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Nutritional Study of Copper and Zinc in Grapes and Commercial Grape Juices from Spain

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This paper presents the levels of copper and zinc determined in a total of 66 samples of the most widely consumed varieties of white and red grapes in Spain, as well as those of 60 samples of grape juice (39 from white varieties and 21 from red ones) chosen from the main commercial brands in the country. Atomic absorption spectroscopy was used as analytical technique, with electrothermal atomization after digestion of the sample with $HNO_3-H_2O_2$ for grapes and with HNO_3 for grape juice. The mean Zn contents obtained (0.0462 mg/100 g in grapes and 0.0460 mg/100 mL in grape juice) are lower than those provided by most of the more commonly used food composition tables. The mean Cu contents were 0.0515 mg/100 g in grapes and 0.0063 mg/100 mL in grape juice. On the basis of these data and the official data on consumption of grapes and grape juice in Spain, the contribution of both products to the recommended daily intake of zinc (15 and 12 mg/day for healthy adult men and women, respectively) is estimated to be approximately 0.1%, whereas for Cu, this supply represents rather more than 0.25% of the established ESADDI (1.5−3 mg/day in adults). The growing popularity of these products in recent years, on the basis of its nutritional properties and beneficial effects, requires additional data, and the present findings are of potential use to food composition tables.

KEYWORDS: **Copper; zinc; grapes; grape juice; dietary intake**

INTRODUCTION

Grapes are the most widely grown fruit in the world, second to oranges, and represent an essential component in the Mediterranean diet and culture. Grape cultivation in the European Union represents 45% of the world's wine-growing area, 60% of the total production, and 70% of the world export (*1*).

The table grapes grown in Spain, with 5.6 million tons (*2*), and grape juice production, estimated to be around 2.5 million hl/year, make up 15.2% of the total production of the European Union, according to data by the Office International de la Vigne et du Vin (*3*). The increase detected in the global market for table grapes is encouraging and should justify the strengthening of measures tending to promote the production and consumption of this fruit, whose beneficial effects on human health have been extensively and sufficiently proved. According to the same source, the improvement in air transport services has played an important role in the increase (6.3% worldwide and 6.5% in Europe compared to 1998), as it has allowed the export of table grapes from the southern to the northern hemispheres in winter and spring (*3*).

Recent studies suggest that the good cardiac health of the inhabitants of the Mediterranean area is due to some substances such as the phenolic compounds found in grapes (*4*−*8*). Likewise, both the grape and its juice can be a source of minerals with important physiological functions in the human organism. Zinc is an essential micronutrient for humans because it activates a large number of enzymes and it has been recognized as a cofactor in the superoxide dismutase enzyme, which is involved in protection against oxidative processes (*9*). In addition, zinc is found in several dehydrogenases, aldolases, peptidases, and phosphatases and is required for deoxyribonucleic and ribonucleic acid synthesis, as well as possibly playing a role in stabilizing plasma membranes (*10*). Zinc balance is closely regulated by homeostatic mechanisms, and its major source is the diet. Thus, several different recommended dietary allowances (RDA) were established for humans by various organizations, such as 15 and 12 mg Zn/day for healthy adult men and women (*11*), respectively; respective ranges of 9.4−13.1 and 6.5−10.3 mg Zn/day for men and women (*12*), and a reference intake of 9.5 mg Zn/day for men and 7.1 mg Zn/day for women (*13*). The composition of the diet has important effects on the bioavailability of the zinc it contains. Interactions have been described with other nutrients, such as proteins, fiber, fitates, and some minerals (*14*−*17*).

Copper is another trace element essetial to the human organism, forming part of many enzymes involved in oxide-

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Table 1. Instrumental Conditions for Cu and Zn Determination in Grapes and Grape Juices by ETAAS

	copper	zinc
wavelength, nm	324.8	213.9
slit width, nm	0.7	0.7
atomization system	L'vov platform	L'vov platform
dry T[a], deg C	110	130
ashing T[a], deg C	1200	600
atomization T[a], deg C	2300	1800
clean T[a],deg C	2600	2600
sample and standard volume, μL	20	10
matrix modifier		6 μg $Mg(NO_3)_2$
background correction	deuterium	deuterium
signal mode	peak area	peak area

reduction processes (*18*). Many epidemiological studies have shown the influence of a low Cu status on particular pathologies, such as hypercholesterolemia, intolerance of glucose, hypertension and even severe deficiencies related to anaemia and osteoporosis (*9*). The estimated safe and adequate daily dietary intake (ESADDI) proposed by the National Research Council (*11*) is 1.5−3 mg/day in adults. Cu deficiencies are infrequent; however, several studies have reported a direct correlation between the dietary Zn/Cu ratio and the incidence of cardiovascular disease (*11*).

At present, the determination of mineral and trace elements has taken on considerable importance in both grapes and their derivates (grape juices, wines, and other alcoholic beverages), not only from the nutritional, but also from the technological point of view. These elements can have a considerable influence on the production process, with, in some cases, negative effects such as oxide-reduction reactions, precipitation, colloidal alterations, formation of gels, or alterations of the organoleptic characteristics (*19*−*21*). The ranges of values found in the literature and in the most commonly used food composition tables vary significantly, which, among other reasons, may be due to insufficient sampling that could lead to nonrepresentative data, lack of experimental data, or analytical methods used with inadequate detection limits (*22*).

In this study, we determined the Cu and Zn levels in samples of the main varieties of (white and red) table grape consumed in Spain, as well as in samples of commercial grape juices. The analytic technique used was atomic absorption spectroscopy with electro thermal atomization (ETAAS) after acid digestion of the samples, and testing of the analytic method's reliability. The results obtained are very useful for compiling food composition tables, given the lack of data on these elements in grapes, in most of them.

MATERIALS AND METHODS

Apparatus. The apparatus used consisted of a Perkin-Elmer 1100B double-beam atomic absorption spectrophotometer (Perkin-Elmer, Norwalk, CT) equipped with a deuterium background corrector with 0.7-nm slit; copper and zinc hollow cathode lamps made by the same manufacturer; a Perkin-Elmer HGA-700 furnace with pyrolytically coated graphite tubes; and a L'vov platform (Perkin-Elmer). The samples were injected manually with a Pipetman micropipet, and 99.999% pure argon was used as internal gas at 300 mL/min flow. The samples were atomized under the conditions shown in **Table 1**. The Ar flow was stopped during atomization to increase sensitivity, and this did not alter the useful life of the tubes. A Selecta digestion block (Selecta SA, Barcelona, Spain) and Pyrex tubes were used for sample mineralization. A Moulinex blender (Moulinex, Bagnolet, France) was used to homogenize the samples.

Reagents. An ammonium molybdate analytical reagent grade (Merck, Darmstadt, Germany) saturated solution was used to precondition the furnace tubes. Magnesium nitrate (Merck) was used as chemical modifier. Merck nitric acid (Suprapure), vanadium pentaoxide (reagent grade) and hydrogen peroxide (reagent grade) were used for sample mineralization. All solutions were prepared with ultrapure water (18 MΩ · cm specific resistivity) obtained by filtering distilled water through a Milli-Q purifier system (Millipore, Gif-sur-Yvette, France) immediately before use. Merck Titrisol copper and zinc standard solutions (1.00 ± 0.002 g) were used to prepare the calibration graphs.

Table 2. Evaluation of the Analytical Characteristics of the Method

	detection limit (pg)	characteristic mass (pg)	recovery (%)	precision (RSD,%)	blank to sample slope ratio
Zn	0.5	0.7	98.7 ± 1.0	3.5	0.999–1.100
Cu	4.0	4.5	99.5 ± 0.5	3.0	0.998–1.000

Sampling. We analyzed a total of 66 samples of the main varieties of table grape consumed in Spain. These varieties were both white (six samples of each of the following varieties: *Alelo*, *Dominga*, *Ohanes*, *Victoria*, *Roseti*, *Diamante*, *Spanish Muscatel*, and *Italia*) and red grapes (six samples of *Cardinal* and *Mariano* and three samples of *Garnacha* and *Palieri*). We also analyzed 60 samples of grape juice from both white (*39*) and red grapes (*21*), chosen from the most widely found and consumed commercial brands in Spain (Spar, Carrefour, Greip, Don Simón, Vivo, Día, Adarve, Casón, Alcampo,), grape musts obtained from mixtures of non declared varieties of grape. All the samples were acquired in markets and department stores and were produced in Spain. Preliminary assays established the appropriate amount of sample for analysis to ensure homogeneity between samples and ensure they were representative (*23*, *24*). The grape samples were properly washed to remove any additional contamination.

Sample Treatment. *Grapes*. Samples were sliced and inedible parts discarded. The remainder was homogenized in a blender and weighed. A 20.0-g sample of the edible portion (without seeds in the varieties that have them) was treated with 25 mL of 65% HNO_3, 5 mL of $HClO_4$ and 3 mL of H_2O_2 in Pyrex tubes placed in the digestion block and heated at 60 °C for 30 min and at 120 °C for 60 min. The solutions were cooled to room temperature, transferred to a calibrated flask, and diluted to a final volume of 10 mL with ultrapure water. All analyses were done in triplicate. Copper and zinc were determined in this solution by ETAAS under the conditions shown in **Table 1**.

Grape Juice. A 5.0-mL portion of sample was treated with 1 mL of 65% (v/v) HNO_3 and 30 μg of V_2O_5 (as catalyst) and heated to 120 °C for 90 min in the digestion block. The solution obtained was then diluted to a final volume of 10 mL with ultrapure water. Copper and zinc were determined by ETAAS, under the conditions shown in **Table 1**. All samples were analyzed in triplicate.

Analytical Characteristics. The detection limit values were calculated for 10 successive injections of the blank using the 3σ criterion (*25*). The characteristic mass was estimated as the amount of analyte producing 0.0044 absorbance units. The standard additions method was used with five arbitrarily chosen samples to detect possible matrix interferences. The slopes of the calibration graphs for spiked samples were similar to the slope of the calibration graph for the standards in acid medium. The Student test was applied to check similarity of slopes (*26*). The accuracy of the method was tested with recovery assays for three determinations in five different samples. Repeatability was tested by calculating the RSD for 10 successive injections in five different, arbitrarily chosen samples. The results obtained are summarized in **Table 2**. Accuracy and precision of the method were also tested in 10 replicate assays with an NBS-certified reference material SRM 1572 *citrus leaves* **(Table 3**).

RESULTS AND DISCUSSION

Copper and zinc concentrations in the grape and grape juice samples analyzed are summarized in **Table 4**, where a broad range of variation can be observed between the different samples—more in the case of copper—undoubtedly due to the strong influence of the type of soil and agricultural procedures

Table 3. Accuracy and Precision of the Method against an NBS-Certified Reference Material (Citrus Leaves SRM 1572)

element	content (μg/g)[a] measured[b]	content (μg/g)[a] certified[b]	accuracy (%)	precision (RSD, %)
Cu	16.40 ± 0.80	16.5 ± 1.0	99.39	4.88
Zn	28.85 ± 1.90	29.0 ± 2.0	99.48	6.58

[a] Based on dry weight. [b] Mean SD, at 95% C. I. interval about the mean (n = 10).

Table 4. Levels of Copper and Zinc in Grapes and Grape Juices

Copper

		n^b	mean	σ_{n-1}	minimum	maximum	variance
grapes[a]	white	48	0.0525	0.02700	0.02604	0.14272	7.3×10^{-4}
(mg/100 g)	red	18	0.0491	0.01374	0.03434	0.07566	1.9×10^{-4}
	total	66	0.0515	0.02391			5.7×10^{-4}
grape juices	white	39	0.00697	0.00552	0.00139	0.02030	3.1×10^{-5}
(mg/100 mL)	red	21	0.00505	0.00094	0.00365	0.00744	8.8×10^{-7}
	total	60	0.00630	0.00456			2.1×10^{-5}

Zinc

		n^b	mean	σ_{n-1}	minimum	maximum	variance
grapes[a]	white	48	0.0455	0.01467	0.0219	0.0790	2.15×10^{-4}
(mg/100 g)	red	18	0.0479	0.06114	0.0400	0.0060	3.74×10^{-5}
	total	66	0.0462	0.01281			1.64×10^{-4}
grape juices	white	39	0.0411	0.01541	0.0240	0.0812	2.37×10^{-4}
(mg/100 mL)	red	21	0.0550	0.00594	0.0465	0.0658	3.53×10^{-5}
	total	60	0.0460	0.01448			2.09×10^{-4}

[a] Refers to fresh weight of the edible fraction. [b] All analyses were done in triplicate

on the contents of these elements in the fruit (*27*). The Cu contents determined by us had mean values of 0.0515 mg/100 g in grapes and 0.00630 mg/100 mL in grape juice, which differ from those provided by the food composition tables. The most commonly used tables give very scarce data on Cu, probably because of a lack of adequate analytical methods at the time of publication or because of the lesser importance of this element in states of deficiency. The data in Spanish tables vary widely. Thus, Rojas (*28*) gives a range between 0.02 and 0.1 mg/100 g in fresh grapes and 0.36 mg/100 g for raisins, whereas Jiménez et al. (*29*) give 90 μg/100 g for fresh grapes and 363 μg/100 g for raisins.

We should also point out that, in general, we found a higher mean concentration (0.052 mg/100 g) in the white varieties, than in the red ones (0.049 mg/100 g), with the highest concentration of Cu **(Figure 1)** in the traditional variety of *Muscatel* grape traditionally consumed in Andalusia.

Considering the main food composition tables in general use, we find that the data proposed by the USDA (*30*) gives values in agreement with those found in our study, but only for American varieties, whereas the European varieties show clearly higher contents in grapes (0.090 mg/100 g) and grape juice (0.011 mg/100 mL). The English tables of the Royal Society of Chemistry (*31*) give mean values for grapes of 0.12 and 0.01 mg/100 mL for juice of peeled, seeded grapes, but 0.02 mg/100 mL for juice of whole grapes, whereas the German tables (*32*) give values of 61.00 μg/MJ in grapes and 40 μg/MJ for grape juice (with a range of 30−60 μg/MJ).

For zinc, the tables of the British Ministry of Agriculture (*31*), which are widely used in Europe, give an overall value of 0.1 mg/100 g for any type of grape, and the same is true for Jiménez et al. (*29*), which is a compilation of several tables. The food composition tables of the German Ministry of Agriculture (*32*) state zinc contents of 82 μg/MJ (with a 35−110 μg/MJ range) for grapes and 0.17 μg/MJ for grape juice (0.10−0.20 μg/MJ range), while the tables of the Spanish Ministry of Health (*33*) state contents of 0.17 mg/100 g. Such variations could be caused by the detection limit and sensitivity of the analytic techniques used. The Zn contents found by our study for both fresh grapes and grape juice are in all cases less than 0.1 mg/100 g, with an average of 0.046 mg/100 g in grapes and 0.046 mg/100 mL in grape juice. The only reference value found in the tables that agrees with our results is the 0.04 mg/100 g of the Spanish Ministry of Agriculture's food composition table for commercial grape juice Spanish Ministry of Agriculture, Fisheries and Food (*34*).

If we examine the results by varieties **(Figure 1),** we can see that the red grapes and juice from red grapes give higher mean Zn contents (0.0479 mg/100 g and 0.055 mg/100 mL, respectively) than white grapes or juices from white grapes (0.045 and 0.041 mg/100 mL respectively), and although the red varieties have higher mean values, the *Dominga* variety has the highest zinc concentration. The data provided by the USDA (*30*) are similar to those obtained by us in both American (0.04 mg/100 g) and European varieties of grape (0.05 mg/100 g). However, the contents for grape juices (0.08/100 mg) are higher than in our case. **Table 5** summarizes in detail the data found in food composition tables on the presence of Cu and Zn in fresh grapes and commercial grape juices. After placing both sample groups in mg/100 g (by means of the density of the grape juice) and after ensuring the homogeneity and normal distribution of the data, we undertook a variance analysis (ANOVA), which showed that there were significant differences ($p < 0.001$) in the copper contents of fresh grapes and commercial grape juice but not in the zinc contents **(Table 6)**. Onianwa et al. (*35*) detected copper contents of 0.001−0.27 ppm in commercial grape juice, in contrast to 0.001−1.02 ppm in fruit juice, and zinc contents of 0.23−1.96 ppm in grape juice and 0.020−1.10 ppm in fruit juice. In Spain, Terrés et al. (*36*) found a mean Zn concentration of 0.3 ± 0.1 μg/g in grapes.

The literature contains some references to enological studies on the same subject, such as Puig-Deu et al. (*37*), who came to the conclusion that the Cu levels in wine-making are highest in the first pressing and decrease as the pressure rises to obtain the juice. The values detected by this author for the *Xarello* variety of grapes from Catalonia (Spain), for example, were 3.31 ± 0.02 mg/L for the first pressing and 0.96 ± 0.12 mg/L at 1 atm pressure. In a study of the main varieties of wine-making grapes from the different French regions, Martin (*38*) found mean zinc contents of 0.44 ± 0.18 mg/L.

It is also interesting to compare the contents of these metals in other foodstuffs, especially in other similar vegetable products. **Table 7** summarizes the concentrations in different fruits and some foodstuffs considered rich in Cu and Zn. According to these data, grapes have similar contents to other vegetable products but differ considerably from animal foodstuffs, nuts, or oysters, in the case of zinc.

In Spain, the official figures on consumption of table grapes estimate 2.3 kg/person/year approximately (*34*), while the total consumption of fresh fruit is some 84.4 kg/person/year. Grape juice consumption is established as 0.4 L/person/year, in contrast to a total of 11.5 l/person/year of fruit and vegetable juices. The consumption of both grapes and grape juice will tend to increase in the coming years, especially because of the intensive publicity campaign explaining the health benefits of both products. According to data by the OIV (*3*), Spain is the third

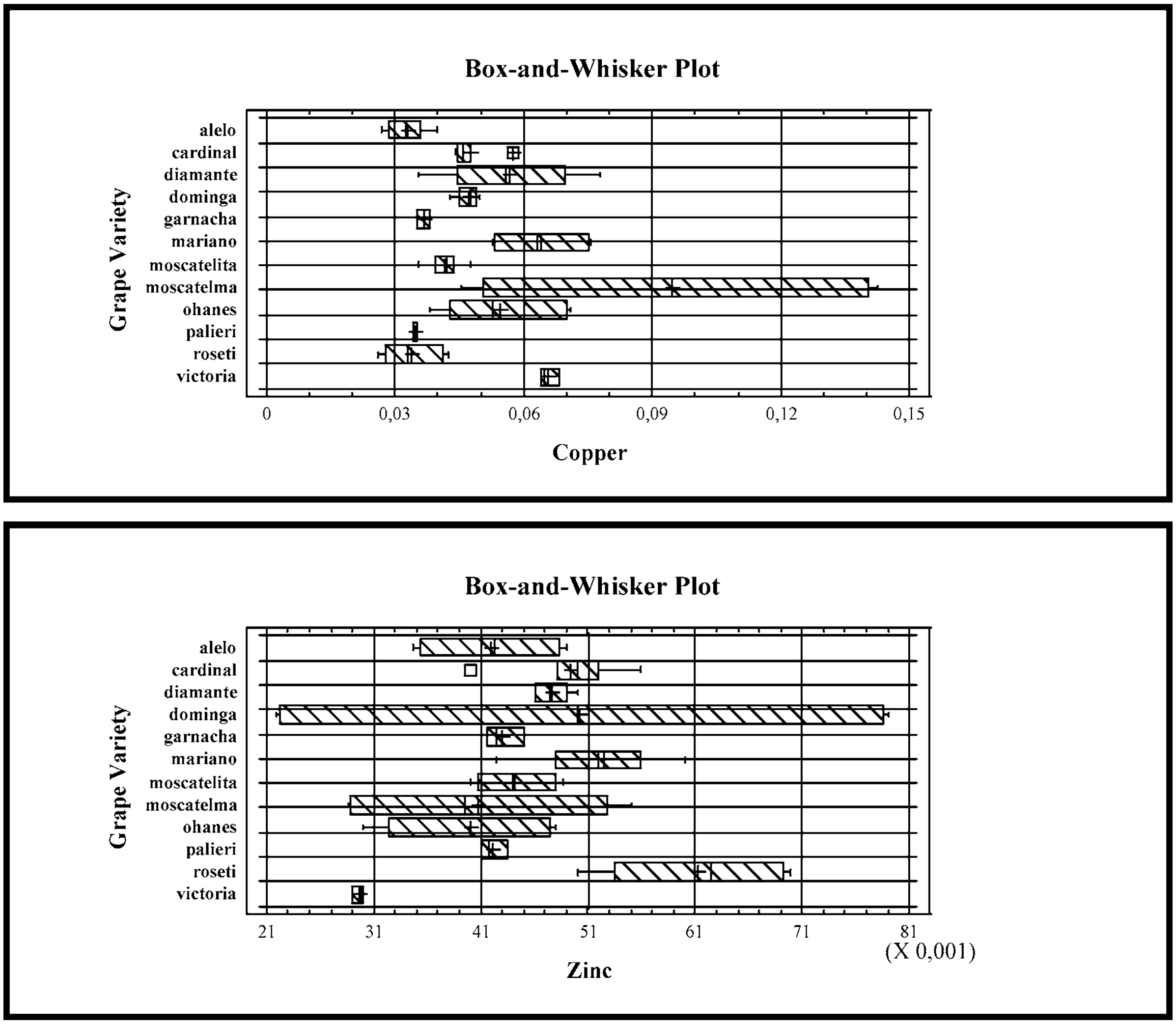

Figure 1. Copper and zinc levels found in the different varieties of grapes analyzed.

Table 5. Copper and Zinc Values in Grapes and Grape Juices Included in the Most Frequently Used Food Composition and Nutrition Tables

	copper (mg/100 g)				zinc (mg/100 g)			
	grapes			grape juices	grape			grape juices
	white	red	raisins		white	red	raisins	
Spanish Ministry of Health (*33*)					0.17			
Jimenez et al. (*29*)	90 μg		363 μg		0.1		0.1	
Mahan et al. (*39*)							0.075	0.075
Mataix and Mañas (*40*)					0.1	0.1	0.1	0.11
Muñoz et al. (*41*)					0.1			
Shils et al. (*9*)		0.047				0.070		
Holland et al. (*31*)	0.12			0.01	0.1			Trazas
Souci et al. (*32*)	61(35–80) μg/MJ			40 μg/MJ	82 (35–110) μg/MJ			0.17 mg/MJ
Varela (*42*)					0.1	0.1		
Rojas (*28*)	0.1	0.1	0.2	0.02				
McCane (*43*)					0.1	0.1		
Spanish Ministry of Agriculture (*34*)					0.08	0.08		0.04
USDA (*30*)	American varieties: 0.040 European varieties: 0.090			0.011	American varieties: 0.04 European varieties: 0.05			0.08

largest consumer of such products in Europe, after Italy and Germany, and consumption is also quite high in the USA. However, as the OIV points out, in many countries, there are no ways of establishing the amounts of grape juice, fresh grapes, and raisins consumed, and the data are scarce, heterogeneous, and generally incomplete.

Finally, regarding intake, and more importantly, the supply provided by consumption of grapes in view of the data provided in this paper, we can say that the present consumption of grapes and grape juice provides less than 0.02% of the recommended daily intake of zinc in the Spanish diet. This in turn represents only 1.7% of the 0.2 mg/day supplied to the Spanish diet by fruit and vegetables, according to the Spanish standard diet (*34*), which is only 85% of the recommended intake of this element.

As established by the data in this paper, the present consumption of grapes and grape juice represents slightly more than

Table 6. Variance Analysis of the Two Elements Studied in Relation to Their Commercialization (grapes/grape juices)

source	sum of squares	Df	mean Square	*F*-ratio	*p*-Value
		zinc			
between groups	0.000299525	1	0.000299525	1.73	0.1915
within groups	0.0210081	124	0.000173621		
total (corr)	0.0213076	125			
		copper			
between groups	0.0639725	1	0.0639725	211.95	0.0000
within groups	0.0365205	124	0.000301822		
total (corr)	0.100493	125			

Table 7. Copper and Zinc Levels in Other Fruits Consumed in Spain

	copper (mg/100 g)	zinc (mg/100 g)		
product	ref 29	ref 29	ref 33	ref 34
orange	0.055	0.17	0.15	0.10
melon	0.060	0.09	0.29	0.10
peach	0.067	0.02		0.02
apple	0.031	0.10	0.13	0.12
apricot	0.088	0.10		0.07
kiwi	0.950	0.11	0.16	
pear	0.113	0.16	0.23	0.23
banana	0.104	0.23	0.21	0.22
strawerries	0.048	0.09	0.22	0.12

0.25% of the ESADDI of copper as defined above. Severe copper deficiency is a rare phenomenon, and although there are not sufficient data to establish the RDAs for this element, the National Research Council (*11*) recommends an ESADDI of 1.5−3 mg/day for adolescents and adults. According to the National Research Council (*11*), the RDAs of zinc are 15 mg/day for adult men and 12 mg/day for adult women, with slightly lower intakes during pregnancy and breast-feeding. According to data by the Spanish Ministry of Agriculture, Fisheries and Food (*34*), based on the latest alimentary survey undertaken in Spain in 1999, the diet only provides 85% of the RDA. We considered that this study contributes new data on the mineral content in grape and grape juice, and our data are potentially useful as supplementary (and previously unavailable) information to current food composition tables and should be useful in calculating dietary intakes of these elements.

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