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Risk Assessment of the Intake of Pb, Cd and Hg by Consumption of Spanish Cheeses

Moreno-Rojas R*, **Sánchez-Segarra PJ, F. Cámara-Martos and Amaro-López MA**

Department of Food Science and Food Technology. University of Cordoba, Campus de Rabanales, Darwin Building C-1, 14040 Córdoba, Spain

Abstract

The presence of heavy metals in foods may pose a risk to human health. The main objective of this paper is to carry out a risk assessment study of lead, cadmium and mercury intake by consumption of Spanish cheeses. In general, the concentration levels found were far from the Provisional Tolerable Weekly Intake (PTWI) for these elements except for mercury in Gata-Hurdes cheese indicating a possible contamination by a unknown source. On the other hand, the concept of toxic density is a powerfull tool which can help to initially assess potential risk, focusing on those cheeses exceeding 100% toxic density.

Keywords: Toxic density; Lead; Mercury; Cadmium; Spanish cheeses; Risk assessment

Introduction

One of the most important goals of nutrition consists of fulfilling the recommended energy intake (2900 Kcal/day for adults between 25 and 50 years) and most of the Recommended Dietary Allowances (RDA) for each micronutrient. Only then, certain long-term deficiency diseases can be prevented such as scurvy, anemia, osteoporisis and pellagra among others. The concept of nutrient density was suggested 20 years ago to define the relationship between the nutrient composition of a food and its caloric content. A high nutrient density points out those foods which are good sources of micro or macronutrients, because such foods contribute to the intake of the essential nutrients while helping to fulfill caloric needs. This concept is especially useful when the caloric intake is low and it is crucial that foods with high nutrient density are included in diet. On the contrary, low nutrient density could lead to situations in which an excess of kilocalories should be consumed to fulfill the need of essential micronutrients causing other pathologies such as overweight and obesity.

However, foods also contain contaminants, biological and chemical hazards such as aflatoxins, pesticides, and heavy metals which threaten human health. We think that the application of the previous concept to these types of substance could be very helpful in order to know the safety or toxicity level of a certain food. Therefore, the concept "toxic density" is proposed [1,2] as a coefficient expressed in percentage terms which measures the relationship between the concentration of a toxic component present in a food and the energetic supply of this food against the Provisional Tolerable Weekly Intake (PTWI) for this component and the required energy for this period of time. Therefore, it is a numeric non-dimensional expression that can measure the convenience or suitability of a given food in the diet if the concentration of the specific toxic component and the energetic supply attributed to this food are known. The benchmark value is 100%, which indicates that, if the food is consumed in a sufficient quantity to fulfil all caloric requirements, it would be within the allowed maximum intake for the toxic component [3]. Values above 100% are related to a considerable toxicology risk in relation to the energy supplied, if the food is consumed [4].

In a recent study carried by our research group [4], this concept was applied to the heavy metal content (Pd, Cd y Hg) in Spanish cheeses belonging to different Protected Designation of Origin (PDO). Spain is the fourth country in Europe after France, Greece and Italy, where cheeses have a quality standard which certificates claim that the product has been manufactured in a specific region under determined raw material and manufacture conditions. Furthermore, consumption of cheese in Spain is relatively high, with 18 Kg person^{-1} year^{-1} or ~ 50 g person^{-1} day^{-1} [5].

Because the high toxicity and the enormous adverse effect of health which can be derived from the chronic intake of heavy metals through diet, risk assessment studies should be carried out. Risk Assessment (RA) provides an estimation of the probability and impact of an adverse health effect attributable to potentially contaminated food [6]. This methodology is the basis used by governments and international organization to set food policies and assess potential risks associated with food consumption. This methodology consists of 4 steps which correspond to: Hazard Identification; Hazard Characterization, Exposure Assessment and Risk Characterization. However, the development of this type of studies, based on probabilistic calculations, requires to know the consumption patterns, the population characteristics in addition to needing great amount of data [7]. From that, we propose the concept of toxic density as method which can help to initially assess potential risk, focusing on those elements exceeding 100% toxic density.

The aim of this work consisted of carrying out a risk assessment study for the intake of Pb, Cd y Hg by consumption of Spanish cheeses belonging to different PDOs by comparing results obtained with values of toxic density for the same samples, and using this concept to independently assess those samples which presented high values of toxic density.

***Corresponding author:** R. Moreno-Rojas, Department of Food Science and Food Technology. University of Cordoba, Campus de Rabanales, Darwin Building C-1, 14040 Córdoba, Spain, Tel: +34 957 21 20 01; Fax: +34 957 21 20 00; E-mail: rafael.moreno@uco.es

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Material and Methods

Samples and mineral content determination

57 varieties of cheese were selected of different PDOs in Spain. Principal characteristics of each analysed cheese (milk type, coagulation, salting and ripening conditions, etc) are shown in Table 1. Four testing set containing all samples were made during the whole period of study. Cheese samples were chopped with a plastic knife, kept in polypropilene packs and refrigerated before being analyzed.

Samples were analysed following the method of Moreno-Rojas et al. [8]. Briefly, a total of 10 g of fresh cheese were taken and to prevent heavy metals loss by volatilization, dried samples were incinerated in a furnace, applying the following mineralization stages: 90 to 250°C (ramp time 1 h, hold time 1 h), 460°C (ramp time 2 h, hold time 6 h) and 460 to 100°C (ramp time 2 h). After cooling, 2 ml nitric acid (2 N) was added and the solutions, after drying on a thermostatic hot plate, were returned to the furnace for a further 1 h at 460°C. Ash was recovered using 5 ml nitric acid 2 N and 20 ml nitric acid 0.1 N

Cheese	Milk	Rennet	Coagulation	Ripenning	Origin
Ahumado de aliva	Cow + Sheep + Goat	Animal	Mixed	Ripened	Cantabria
Afuega´l Pitu	Cow	Lactic ferments	Lactic	Fresh	Asturias
Aracena	Goat	Animal	Enzimatic	Ripened	Andalucía
Arzua	Cow	Animal	Mixed	Ripened	Galicia
Los balanchares	Goat	Animal	Enzimatic	Ripened	Andalucía
Beyos	Cow + Goat	Lactic ferments	Lactic	Ripened	Asturias
Burgos	Sheep + Cow	Cl_2Ca + animal	Enzimatic	Fresh	Castilla-Leon
Cabrales	Cow + Sheep + Goat	Animal	Mixed	Ripened	Asturias
Cadiz	Goat	Animal	Enzimatic	Fresh	Andalucía
Cantabria	Cow	Lactic ferments + Cl_2Ca + animal	Enzimatic	Ripened	Cantabria
Casin	Cow	Animal	Enzimatic	Ripened	Asturias
Castellano	Sheep	Animal	Enzimatic	Ripened	Castilla-León
Cebreiro	Cow	Lactic ferments	Lactic	Ripened	Galicia
Conejero	Goat	Animal	Enzimatic	Fresh	Islas Canarias
Flor de guia	Goat + Cow + Sheep	Vegetable	Enzimatic	Ripened	Islas Canarias
Friol	Cow	Animal	Mixed	Ripened	Galicia
Gamonedo	Cow + Sheep + Goat	Animal	Mixed	Ripened	Asturias
Garrotxa	Goat	Lactic ferments + Cl_2Ca + animal	Mixed	Ripened	Cataluña
Gata-hurdes	Goat	Animal	Enzimatic	Ripened	Extremadura
Gaztazarra	Sheep	Animal	Enzimatic	Ripened	País Vasco
Genestoso	Cow	Lactic ferments + Cl_2Ca + animal	Mixed	Ripened	Asturias
Grazalema	Goat	Animal	Enzimatic	Ripened	Andalucía
Herreño	Goat + Cow + Sheep	Animal	Enzimatic	Fresh	Islas Canarias
Ibérico	Cow + Sheep + Goat	Animal	Enzimatic	Ripened	Castilla
Ibores	Goat	Animal	Enzimatic	Ripened	Extremadura
Idiazabal	Sheep	Animal	Enzimatic	Ripened	Navarra
Mahon	Cow	Animal	Enzimatic	Fresh	Islas Baleares
Majorero	Goat	Animal	Enzimatic	Ripened	Islas Canarias
Manchego	Sheep	Animal	Enzimatic	Ripened	Castilla-La Mancha
De Murcia	Goat	Cl_2Ca + animal	Enzimatic	Fresh	Murcia
De Murcia al vino	Goat	Lactic ferments + Cl_2Ca + animal	Enzimatic	Ripened	Murcia
Palmero	Goat	Animal	Enzimatic	Ripened	Islas Canarias
Del páramo leonés	Sheep	Lactic ferments + Cl_2Ca + animal	Mixed	Ripened	Castilla-León
Pasiego	Cow	Animal	Enzimatic	Fresh	Cantabria
Los Pedroches	Sheep	Vegetable	Enzimatic	Ripened	Andalucía
La Peral	Cow	Lactic ferments + Cl_2Ca + animal	Enzimatic	Ripened	Asturias
Peñamellera	Cow + Sheep + Goat	Lactic ferments + Cl_2Ca + animal	Enzimatic	Ripened	Asturias
Picón	Cow + Sheep + Goat	Animal	Mixed	Ripened	Cantabria
Porrua	Cow + Sheep	Animal	Enzimatic	Fresh	Asturias
Quesuco	Cow + Sheep	Cl_2Ca + animal	Enzimatic	Ripened	Cantabria
Roncal	Sheep	Animal	Enzimatic	Ripened	Navarra

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Ronkari	Sheep	Animal	Enzimatic	Ripened	País Vasco
Queso de Ronda	Goat	Animal	Enzimatic	Fresh	Andalucía
San Simón	Cow	Animal	Enzimatic	Ripened	Galicia
La serena	Sheep	Vegetable	Enzimatic	Ripened	Extremadura
Serrat	Sheep	Animal	Enzimatic	Ripened	Cataluña
Servilleta	Goat + Sheep	Vegetal	Enzimatic	Fresh	Valencia
Tenerife	Goat	Animal	Enzimatic	Fresh	Islas Canarias
Tetilla	Cow	Animal	Mixed	Ripened	Galicia
La tercia	Goat	Animal	Enzimatic	Ripened	Castilla-León
Tietar	Goat	Animal	Enzimatic	Fresh	Castilla-León
Torta del casar	Sheep	Vegetable	Enzimatic	Ripened	Extremadura
Tronchon	Sheep + Goat	Animal	Enzimatic	Ripened	Cataluña
Tupi	Sheep	Animal	Enzimatic	Ripened	Cataluña
Ulloa	Cow	Animal	Enzimatic	Ripened	Galicia
Vidiago	Cow	Lactic ferments + Cl_2Ca + animal	Enzimatic	Ripened	Asturias
Zamorano	Sheep	Animal	Enzimatic	Ripened	Castilla-León

Table 1: Brief description of general characteristics of the cheese sample studied.

in a 25 ml volumetric flask and stored in polypropylene flasks under refrigeration.

Determination of Pb and Cd total contents was performed using a Perkin-Elmer model 2100 atomic absorption spectrophotometer connected to a Perkin-Elmer HGA-700 graphite furnace running an M-2100 Multielement Program Software. Argon was used as internal and external gas with a hollow cathode lamp for Pb and Cd and a deuterium lamp as a background corrector. Graphite pyrolytically coated tubes with L'vov platform were employed. A study of standard additions was carried out to prevent heavy metals loss and to corroborate the linear calibration of the apparatus. With regard to the chemical modifier, 2 g $NH_4H_2P0_4$ + 0.2 g $Mg(N0_3)\cdot 6H_2O$ in 100ml deionized H_2O were used but no beneficial effect was observed.

For the determination of total Hg a wet digestion method was used. A quantity of 0.5 g freeze-dried of sample was weighed into Teflon® flasks and 3 mL of nitric acid (68%) and 0.5 mL of hydrogen peroxide (30%) mixture was added. The flasks were heated under reflux in a microwave oven (MAS-2000 CEM). After cooling the solution was brought to 15 mL with desionized water prior to add 3 drops of potassium permanganate (5%) to favour pre-reduction of Hg. Final solutions were transferred to polypropylene flasks.

Total Hg was determined by a cold vapour technique adapted to a Flow Injection Atomic Spectroscopy system (FIAS 400 Perkin-Elmer) with a wavelength of 253.7 nm, split of 0.7 nm, Ar as a carrier gas and BH_4Na-NaOH of reductor agent. The analytical methodologies were validated using the certified reference material, BCR-150 and BCR-151 from Community Bureau of Reference. The results of these analyses (Pb: 1.05 ± 0.035, Cd: 22.5 ± 0.7, Hg: 103.4 ± 3.1) were in a good agreement with the reference values (Pb: 1.0 ± 0.04, Cd: 21.8 ± 1.4, Hg: 101 ± 10). For each element being determined, the analyses included triplicate of samples.

Statistical analysis

The exposure assessment model was developed to estimate the intake level for each metal derived from consumption of Spanish cheese. For that, concentration levels for heavy metal species were combined with the consumption level of Spanish cheeses (18 Kg person^{-1} year^{-1} or ~ 50 g person^{-1} day^{-1}) provided by the Spanish Consumption Ministry [5] which does not distinguish the type of cheese, so an only consumption value was used to estimate the intake.

The exposure assessment model followed a probabilistic approach including probability distributions for calculations. Therefore, probability distributions describing the metal concentration data were fitted by using the method of moments. Concentration data from different cheeses were grouped into one single set in order to give a representative level of exposure to each heavy metal by consumption of Spanish cheeses. By doing so, the exposure variability could be considered providing results of intake for the whole Spanish population. The assumption here was that all cheeses are consumed by Spanish population in the same proportion. Particularly, as exception, those cheeses presenting high toxic density, above 120 %, were also studied in an individual exposure assessment model to provide more detailed information.

The exposure model did not include a separation between the variability and uncertainty of input variables (so-called first order model). The fitting to data was assessed by using different statistical tests such as kolmogorov-Smirnov test and Chi-square test. In addition, a visual analysis was equally considered to assess the fitting of the probability distributions to concentration data. The model was simulated using a simulation technique implemented in Excel Microsoft © software. The simulation was run using 10,000 iterations for each heavy metal specie and the value of the seed used for the Random Number Generator on which the simulation was based to single value in all simulation carried out in order to make results comparable.

Results and Discussion

Statistical tests applied to log-transformed concentration data (i.e., Kolmogorov-Smirnov test and Chi-square test) indicated that the most suitable probability distribution to represent metal concentrations was the normal distribution which was defined by mean and standard deviation of log-transformed data. Similarly, the visual analysis indicated that these data when represented in bar histogram showed bell-shaped-like aspect confirming the normal assumption for concentration data. The parameters mean and standard deviation for

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the fitted normal distributions correspond to 1.1 and 0.44 log μg/kg for Hg; 0.6 and 0.27 log μg/kg for Cd; and 1.4 and 0.25 log μg/kg for Pb.

In order to estimate the Pb intake level, the fitted normal distribution to concentration data was combined with the consumption level for cheese. This model was simulated according to description given in materials and methods. As higher heavy meal levels than those observed in our survey are also possible in reality, distributions were not truncated at any maximum value. Nevertheless, resultant simulated values were situated in a realistic concentration range. The simulated distribution shown in Figure 1 represents the distribution of intake level for Pb in the Spanish population derived from Spanish cheese consumption. In that Figure, it can be observed that intake levels ranged between -1.70 and 0.11 log μg kg^{-1} body weight week^{-1}. The average corresponded to -0.80 log μg kg^{-1} body weight week^{-1}, and the 95th percentile was -0.36 log μg kg^{-1} body weight week^{-1} which means that 5 % Spanish population was exposed to level above this intake level.

Assuming that Pb contamination in foods is unavoidable, based on previous data, it can be stated that 5 % population would be exposed to Pb levels above 0.44 μg kg^{-1} body weight week^{-1} (derived from the logarithmic transformation of the earlier percentile). To calculate that, a daily consumption of 50 g (4 or 5 pieces) of cheese for a person with body weight of 60 Kg was used. That amount is far from the Provisional Tolerable Weekly Intake (PTWI) for this element which corresponds to 25 μg kg^{-1} body weight week^{-1} [9]. These results are in agreement with values of toxic density obtained for all cheeses in which none cheese exceeded 50% [4].

Regarding Cd, intake levels ranged between -2.63 and -0.70 log μg kg^{-1} body weight week^{-1} (Figure 2). The average corresponded to -1.66 log μg kg^{-1} body weight week^{-1}, and the 95th percentile was -1.19 log μg kg^{-1} body weight week^{-1}, which means that 5 % Spanish population was exposed to level above 0.06 μg kg^{-1} body weight week^{-1}. Again this amount is still below the PTWI established for Cd, 7μg kg^{-1} body weight week^{-1} [3]. These results are in agreement with previous studies [10, 11] which suggest a low content of Cd in dairy products due to, in part, the negligible transfer of Cd from feed into milk.

However, in the present study, some cheeses presented a value of toxic density above 100%, and especially for Porrúa (132%) cheese.

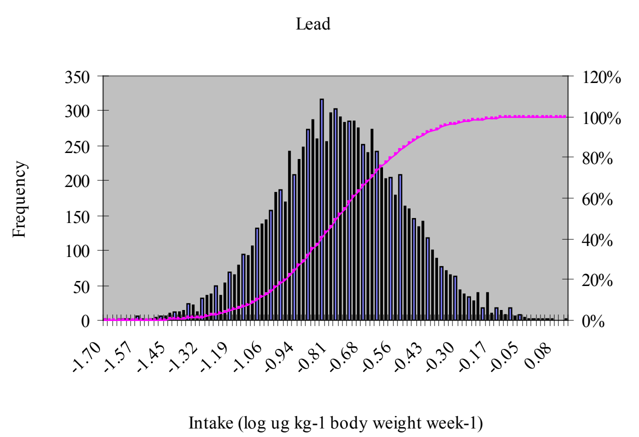

Figure 1: Similuted distributions of the lead intake by consumption of Spanish cheeses.

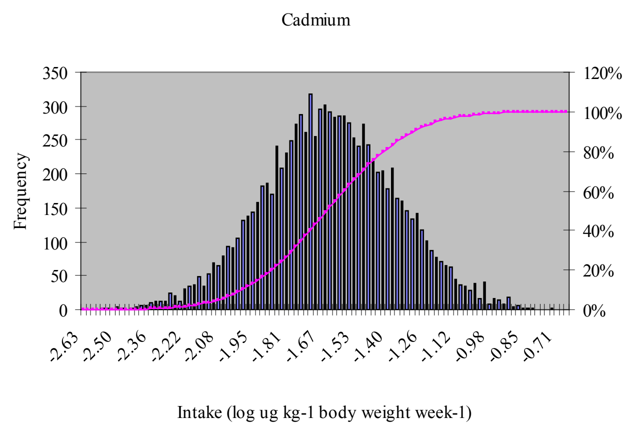

Figure 2: Similuted distributions of the cadmium intake by consumption of Spanish cheeses.

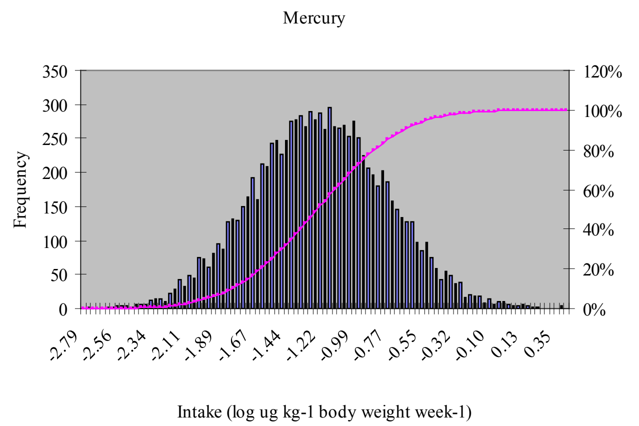

Figure 3: Similuted distributions of the mercury intake by consumption of Spanish cheeses.

From that, and because the toxicity of Cd is 1000 times higher than Pb being able to cause changes in many enzymes dependent on Zn, hypertension, arteriosclerosis and growth inhibition [12], we decided to carry out a individual study for this type of cheese. Results from the individual exposure assessment model for Porrua cheese indicated that the 95th percentile was slightly higher (i.e. 0.08 μg kg^{-1} body weight week^{-1}) than the value obtained for model including all cheeses. Nonetheless, this value is still far from the PTWI for Cd due to insignificant Cd contamination in dairy products.

Finally although Hg^0 is not a common food contaminant given that it is only absorbed in small proportions, organomercury (especially methylmercury) could be present as food contaminant, posing a serious health risk. In our study, intake levels ranged between -2.77 and 0.41 log μg kg^{-1} body weight week^{-1}(Figure 3). The average corresponded to -1.23 log μg kg^{-1} body weight week^{-1}, and the 95th percentile was -0.51 log μg kg^{-1} body weight week^{-1} which means that 5 % Spanish population was exposed to level above 0.31 μg kg^{-1} body weight week^{-1}. Again, values fell below the safety level for this heavy metal. A PTWI for total mercury of 5 μg kg^{-1} body weight week^{-1} has been established, of which no more than 3.3 g kg^{-1} body weight week^{-1} should be present as methylmercury [13].

On the basis of the values of toxic density obtained for our cheeses [4], most them were lower than 50%, excepting for some varieties which had slightly higher values such as Aracena (77.78%) and Herreño (82.80%) cheeses. However there was a sample which presented very high values of toxic density, Gata-Hurdes cheese (325%), indicating a possible contamination by an unknown source. For that, a risk assessment study was specifically carried out for this type of cheese.

Results from the individual exposure assessment model for Gata-Hurdes cheese indicated that the 95th percentile was much higher (i.e. 1.65 μg kg^{-1} body weight $week^{-1}$) than that obtained for model including all cheeses. Similarly, maximum and mean value showed higher values (i.e. 2.59 and 1.19 μg kg^{-1} body weight $week^{-1}$, respectively) than those obtained from the general exposure model, suggesting that this type of cheese could more contribute to Hg intake thereby corroborating the results for the toxic density. Levels were quite close to the established PTWI for Hg, even though this fact was more relevant for the 95th percentile and maximum value.

Conclusions

Toxicological evaluation of Spanish cheeses using methodological tools such as risk assessment models and toxic density showed that cheese is generally a safe food with respect to Pb, Cd and Hg. Nonetheless, a more specific toxicological evaluation showed that Hg was significantly high, especially for Gata-Hurdes cheese. This result corroborates the obtained toxic density value while highlighting the importance of this concept as tool to assess safety level in foods.

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