1 **PROFILING OF DIFFERENT BIOACTIVE COMPOUNDS IN**

2 **FUNCTIONAL DRINKS BY HPLC[★]**

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10 11 12 **Abstract**

13 In the present work, an HPLC method is proposed to simultaneously detect and quantify
14 water and fat soluble vitamins, phenolic compounds, carotenoids and chlorophylls in a
15 single run, by using an ultradeactivated C18 column and gradient separation using
16 trifluoroacetic acid, water and methanol. It is shown that the HPLC method provides
17 baseline separation of all these compounds with good resolution values in 40 min.
18 Moreover, other figures of merit of the method show a good linear response and low
19 detection limits for all the compounds considered in the present study. Furthermore, the
20 usefulness of this method is demonstrated via its successful application to the analysis
21 of different beverages from different natural origin (orange, strawberry, apple, peach
22 pineapple, plum and blackcurrant juices, soybean milk, beers) without the need of any
23 previous sample preparation. A good correlation is also found by comparing the total
24 phenol content (measured by Folin-Ciocalteu method) with the sum of total phenolic
25 compounds obtained using the proposed HPLC method. By using statistical tools, the
26 main compounds associated with antioxidant activity of the extracts (measured by
27 DPPH radical scavenging) were assessed.

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29 Keywords: functional foods, water soluble vitamins, fat soluble vitamins, phenolic
30 compounds, HPLC-DAD, Folin-Ciocalteu, Antioxidant activity, PLS.

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1.- Introduction

Nowadays, the consumers' demand of new functional foods including those enriched with vitamins is clearly rising. Usually mixtures of different vitamins are added to food products, whose complex composition make the analysis and quantification of vitamins and other biologically active compounds difficult. The analysis of such a mixture by conventional methods usually involves tedious and time-consuming procedures, since it requires the use of numerous analytical and/or sample preparation protocols.

Vitamins are a structurally heterogeneous group of essential food constituents that are required in small amounts for normal growth and maintenance of human health. They are generally divided in two main groups: lipid- and water-soluble vitamins. Until recently [1], this classification has been used to determine the type of analytical method applied, being the most common approach the analysis of each vitamin individually (mostly by using HPLC) or even a multiple water- or fat- soluble vitamins determination [2-8]. Nevertheless, some methods have been developed that combine the analysis of water and fat soluble vitamins in one step such as using TLC [9] or HPLC [1]. Thus, in the work developed by Kledjus et al. [1], the simultaneous determination of water- and fat-soluble vitamins in pharmaceutical preparations and food samples was achieved after a careful optimisation of all the factors influencing the separation such as type of column, pH, temperature, gradient elution and so on.

Even considering the enormous interest of vitamins as micronutrients in food samples, there exist other compounds with known biological activity that can, at the end, have a similar or even greater contribution in the functional activity of a food and/or beverage. These compounds (e.g., polyphenols, carotenoids, chlorophylls, etc) can play an important role in the associated bioactivity of a food product and, therefore, their determination can also be of great importance for food and nutrition studies. These food components have the common property of being labile and so degraded by light, heat or oxygen, also they are extremely complex, with dozens of very similar components or isomers which makes its separation extremely difficult as next discussed.

The basic feature of all polyphenols is the presence of one or more hydroxylated aromatic rings, which seemed to be, in fact, responsible for their properties as radical

scavengers [10-16]. Some analytical strategies have been reported [17] for the determination of polyphenols. Their presence may interfere in the analysis of vitamins, so usually a clean up step is needed, namely solid-phase extraction, liquid extraction, etc. In spite of this difficulty, some analytical methods based on HPLC have already been developed to quantify some phenolic compounds along with some vitamins [18, 19]. Carotenoids can have many physiological functions. Given their structure, carotenoids are efficient free-radical scavengers, and they enhance the immune system [20-26]. On the other hand, some biological effects have also been associated to chlorophylls [20]. The analysis of carotenoids and chlorophylls is commonly done by HPLC and several methods have been developed to simultaneously determine these type of compounds [27-29].

The goal of this work was to develop an HPLC method (using diode array detection) for the simultaneous separation, detection and quantification of water- and fat-soluble vitamins, phenolic compounds, chlorophylls and carotenes in a single run. The developed method should allow obtaining a “bioactive profile” of different food matrices. The usefulness of the HPLC method was corroborated through its application to the analysis of several commercial drinks, some of them fortified with vitamins. Moreover, based on statistical analysis, it was possible to associate the antioxidant activity of the functional beverages to their content on some target compounds.

2.- Experimental

2.1.- Chemicals and reagents

Trifluoroacetic acid (TFA) was purchased from Scharlau Chemie (Barcelona, Spain). All vitamins, fat- (α -tocopherol, menadione, cholecalciferol, and α -tocopherol succinate) and water soluble vitamins (thiamine, niacine, folic acid, pantothenic acid, ascorbic acid, riboflavin, cobalamine) were from Accustandard (New Haven, CT, USA). All standard phenolic compounds (caffeic acid, coumaric acid, catechin, catechol, cyanidine chloride, gallic acid, genistein, hesperidin and quercetin) were purchased from Extrasynthese (Genay, France) and chlorophylls and zeaxanthin from DHI (Hørsholm, Denmark). Milli-Q water was obtained using a Millipore purification system (Millipore), methanol of HPLC grade was purchased from LabScan (Dublin, Ireland).

Standards solutions (3 mg/ml) were prepared using solutions of 0.01% TFA in water (polar vitamins, caffeic acid and gallic acid), methanol:water (the rest of phenolic compounds) or methanol (pigments and fat soluble vitamins) and stored in darkness under freezing conditions (<-20°C).

2.2.- HPLC conditions

The method developed in the present work was modified from Klejdus et al [1]. Analytes were separated on a ACE-100Å C18 (150 mm × 4.6 mm, 3 µm particle size) (Advanced Chromatographic Technologies, Aberdeen, UK) in a single run using combined isocratic and linear gradient elution with a mobile phase consisting of 0.010% trifluoroacetic acid (solvent A, pH=3.9) and methanol (solvent B) at the flow rate of 0.7 ml/min using an Agilent 1100 HPLC apparatus. The gradient profile (A%:B%) started at 96:4 and increased up to 95:5 in the first 4 min, then linearly changed up to 2:98 during the next 7 min, then it was constant in the next 6 min, increased up to 0:100 in 2 min, then constant until a total analysis time of 38 min and finally linearly increased up to 96:4 to reach initial conditions. Different wavelengths were selected for monitoring the HPLC separation: 280, 350, 450 and 660 nm.

Direct injection of pure vitamin standards was done by dissolving them in TFA 0.01% or 100% methanol depending on the polarity of the vitamin. For polyphenols, all samples were injected dissolved in hydromethanolic solutions.

2.3.- Food samples

Different commercial drinks enriched in vitamins were analyzed along with two beer samples, namely:

- a) Orange Juice: sterilized canned orange juice.
- b) Soy-orange drink: mixture of orange juice with “soybean milk” with vitamin E.
- c) Strawberry Yogurt: drinkable yogurt with strawberry juice.
- d) Multifruit-Milk Juice: partially fermented milk with *Bifidus*, mixed with peach, apple and orange juices enriched in vitamins A, C and E.

- e) Antioxidant Juices: mixture of juices rich in antioxidants, two flavours were analyzed: orange+raspberry+acerola (Antiox 1) and pineapple+plum+blackcurrant (Antiox 2)
- f) Beer: beer pilsner type with 5.4% alcohol, as declared in label
- g) Light Beer: light beer pilsner type with 0.0% alcohol, as declared in label.
- h) Fruit purée: mixture of different fruit purees and juices, include apple, carrot, pineapple, passion fruit and sweet corn.

The only clean up used was filtering through a 0.45 µm nylon syringe filter (Symta, Madrid, Spain). Fruity samples were diluted 1:1 with water. Beer was degassed using an ultrasonic bath to avoid CO₂ to enter into the chromatographic system.

2.4.- Antioxidant activity

The antioxidant activity of beverages was measured using the DPPH method. All the measurements were carried out according to a procedure previously used in juices by Klimczak et al. [30]. Briefly, 0.1 mL of juice sample (filtered using syringe nylon filter of 0.45 µm and diluted with distilled water 10:1) was added to 2.46 mL of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH[•]; 0.025 g·L⁻¹ in 50% ethanol) and mixed by vortex for 1 min. The absorbance of the samples was measured at 515 nm every 5 min for 30 min using the spectrophotometer Genesys (Thermo Finnigan, Madrid, Spain). For each sample, three separate determinations were carried out. The percentage of DPPH, which was scavenged (%DPPH_{sc}) was calculated as in [30].

2.5.- Total polyphenol content

Total polyphenols were determined using the Folin–Ciocalteu method [31]. Previously, a precipitation of proteins was carried out using HCl 1,48% and centrifugation. The method consists of mixing 2 ml of Na₂CO₃ (2% w/v in water) with 100 µl of the supernatant; after 3 minutes of reaction, 50 µl of Folin reagent (Scharlau Chimie, Barcelona, Spain) was added. After 30 min of incubation the absorbance was measured at 750 nm. The standard calibration curves were prepared using pure caffeic

acid (Sigma, Madrid, Spain) and caffeic acid + BSA (Bovine serum albumin, Sigma, Madrid, Spain). The results were expressed in mg of caffeic acid equivalents per liter of beverage.

2.6.- Statistical analysis

In order to correlate the antioxidant activity found in the beverages with the different compounds detected using the HPLC method, two statistical strategies were used, namely: Partial Least Squares (PLS) regression and Forward Stepwise Multiple Linear Regression (FSMLR), were tested to predict the antioxidant activity of the samples using the complete profile of components of HPLC analysis or considering only a few selected components, respectively. The STATISTICA program for Windows, version 7.1, was used for data processing (StatSoft, Tulsa, OK, USA (2006), www.statsoft.com).

3.- Results

As mentioned, the method developed in the present work has been optimized based on the work done by Kledjus et al [1]; the most important modifications of the method were in the phase gradient and in the selected analytical column. The main challenge encountered for the simultaneous determination of water- and fat-soluble vitamins, phenolic compounds, carotenoids and chlorophylls was the different physico-chemical properties of the compounds tested. As mentioned under Experimental section, 5 water soluble vitamins and 2 forms of fat-soluble vitamins were used as standards along with 8 phenolic compounds (corresponding to 7 families of polyphenols), zeaxanthin and chlorophyll-a. To be able to separate such a complex mixture composed of polar and non polar analytes in a single run, the selection of the chromatographic column, among other parameters, is crucial. Therefore, an ultra inert HPLC column whose main features are high efficiency and low silanol activity was selected in the present work. Besides, this column allows working under highly acidic conditions without deterioration of the separation achieved.

Another important challenge is the selection of the most suitable wavelength for simultaneous detection and quantification of all the compounds. In this method, the use

of a diode array detector allowed us to select 4 different wavelengths according to the spectral characteristics of all compounds tested. Thus, 280 nm was chosen as the most suitable detection wavelength for simultaneous vitamin-polyphenol-pigment determination [1], while 350 nm was selected for flavones, flavonols, chalcones and aurones, 450 nm for carotenoids, chlorophylls and anthocyanins, and 660 nm for chlorophylls.

By optimizing the separation conditions it was possible to achieve the elution of all the standards in a single run with a good resolution. As can be seen in Figure 1, water-soluble vitamins elute from minutes 2 to 10 while phenolics elute between min 6 and 14. Fat-soluble vitamins as well as photosynthetic pigments are eluted in the last part of the chromatogram, that is, after 20 minutes. Interestingly, with the information provided by both, retention time and spectra of each compound, a profile of the bioactive components of a food sample can be obtained using this method.

3.1.- Figures of merit

The HPLC method was evaluated in terms of linear response range, limit of detection (LOD), limit of quantification (LOQ) and precision, obtaining the results shown in Table 1. External calibration was used to perform the quantification of the compounds.

Moreover, $RSD_{n=3}$ values obtained for intra-day repeatability were better than 3.4% and 1.9% for peak areas and migration time, respectively. On the other hand, RSD values obtained for three different days were better than 6.7% and 2.4% for peak areas and migration time, respectively, assuring an adequate quantitation of the analytes.

As can be seen in Table 1, all studied standards provide a good linear response. Thus, the widest linear response was provided by pyridoxine, while the anthocyanin (cyanidine chloride) provide the narrowest linear response and the worse linear adjustment (R^2). The limit of detection (LOD) and limit of quantification (LOQ) were calculated according to IUPAC [32]:

$$x_L = \overline{x_{bl}} + k \times S_{bl} \quad \text{Equation 2}$$

where: x_L is the area limit, $\overline{x_{bl}}$ is the mean of the blank areas, k is the numerical factor used ($k=3$ for limit of detection LOD, $k=10$ for limit of quantification LOQ) and S_{bl} is the standard deviation of blanks.

All calibration curves were obtained using 280 nm despite it is not the maximum wavelength of absorbance for all compounds, i.e., zeaxanthin and chlorophyll. However, the selection of a single wavelength to quantify at the same time all the compounds present in functional drinks can be useful for both, routine analysis and profiling of bioactive compounds using a method as simple as possible that can be run in a HPLC with single wavelength detector.

3.2.- Total phenolic content and antioxidant activity

In our study, antioxidant activity of beverages was determined using DPPH free radical scavenging and concentration of total polyphenols was determined using the Folin-Ciocalteu method. Both assays have proven their efficacy in juices [30]. Table 2 shows the mean of the results obtained in each assay (analysis were done by triplicate).

In terms of antioxidant activity, the best results were obtained with both, Antiox 1 and Antiox 2 beverages while the worse results were obtained with beer and the multifruit-milk beverage. This behavior can somehow be correlated to their total phenolic content since Antiox 1 was the drink with higher phenolic content while the multifruit-milk beverage was the lowest. Despite these results, there is not a clear relationship among the total phenolic content and the radical scavenging activity, as can be observed in Figure 2A. This lack of relationship could be associated with the presence of vitamins (with antioxidant properties) in the drinks (i.e. vitamins C and E), which do not contribute to the total phenolic measurement but can have synergistic effects with the antioxidant activity of phenolic compounds [33].

3.3.- Food samples analysis

The chromatograms obtained for all beverages (after dilution 1:1 with water) can be seen in Figure 3. In general, good resolution was achieved. As can be seen, the amount

of fat soluble vitamins and pigments was not very significant, because the non-polar zone of the chromatograms is almost empty. The quantification of vitamins and polyphenols detected is shown in Table 3. In general terms, there exist a good correlation between the quantification of total phenolic compounds using Folin-Ciocalteu method and the total phenolic compounds quantified using the present HPLC-DAD method, as can be seen in Figure 2B.

The main phenolic compounds present in orange juice are flavanones, as expected [34-36], but the vitamin C content of this juice was very low, probably due to the sterilization step during its manufacture. In fact, concentration of vitamin C is a significant indicator of orange juice quality and, therefore, how it has been processed [37, 38].

The analysis of the soy-orange drink reveals a high similarity with the pure orange juice but with two main differences: vitamin E and isoflavones. Vitamin E has been detected in the soy-orange drink, probably being incorporated as additive E-307 (α -tocopherol) to avoid lipid peroxidation of the fat content of “soy-milk” [39]. As for the isoflavones, their origin come clearly from the soy-milk [40-42].

Both Antiox drinks present a high content in ascorbic acid and the highest concentration of anthocyanins. In spite of it, these beverages showed quite different profiles. Antiox 1 possesses the highest content in thiamine and flavanones that can be related to both, its antioxidant activity and its high total phenolic content. The phenolic compounds observed in both beverages were closely related with those phenolic compound that are typically detected in fruits, i.e., flavanones and flavonols in oranges [30], anthocyanins in berries [43], acerolas [44] and plums [45] or cinnamic acids and flavan-3-ols in pineapples [46, 47].

Qualitative profiles of beers is quite similar being the main differences quantitative. Both present an important content of vitamins B, which is higher in the “traditional beer”; in fact, “light beer” shows lower content of most of the compounds analyzed. The content of vitamin C could be related with the addition of E-300, ascorbic acid, as additive (as declared in label). In terms of phenolic compounds, the main families are the phenolic acids and flavanones, but in light beer the presence of benzenediols could be detected. The composition of beers obtained using this method is in accordance with previously published works [48].

It is important to remark the presence of tocopherol in milky drinks (multifruit and strawberry). Vitamin E is stabilized by the non polar fraction of the milk. The strawberry yogurt is the only beverage lacking ascorbic acid. These beverages possess an important content of thiamine, and their phenolic profile corresponds with previously published profiles of fruits that take part in the mixtures [10, 36, 43, 49, 50].

Regarding photosynthetic pigments (carotenoids and chlorophylls), none of the previously discussed samples contained such compounds above their detection limit. In order to prove the efficacy of the HPLC method in real samples, a fruit purée was analyzed. In this case the compounds detected were similar to those detected in the multifruit juice, but in this case a certain amount of zeaxanthin was found. Zeaxanthin is a typical compound found in corn, *Zea mays* [51].

3.4.- Statistical analysis

In order to correlate the composition of beverages with its antioxidant activity, two statistical strategies were used: partial least squares (PLS) regression and forward stepwise multiple linear regression (FSMLR).

By using the PLS analysis four principal components could be assessed, but only using the first one the model ($\hat{y} = b_0 + \sum_{i=1}^p b_i X_i$) could explain 91.1% of variability ($R^2=0.911$). Figure 4A shows the predicted values for antioxidant activity (ActAntOx), obtained from PLS regression with all the components of the chromatographic profile and 1 component, versus the observed values. As can be seen, the fit for the predictions of ActAntOx can be considered adequate. From the standardised regression coefficients, the most important variables were the anthocyanins eluted at 11.7 and 11.8 min (with coefficients of correlation of 0.93 and 0.91, respectively), the benzenediol eluted at 9.3 min (0.74), flavanones eluted between 13.2 and 14.2 min (0.74) and sum of flavanones (0.73). The root-mean-square error of this calibration was RMSEC=6.32, this value is

defined by the equation: $RMSEC = \sqrt{\frac{\sum (y_i - \hat{y}_i)^2}{n}}$ where n is the number of samples (in this study $n=8$), y_i is the true ActAntOx.

A relative error, $RE = \frac{RMSEC}{\bar{y}_i}$, of 11.5% was obtained in this case. Using this model

the difference among the predicted value of antioxidant activity and the observed is below 5 for all beverages, except for the multifruit-milk drink, whose difference is 15. The reason of this difference can be due to the low amount of phenolic compounds present in this sample.

By using the FSMLR, an equation $\hat{y} = b_0 + \sum_{i=1}^p b_i X_i$ is assumed, where b_0 is the intercept of the model, b_i is the regression coefficient for the compound (X_i), p is the number of compounds in the model, and $\hat{y}_i$ is the radical scavenging activity calculated with the model. Values of 4.0 and 3.9 were used for F-to-enter and F-to-Remove, respectively, and a limit of 10 steps was fixed. The *Multiple Regression* module of STATISTICA software was used for calculation. The selected variables were the compounds that eluted between 11.6 and 11.8 min. The value of determination coefficient was $R^2=0.978$, and the value of the root-mean-square error of calibration was $RMSEC=3.09$. A relative error of $RE=5.6\%$ was obtained in this case. Fig. 4B shows the predicted values obtained using the fitted regression equation versus the observed values for antioxidant activity. As can be seen, the fit for the predictions of DPPH radical scavenging activity can be considered appropriate. With this statistical procedure the differences of all the predicted values with the observed antioxidant activities are below 7.

4.- Conclusions

The results obtained for the simultaneous determination of polyphenols, carotenoids, chlorophylls and water and fat soluble vitamins using HPLC-DAD confirm that the proposed method can be successfully applied to the routine analysis of these kind of compounds and to obtain a clear profiling of the bioactive compounds in a functional food sample. All vitamins, whether fat or water soluble, phenolic compounds and photosynthesis-related pigments were baseline separated with good resolution values and good linear response. The analytical method was applied to the determination of vitamin and polyphenols of functional drinks by direct injection with the only cleanup of a filtration step. A good correlation could be found by comparing the total phenol content measured by Folin-Ciocalteu method with the sum of phenolic compounds

detected by HPLC-DAD. By using statistical tools the main compounds associated with antioxidant activity of the functional drinks (measured by DPPH radical scavenging) belonged to the anthocyanin and flavanone families. Easy prediction of the antioxidant activity of a functional beverage can be obtained by knowing the composition in terms of these families of compounds.

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Tables

Table 1.- Quantification limits and calibration curves of studied standards

Compound		calibration curve [†]	R ²	RT (min)	Limits [♦]	
					LOD (ng)	LOQ (ng)
Vitamins	Ascorbic acid (vitamin C)	y = 344.30x + 75.16	0.9999	2.6; 2.8 [‡]	5	45
	Thiamine (vitamin B1)	y = 126.36x – 60.30	0.9998	3.2	32	161
	Niacin (vitamin B3)	y = 240.94x + 15.98	0.9999	5.8	8	59
	Pyridoxine (vitamin B6)	y = 2316.43x + 21.20	0.99999	5.9	0.2	3
	Cobalamine (vitamin B12)	y = 670.94x – 38.80	0.9996	10.2	251	690
	Cholecalciferol (vitamin D3)	y = 241.71x + 335.84	0.9995	27.4	14	825
	Riboflavin (vitamin B2)	y = 98.85x + 156.21	0.9991	11.5	44	304
	α-tocopherol (vitamin E)	y = 328.58x + 0.07	0.9999	27.2	2	12
Polyphenols	Gallic acid (phenolic acids)	y = 559.18x + 277.70	0.9988	6.6; 8.1 [‡]	5	31
	Catechin (flavan-3-ols)	y = 479.12x - 15.86	0.9999	10.7	146	401
	Catechol (benzenediols)	y = 1927.81x + 31.03	0.9999	11.0	2	19
	p-Coumaric acid (cinnamic acids)	y = 5424.26x + 132.80	0.9999	12.1	0.5	5
	Hesperidin (flavanones)	y = 533.71x + 11.01	0.9999	12.2	5	55
	Cyanidin Chloride (anthocyanins)	y = 1421.36x – 10.50	0.9966	12.3	3	9
	Quercetin (flavonols)	y = 1870.91x – 14.99	0.9999	13.2	4	10
	Genistein (isoflavones)	y = 743.36x + 907.10	0.9988	13.6	5	58
	Chlorophyll-a	y = 0.047x + 1.062	0.9930	33.5	82	110
	Zeaxanthin	y = 0.625x + 75.253	0.9938	23.1	8	9

[†]- $\text{Area}_{280\text{ nm}} = \text{slope} \times [\text{injected ng}] + \text{constant term}$

[‡]- Isomers presence in standards

[♦]- LOD and LOQ calculated as Equation 1, using k=3 for LOD and k=10 for LOQ

Table 2.- Total phenolic content and antioxidant activity of beverages used for vitamin-pigments analysis

Product	Total Phenolic Content [†]	Antioxidant activity [‡]
Antiox 1	3085.14 ± 8.56	96.22 ± 4.81
Antiox 2	309.24 ± 2.48	78.37 ± 1.16
Beer 1	865.42 ± 10.46	32.33 ± 0.76
Beer 2	689.73 ± 3.64	41.54 ± 1.05
Multifruit	113.16 ± 4.35	33.50 ± 1.49
Soy-Orange	943.04 ± 57.50	42.83 ± 3.28
Yogurt	376.20 ± 8.30	55.11 ± 2.75
Orange Juice	808.37 ± 12.18	58.72 ± 1.16

[†]- Results of phenolic content expressed as caffeic acid equivalents

[‡]- Results of antioxidant activity expressed as % of DPPH radical scavenged

Table 3.- Identification and quantification of vitamins and polyphenols

Product	Compound	ng/ml drink	Standard dev.
Antiox 1	Ascorbic acid	133.44	0.30
	Thiamine	1486.49	21.01
	Benzenediols	22.60	1.03
	Flavanones	1729.46	22.80
	Anthocyanins	92.74	1.42
	Flavonols	34.11	1.05
Antiox 2	Ascorbic acid	93.89	0.18
	Niacin	44.13	0.37
	Pyridoxine	U.Q.	--
	Phenolic acids	U.Q.	--
	Cinnamic acids	12.24	0.04
	Flavanones	56.83	1.11
	Flavonols	U.Q.	--

	Flavan-3-ols	9.70	0.91
	Anthocyanins	63.46	1.26
Multifruit-milk	Ascorbic acid	92.85	1.07
	Thiamine	901.80	7.81
	Niacin	44.63	0.15
	Piridoxine	3.52	1.02
	Benzenediols	U.Q.	--
	Phenolic acids	U.Q.	--
	Cinnamic acids	U.Q.	--
	Tocopherol	13.87	0.18
Orange-Soy	Ascorbic acid	193.09	0.95
	Thiamine	U.Q.	--
	Niacin	36.08	0.66
	Piridoxine	6.43	0.32
	Cinnamic acids	U.Q.	--
	Flavanones	168.92	0.82
	Isoflavones	154.81	1.31
	Flavan-3-ols	18.02	1.49
	Tocopherol	14.29	0.58
Strawberry yogurt	Thiamine	121.56	0.83
	Niacin	262.41	0.25
	Piridoxine	3.40	0.11
	Cinnamic acids	13.87	0.20
	Flavanones	55.62	0.22
	Phenolic acids	U.Q.	--
	Anthocyanins	11.55	1.05
	Tocopherol	20.78	1.31
Orange Juice	Ascorbic acid	14.75	0.79
	Thiamine	266.62	5.57
	Niacin	U.Q.	--
	Cinnamic acids	U.Q.	--
	Flavanones	266.69	4.20
	Benzenediols	U.Q.	--
	Flavan-3-ols	13.12	0.49
Beer 1	Ascorbic	62.82	2.28
	Thiamine	789.20	10.32
	Niacin	226.58	5.47
	Folic ac.	U.Q.	--
	Phenolic acids	315.91	6.48
	Flavanones	82.13	0.54
Beer 2 (light)	Ascorbic	46.91	1.47
	Thiamine	39.45	1.14
	Niacin	76.42	2.23
	Folic ac.	U.Q.	--
	Phenolic acids	172.43	4.59
	Flavanones	73.24	2.84
	Benzenediols	25.57	1.69
Fruit Purée	Ascorbic acid	94.12	2.71
	Thiamine	970.80	17.94
	Niacin	53.08	1.52
	Piridoxine	12.21	0.92
	Benzenediols	U.Q.	--
	Phenolic acids	87.24	2.47
	Cinnamic acids	10.14	1.14
	Zeaxanthin	24.2	5.83

U.Q.- Under Quantification limit, but above Detection Limit.

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Figure captions

Figure 1.- Chromatograms of standards at 280 nm as detection wavelength. (A) water soluble vitamins and phenolic compounds, and (B) photosynthetic pigments and fat soluble vitamins.

Figure 2.- Comparison among the total polyphenol content measured by Folin-Ciocalteu method and percentage of DPPH scavenged (A) and the total phenol content and the sum of quantified phenolic compounds measured by present HPLC method (B).

Figure 3.- Chromatograms of the different beverages analyzed. Codes used for identification: IF: isoflavone, FLA: Flavanone, FOL: Flavonol, BPN: Biphenol, F3O: Flavan-3-ol, PA: phenolic acid, CA: cinnamic acid, AN: anthocyan, ZX: Zeaxanthin, and vitamins are named as seen in Table 1

Figure 4.- Correlation between the predicted values obtained using PLS using one component (A), FSMLR (B) and the observed values for DPPH radical scavenging in the 8 samples

Fig 1.A

Fig 1B

Fig 2

Fig 3

Fig 4