were gathered, rather than on the length of aging. In any case, all these samples prove to have a high antioxidant power, comparable with that of commercial samples A, B and C.

All the data at our disposal were used to carry out the principal components analysis (PCA), in order to bring out the variables that most influence the variability of the samples. Table 7 contains the loadings for all variables for the first and second components, which were used to select the most important uncorrelated variables. As regards phenol composition and antioxidant activity, since all these characteristics proved to be intercorrelated, the variable "total phenols" was chosen, both because it has a greater influence on the first component, and because it is the simplest analysis to carry out. For the other composition characteristics, the variable ethanol – which is important as regards the first principal component (PC1) – was chosen, together with pH, sucrose and glucose, which reveal high loadings for the second principal component (PC2). After the choice of variables, PCA was again performed, using the data

Table 7
Loadings for all variables for the first and second principal components

Variable	PC1	PC2
pH	0.029	0.402
Sucrose	−0.135	0.470
Glucose	0.155	−0.531
Fructose	0.168	−0.515
Ethanol	−0.215	−0.115
Total phenols	0.449	0.086
Non-tannin phenolics	0.423	0.034
Total tannins	0.398	0.116
$1/I_{50}$	0.441	0.142
TE	0.387	0.117

from all the samples. The first two components taken together explain 71% of the total variance. Negative PC2 values correspond to the samples with a higher total phenols content, which are also those with a greater antioxidant power. Positive PC1 values correspond to the samples taken from the earliest years, which have a higher glucose content; vice-versa, negative values correspond to the younger samples, characterized by a low glucose content.

The variables chosen for PCA were also utilized to carry out a cluster analysis using the *k*-means method and forcing the allocation of samples to three groups. In Table 8 the *F*-ratio values obtained for each variable are reported; from these it can be noticed that total phenols (and therefore antioxidant activity) are the compounds which best permit the classification of nocino liqueurs. The three groups identified from the cluster analysis are shown in Table 9.

Table 8
F-ratio values obtained for each variable by cluster analysis

Variable	*F*-ratio
Total phenols	95.200
pH	8.024
Sucrose	1.656
Glucose	2.827
Ethanol	2.112

Table 9
Allocation of nocino samples in the three groups identified from the cluster analysis

Cluster	Samples
1	I, L HM2, HM3
2	D, E, F, G, H 1978, 1980, 1982, 1986, 1990, 1995
3	A, B, C HM1, 1977, 1983, 1985, 1988, 1991, 1993, 1996, 1997, 1998, 1999, 2000

4. Conclusions

This work has allowed us to acquire some knowledge as to the phenol composition and antioxidant activities of nocino, a liqueur on which no scientific research had previously been done. Its antioxidant activity proved to be directly correlated with its total phenol content, together with its content in non-tannin phenolics and total tannins. The study of samples aged for different lengths of time showed that the characteristics considered remain substantially unchanged during the aging process, even if this lasts many years. It has also come to our notice that liqueurs varying considerably as regards phenolic profiles and antioxidant activity can be found in the shops under the name "nocino".

The results obtained suggest that it might be interesting to study the technological variables of the production process, in order to gain information which may prove useful in order to standardize the most interesting characteristics of nocino.

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