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Metals in wheat flour; comparative study and safety control

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Abstract

Cereal industry and its derived products have a big economic and social importance worldwide. Therefore, as wheat flour is a commodity for all bread and bakery industry, it is safety is of high nutrition and toxicological interest. In this investigation we intend to study and determine the content of twelve metals in 50 samples of wheat flour coming from a wheat flour industry. Macro elements sodium, potassium magnesium and calcium, micro elements manganese, iron, copper, zinc, chrome and nickel as well as toxic trace elements cadmium and lead have been analysed. The estimated diary intake of each metal and their contribution in percentage terms to the RDI (macro and micro elements) and to the PTWI (toxic elements) has been determined. Contribution of Cd and Pb to the PTWI was very low, a fact that shows safety in this wheat flour concerning toxic metals.

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Key words: *Wheat flour. Toxic metals. ICP. Cluster analysis. Intake.*

METALES EN HARINA DE TRIGO; ESTUDIO COMPARATIVO Y CONTROL DE SU SEGURIDAD

Resumen

La industria de los cereales y sus derivados tiene una gran importancia económica y social en todo el mundo. Por ello, la seguridad de las harinas como materias primas de todas las industrias de panadería, bollería y repostería es de sumo interés nutricional y toxicológico. En este trabajo nos hemos propuesto estudiar y determinar el contenido de doce metales en 50 muestras de harina de trigo procedentes de una industria harinera. Se analizaron los macroelementos sodio, potasio, magnesio y calcio, los microelementos manganeso, hierro, cobre, zinc, cromo y níquel y los elementos traza tóxicos cadmio y plomo. Se determinó la ingesta diaria estimada de cada metal y su contribución porcentual a las IDRs (macro y microelementos) y a las PTWIs (elementos tóxicos). La contribución de las PTWIs para el Cd y Pb fue muy baja, lo que demuestra la seguridad de estas harinas en relación con estos metales tóxicos.

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Palabras clave: *Harina de trigo. Metales tóxicos. ICP. Análisis cluster. Ingesta.*

Abbreviations

FAO/WHO: Food and Agriculture Organization/ World Health Organization.
HNO_3: Nitric acid.
NIST: National Institute of Standard and Technology.
PTWI: Provisional Tolerable Weekly Intake.
EFSA: European Food Safety Authorit.
DEC: Daily Estimated Consumption.
RDA: Recommended Dietary Allowances.
NSI: National Statistics Institute.
ICP: Inductively Coupled Plasma.
EDI: Estimated Daily Intake.
TDI: Tolerable Daily Intake.
MOS: Margin of Safety.
TWI: Tolerable Weekly Intake.

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Introduction

Cereals are the main source of food in many countries. Concerning human diet, the most important cereals are wheat (*Titicum*), rice (*Oryza sativa*), oats (*Avena sativa*), barley (*Ordeum vulgare*), rye (*Secale cereale*), corn (*Zea mays*) and millet (*Panicum miliaceum*)[1]. Among them, wheat is one of the most consumed[2] and spread. Together with rice it is the basic food of 4/5 parts of the world population. [3] Cultivation of wheat dates back to antiquity, when its harvest was essential for human diet. Today it highly contributes to the economy and the social structure of a country. The main producers are China, European Union, India and the USA.[4]

Most of the cultivated varieties of wheat can be classified depending on their usefulness, but among them hard wheat *(Triticum durum)* and soft wheat *(Triticum aestivum)* are the most important. Hard wheat is usually used for manufacture of diet pastries in Europe, mostly cultivated in dry areas and richer in proteins, water and calcium than soft wheat. On the other hand, soft wheat can be cultivated in hot or temperate areas and, when it breaks, the grain is harder at edge than in the middle, where it is floury. It is mostly used for bread manufacture in Europe and contains more fat, starch, iron, phosphorus and vitamin B than hard wheat.[3]

Nowadays, more than wheat itself, its derived products are especially relevant, from which wheat flour is the one to be stood out. Wheat flour is the irreplaceable raw material of a group of basic and essential food in a balanced diet like bread, pastries and cookies[2] as well as pizzas, sponge cakes and other starchy products.[4] Generally speaking, cereals are necessary for a healthy diet and nowadays a diary consumption of a media between 4 and 6 portions of cereals derived products is recommended due to their content in fibre, trace minerals and vitamins, which are supposed to prevent various diseases.[3] All cereal derived products are rich in carbohydrates, and therefore, the base of a well-balanced and healthy diet. This is reason for the importance of this group of food.

Manufacture of soft wheat flour in Spain has been growing gradually in the last years and it is mainly for domestic market, because it is enough to cover human soft wheat consumption in the country. Nevertheless, the Spanish wheat flour manufacture has maintained a stable export level which moves between 6% and 7% of total production. The same way, according to the data of the *National Statistics Institute* (NSI), total consumption of wheat flour in Spain has also been increasing during the last 15 years, due not only to the population growth, but also to an increase of the consumption per inhabitant a year. However the Canary Islands can be considered as a region with a great consumption of wheat flour, because the diary intake of this product per inhabitant is significantly higher than the national level.

From a technological point of view, quality of wheat flour is based on obtaining a final product with excellent organoleptic properties as flavour, colour and smell, as well as an appropriate level of humidity, a correct content of ash, an ideal particles size and absence of foreign substances.[5]

Some metals are essential for the development of life due to their importance in many of the biochemical reactions which are specific in human beings[6] and play a very important role at a pathological level, because their shortfall could produce certain diseases or reduce life expectancy, delay the growth or cause alterations in reproduction. On the other hand, minerals contribute to the flavour, the enzymatic reactions, the texture and the colour of food.[7]

In this group of elements are race elements or micro elements like iron, zinc, manganese and copper, between others, which have a physiological function in the organism and are daily necessary in different quantities between milligrams and micrograms. Apart from those, there are the macro elements, which are essential for normal life and necessary in higher quantities, more specifically in grams, like sodium, potassium, calcium and magnesium.

Minerals constitute between 1 and 3 per cent of the weight of a cereal grain and concentrate more in the external areas of the wheat grain.[5] From a nutritional and toxicological point of view, their presence is very important. The metallic content is very variable and will depend on the variety, the type of land where it has been cultivated, the fertilization that has been used and the weather.[8]

On the other hand, cereals do also contain heavy metals which, on the contrary, are not essential for the organism, and penetrate through the ground, the air and the water.[9] The importance of these routes of penetration depends on the type of ground and where it is placed and the agricultural practices that have been used. Concerning wheat flour, the content of heavy metals like lead and cadmium is usually very low.[7] The main characteristics of these trace elements are that they are not biodegradable, have no biological function, are able to accumulate in the organism and generate dysfunctions in the biological system, causing therefore serious problems to the human health. For this reason content of these toxic elements in cereals should be under control.

Material and methods

Samples

Sampling has been done in a wheat flour industry in the Canary Islands during April 2011 where 50 samples of two different wheat flours have been taken. The first one is a bread flour (A) made of three types of wheat of different origin, one coming from Canada and the two others from France. The other sample is a medium strength wheat flour (B) made of another mixing of wheat flours, one from Sweden, two from France, one from Canada and another one from Germany.

Samples treatment

Using plastic material to prevent contamination of metals, 10 grams of sample has been weighted in porcelain capsules. After that the samples were dried during 24 hours to remove the humidity of the wheat flour and approximately 2 ml of 65% concentrated HNO_3 were added in order to make a digestion previous to incineration. Wheat flour is a *complex master* so a good preparation of the sample is required.

Afterward, the sample was incinerated in an oven under a temperature that was gradually increased in 10º C every 60 minutes until the wished final temperature of 450º C was reached, 48 hours later.[10,11] The resultant ashes were subjected to another digestion with some drops of 65% concentrated HNO_3 in order to help oxidation of the organic matter that could last, removing then the rests of nitric through evaporation by means of a hot plate. After that, it was introduced again into the oven and, following the same previous process, white ashes were obtained. These white ashes were filtered in order to avoid residues that could affect the analytic instrument and then dissolved with 1.5% HNO_3 up to a volume of 25 ml.[12]

Analytic process to determine macro, micro and trace elements

Macro (Na, K, Mg and Ca), micro (Mn, Fe, Cu, Zn, Cr and Ni) and trace toxic elements (Cd and Pb) were determined by inductively coupled plasma optical spectrophotometry (ICP) by means of the Thermo Scientific ICAP 6300 spectrophotometer. Concentration was calculated through extrapolation on calibration straights made from model solutions of already known concentrations and the quantification limit for each of the analysed metal is 0.010 mg/l.

Quality control

The quality control for the analysing method that was used to determine the studied metals was made by means of certified referenced materials MRC-1515 Apple Leaves and MRC-1573a Tomato Leaves of the National Institute of Standards and Technology (NIST). The recuperation percentages obtained in this study for each referenced material were all over 94%.

Statistic analysis

The statistic analysis in this study was made with the data analysis program SPSS Inc., version 19.0. In order to determinate if there were significant differences between both wheat flours concerning their mineral content, first of all, a normality study was made. Therefore the *Kolmogorov-Smirnov* test was used to compare the samples and determine if they followed or not a normal distribution. The statistic *Levene* test on homogeneity of the variances was also done.[11] Once these tests were finished, it could be observed that there was no normality and therefore, the types of wheat flour did not meet the conditions to be subjected to parametric tests. Therefore a non-parametric *Kruskal-Wallis* test was used to verify the significant differences between the two types of wheat flour. On the other hand, a *cluster analysis* was made by means of a hierarchical distribution in order to check the distribution level of the analyzed wheat flour depending on their content of metals (Ca, Cd, K and Zn) where significant differences between both samples could be observed.

Table I
Medium levels of mineral elements in samples of two types of whole wheat flour (medium ± σ = mg/kg fresh weight)

Type of wheat flour	*Wheat flour A*	*Wheat flour B*
Macro elements		
Na	126.5 ± 62.98	169.4 ± 56.36
K	933.9 ± 97.50	761.1 ± 68.96
Ca	200.6 ± 14.94	179.4 ± 10.37
Mg	226.7 ± 10.54	226.4 ± 6.632
Micro elements		
Mn	4.309 ± 0.187	4.414 ± 0.171
Fe	8.631 ± 1.717	8.168 ± 0.662
Cu	2.275 ± 1.553	2.866 ± 0.981
Zn	6.154 ± 0.313	6.314 ± 0.211
Cr	0.132 ± 0.130	0.079 ± 0.067
Ni	0.080 ± 0.059	0.088 ± 0.030
Trace toxic elements		
Cd	0.027 ± 0.002	0.023 ± 0.002
Pb	0.037 ± 0.013	0.056 ± 0.045

σ = standard deviation; wheat flour A = "bakery"; wheat flour B = "medium strength".

Results and discussion

Mineral composition of both types of wheat flour

Macro elements: sodium, potassium, magnesium and calcium

Table 1 shows the media concentration of the analysed macro elements, their standard deviation and the margin between minimum and maximum.

First of all, the highest content of sodium can be found in the wheat flour type B of medium strength (169.4 ± 56.36 mg/kg), although there are no big differences between both types of wheat flour. On the other hand, stands out that in type A (bread flour) levels of potassium (933.9 ± 97.50 mg/kg) and calcium (220.6 ± 14.94 mg/kg) are higher, being these macro elements the main difference between both types.

For both of them concentration of magnesium is similar (226.7 ± 10.54 mg/kg and 226.4 ± 6.632 mg/kg respectively), so there have not been found significant differences between wheat flour type A and type B concerning this macro element.

The content of each macro element follows the sequence K > Mg > Ca > Na in both of the analyzed types of wheat flour. The most found mineral is potassium (K), followed by magnesium and calcium, a fact that matches other results found in bibliography.[1]

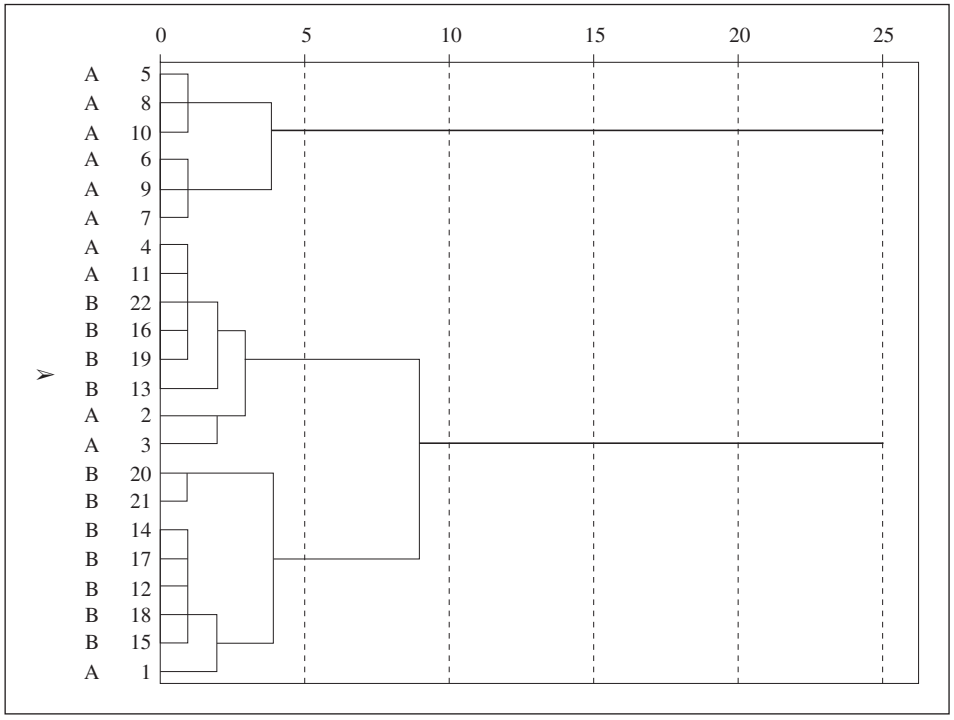

Fig. 1.—Cluster analysis of the wheat flour samples.

Micro elements: manganese, iron, copper, zinc, chrome and nickel

The same way results for micro elements have been analysed and descriptive data are shown in table I.

In this case it is important to stand out that content of manganese, copper, zinc and nickel were found in wheat flour type B (medium strength); however, wheat flour type A (bread flour) has a higher content of iron and chrome (8.631 ± 1.717 mg/kg and 0.132 ± 0.130 mg/kg respectively).

Significant differences in this group of trace elements were found only for zinc.

The content of micro elements is in the following order: Fe > Zn > Mn > Cu > Cr > Ni. The main trace element is Fe, as it can also be found in the bibliography.[1]

Toxic trace elements: cadmium and lead

Wheat flour type A has a slight higher content of cadmium (0.027 ± 0.002 mg/kg), but the contrary happens with lead, whose medium content is higher (0.056 ± 0.045 Pb/kg) in wheat flour type B. Results can be checked in table 1.

Results of the *Kruskal-Wallis* test show that for cadmium there have been found significant differences between both samples, the contrary than for lead. There has to be taken into account that the concentration of lead in both types of wheat flour was sometimes lower than the quantification limit (0.010 mg/l).

On the other hand, in both cases levels do not exceed the maximum permitted by Regulation (CE) num. 1881/2006 that fixes a limit of some contaminants in food, specifically in cereals like wheat. The maximum limit permitted for cadmium and lead is 0.20 mg/kg fresh weight. This fact allows us to confirm that the analysed wheat flour is safe concerning these two toxic metals that have been analyzed.

Figure 1 shows the dendrogram obtained through the cluster analysis, in which it can be observed that there is a clear difference between the analyzed wheat flour, clearly divided into two groups. A first group where only type A wheat flour (bread flour) can be found and a second group mainly formed by type B wheat flour (medium strength) and some type A, whose values of the parameters studied in the cluster analysis (Ca, Cd, K and Zn) are closer to medium strength wheat flour than to bread flour.

Comparison of our results with other author's results

Macro elements: sodium, potassium, magnesium and calcium

Table II shows the results obtained by other authors in the content of sodium, potassium, magnesium and calcium in whole wheat samples and those obtained in the present study.

The medium levels of the determined macro elements in both types of wheat flour are quite lower in the case of sodium and potassium, slightly lower in the case of magnesium and similar in the case of calcium in comparison with other authors.[6, 13, 14]

Table II
Comparison of the obtained results for macro and micro elements with other author's results (mg/kg fresh weight)

Origin	*Na*	*K*	*Mg*	*Ca*	*Mn*	*Fe*	*Cn*	*Zn*	*Cr*	*Ni*	*Reference (year)*
Spain	–	–	–	–	–	–	–	–	0.03-0.09	0.21-0.29	González et al.,[7] 2001
India	–	–	–	–	18.6-49.4	–	3.26-6.05	17.1-27.2	–	0.48-2.22	Roychowdhury et al.[15], 2003
Italy	–	–	–	–	11.8-18.7	36.9-38.0	5.11-8.82	87.5-90.6	–	–	Locatelli,[16] 2004
Brazil	–	–	–	–	15.0	–	0.9	5.0	0.022	0.062	Santos et al.,[17] 2004
India	362	1900	–	160	29.7	43.8	–	20.3	0.69	–	Singh & Garg,[6] 2006
China	–	1018-1495	233-504	180-318	4.9-10.5	5.5-15.7	1.5-2.8	7.2-12.7	–	–	Tang et al.,[13] 2008
Brazil	–	–	289-312	–	2.2-2.5	–	0.88-0.92	2.9-3.2	0.083-0.091	–	Nardi et al.,[14] 2009
Spain	126.5-169.4	761.1-933.9	179.43-200.6	226.4-226.7	4.31-4.41	8.17-8.63	2.28-2.86	6.15-6.31	0.08-0.13	0.08-0.09	Current study, 2011

Micro elements: manganese, iron, copper, zinc, chrome and nickel

The comparison between the results of the analysed micro elements obtained in this study and those of other authors can be checked in table II.

In the case of manganese, the medium concentrations determined in this study (2011) are quite lower than those of other authors,[6, 13, 15, 16] except than the data published by Nardi et al.,[14] where they obtained approximately half of the content than we did.

The values of iron determined in wheat flour of Tenerife (Spain) are within the range obtained by Tang et al.,[13] but much lower than those provided by other authors.[6,16]

In the case of copper, the results obtained are similar to those of Tang et al.,[13] higher than those obtained by Nardi et al.[14] and Santos et al.[17] and, finally, lower than those found by Roychowdhury et al.,[15] and Locatelli.[16]

The concentrations of zinc in both analyzed wheat flour found in this study (2011) are quite lower than other results published by other authors,[6,15,16] slightly lower than those obtained by Tang et al.,[13] and higher than those found by Nardi et al.[14] and Santos et al.[17]

Comparing the values of chrome in whole wheat obtained in this study, it can be observed that they are similar to those of other authors[6,7,14] and higher than those found by Santos et al.[17]

Excepting Santos et al.,[17] who publish in their study concentrations that are slightly lower than those in our study, data of nickel in both types wheat flour are quite lower than those obtained by other authors.[7, 15]

Toxic trace elements: cadmium and lead

Table III shows concentrations of cadmium and lead in whole wheat obtained by other authors, as well as those found in this study.

All studies that were found publish concentrations of cadmium between 0.006-0.24 mg/kg in the whole wheat analyzed. In this study it has been found a medium content within this margin. In this case, the reason for this value is due the fact that concentrations of heavy metals in wheat flour are usually very low.[7] Levels of cadmium vary depending on the part of the grain, but the highest concentrations have been found in the bran and the wheat germ (approximately 0.1- 0.3 mg Cd/kg), while levels in wheat flour usually move between 0.02 and 0.05 mg/kg.[16] Therefore, it can be confirmed that the results of this study are within the margin of those commonly found in this kind of products.

After having compared our contents of lead with those of various authors, it can be assumed that they are similar to those purposed by Zhang et al.,[19] much higher than those found by Santos et al.,[17] and lower than the results of Locatelli.[16]

In conclusion the content of minerals in whole wheat flour is very variable, because it depends on facts like the geographic origin of the grain, the wheat texture

Table III
Comparison of the obtained results for toxic trace elements with other author's results (mg/kg fresh weight)

Origin	*Cd*	*Pb*	*Reference (year)*
Colorado (U.S.A.)	0.050	–	Lorenz et al.,[30] 1995
China	0.0157	0.0351	Zhang et al.,[19] 1998
China	0.11-0.24	–	Zhang et al.,[31] 2000
Italy	0.04-0.08	0.49-0.89	Locatelli,[16] 2004
Rio de Janeiro (Brazil)	0.006	0.0002	Santos et al.,[17] 2004
Brazil	0.009-0.016	–	Araujo et al.,[4] 2008
Taiwan	0.017-0.0184	–	Chen & Jiang,[32] 2009
Tenerife (Spain)	0.023-0.027	0.037-0.056	Current study, 2011

Table IV
Dietetic consumption of macro and micro elements and their percentage contribtuion to a consumption of 3.5 g/day of whole wheat flour

	RDA (mg/day; µg/día)*		*EDI (mg/day; µg/día*)*		*Percentage contribution to consumption (%)*			
			Wheat flour A	*Wheat flour B*	*Wheat flour A*		*Wheat flour B*	
	Men	*Women*			*Men*	*Women*	*Men*	*Women*
Macro element								
Na	1,500	1,500	0.443	0.593	0.030	0.030	0.040	0.040
K	3,100	3,100	3.269	2.664	0.105	0.105	0.086	0.086
Mg	350	300	0.794	0.792	0.227	0.265	0.226	0.264
Ca	900	1,000	0.702	0.628	0.078	0.070	0.070	0.063
Essential trace element								
Mn	2.3	1.8	0.015	0.015	0.652	0.833	0.652	0.833
Fe	9.0	18.0	0.030	0.029	0.333	0.167	0.322	0.161
Cu	1.1	1.1	0.008	0.010	0.727	0.909	0.727	0.909
Zn	9.5	7.0	0.022	0.022	0.232	0.314	0.232	0.314
Cr*	35.0	25.0	0.463	0.280	1.323	1.852	0.800	1.120

Wheat flour type A = "bakery"; wheat flour type B = "medium strength".

and the characteristics of the technological process implied in the milling phase.[7] Other factors that do also have an influence in trace elements concentration of the cereal are the following: the type of cultivation, the ground conditions, and the weather conditions during the growth and the use of fertilizers.[8]

Comparing the data shown by the authors in the food composition tables[20,21,22,23,24] with our study, it can be considered as a relevant result that our levels of sodium were quite higher. On the other hand, in this study lower levels of K, Mn and Fe and similar levels of Ca, Mg, Cu and Zn were found. As it can be observed, cereals constitute an important food commodity due to their essential origin of minerals.[7]

Estimate daily consumption and percentage contribution to the recommended values

Table IV shows the intake of each of the analyzed macro and micro elements for each type of wheat flour, so the dietetic contribution of this food to the Spanish population between 17 and 60 years, men and women, can be estimated. In order to calculate the Daily Estimated Consumption (DEC) it has been taken a medium consumption of 3.5 g/day of wheat, published in the "Spanish diet model for determination of consumers exposure to chemical substances".[25] The Reference Dietetic Consumption for the Spanish population has been used to know the contribution.[26]

From a quantitative point of view, table 4 shows that Estimated Daily Intake (EDI) of each macro and microelement were similar for both wheat flour, excepting sodium, potassium, calcium and chrome. In the case of sodium, the EDI in wheat flour type B is higher than in type A. On the other hand, wheat flour type A had the highest EDI of calcium, potassium and chrome.

Among the analyzed macro elements, potassium has the highest EDI, but magnesium had the biggest contribution to consumption in both types of wheat flour and for the whole population. The contribution of the rest

Table V
Toxicological assessment of lead and cadmium consumption depending on the type of whole wheat flour

	Wheat flour A			*Wheat flour B*		
	Cd[a]	*Cd*[b]	*Pb*[a,b]	*Cd*[a]	*Cd*[b]	*Pb*[a,b]
Mean (μg/kg)	27.26	27.26	37.48	23.63	23.63	55.61
Consumption (μg/week)	0.668	0.668	0.918	0.579	0.579	1.362
MOS (pers. 65 kg)	564.4	243.3	1770.2	651.1	280.7	1193.1
MOS (pers. 70 kg)	607.8	261.9	1906.3	701.2	302.2	1284.9
MOS (pers. 75 kg)	651.2	280.7	2042.5	751.3	323.8	1376.7

[a]Refered to FAO/OMS.
[b]Refered EFSA.

of macro elements were similar in both types of wheat flour, both for men and women and had the following sequence: K > Ca > Na.

In the case of micro elements it is important to stand out that there are no differences between both types of wheat flour concerning contribution to the Recommended Dietary Allowances (RDA), excepting chrome: type A has slightly higher levels. On the other hand, it can be observed that between male and female there are differences in all estimated micro elements, because the RDA varies significantly from one sex to the other. Only in the case of iron, the contribution is lower for women, because their RDA varies significantly from the men's one. This fact is not positive, especially for pre-menopausal women.

Concerning this group of minerals, iron was the one that reached the highest DEC, followed by zinc, manganese, copper and chrome. The element that mostly contributed to consumption was chrome, followed by copper, manganese, iron and zinc.

In the case of nickel, the EDI could not be determined, because the RDA for this micro element could not be found, so its percentage contribution cannot be known.

Toxicological assessment of cadmium and lead consumption

In order to assess the consumption of cadmium and lead from a toxicological point of view, a Margin of Safety (MOS) has been used, being it the relation between an acceptable dose for health, in this case Provisional Tolerable Weekly Intake (PTWI) or Tolerable Weekly Intake (TWI), and the consumption and Tolerable Daily Intake (TDI). Once this value has been obtained, it will be concluded if there is a risk or not. If the resulting MOS < 1, there is risk; MOS = 1 means a reason to worry; MOS > 1 and < 10 means no theoretic risk, but reason to worry and if MOS > 10 there is no risk at all.[27] It has been considered a consumption of 3.5 grams/person/day of whole wheat flour and a PTWI for cadmium and lead of 5.8 and 25 μg/kg body weight/week respectively, established by the mixed committee Food and Agriculture Organization/World Health Organization (FAO/WHO).[28,29] Furthermore, the EFSA (European Food Safety Authority) has established a TWI for cadmium in 2009 of 2.5 μg/kg body weight/week.[29]

The safety margins of cadmium that was calculated using the PTWI established by the mixed committee FAO/WHO and those calculated by means of the TWI fixed by the EFSA are shown in table V. In addition, safety margins for lead have been calculated the same way (table V). In both cases the risk has been studied depending on the type of wheat flour that has been consumed. As it can be observed, for both elements MOS is much higher than 10, so there is no risk for the health of consumers.

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