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Detection of Aflatoxin M1, Heavy metals (As, Pb, Hg, Cd, Al) and Polychlorinated biphenyls in demineralized whey powder produced from sweet whey

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Abstract

In this study, aflatoxin M1 (AFM1), heavy metals (As, Pb, Hg, Cd, Al) and polychlorinated biphenyls (PCBs), were evaluated in 90% demineralized whey powder (DWP-90), produced from sweet whey. For this purpose, 12 DWP-90 samples (400g/package) collected during 6 months. AFM1 was found in 100% of the samples. The average, maximum and minimum amounts (45.7, 11 and 99ppt respectively) were less than maximum level (1000ppt) according to Iranian national standard. AFM1 content was less than maximum limit according to Codex and EC standards for milk (50ppt). The average concentrations of Pb, Cd, As, Hg, Al and PCBs were 18, 1, <20, 10, 2679 and <6 ppb respectively (less than maximum limits in Codex and EC standards, except for Hg and Al). Heavy metals and PCBs content in DWP-90 compared with natural mineral waters in Codex. So PCBs and AFM1 were not a safety risk in DWP-90.

Key words : Aflatoxin M1, Heavy metals, Polychlorinated biphenyls, Demineralized whey powder.

INTRODUCTION

Whey can be categorized into two main groups, namely sweet and sour whey [1] both types can be spray-dried but requires different types of spray dryers as well as different handling techniques. Sweet whey is easier to dry than acid whey. This by-product is the leftover milk components after making cheese by enzymatic method and contains the minerals found in milk. Sweet whey usually used to produce demineralized whey powder (DWP). The processing steps involves preheating, demineralization, concentration, flash cooling, pre-crystallization, spray drying, cooling in a vibrated fluid bed and packaging. DWP-90 or Demi90 is a kind of whey powder in which about 70-90% of its' minerals are removed by ion-exchanging before drying. Whey powder, especially in demineralized form, is a good nutritional supplement added to infant formulations. The high amount of minerals in whey protein powder will increase the osmolarity and estimated renal solute load (ERSL) of final product, which leads to some problems for kidney of infants. The maximum acceptable osmolarity of infant milk powder is 40 mOsm based on Iranian national standard [2]. Contaminants like heavy metals, Aflatoxin M_1 (AFM_1), dioxins, polychlorinated biphenyls (PCBs), Nitrate and Nitrite are considered as greatest concerns for Demi90 used in medical industries and infant milk powder production in which using raw materials with less contaminants is very important stage. AFM_1 is a hepato-carcinogen found in milk of animals that have consumed feeds contaminated with Aflatoxin B_1 (AFB_1), the main metabolite produced by fungi of the genus *Aspergillus*, particularly *A. flavus, A. parasiticus* and *A. nomius* [3]. The Maximum limit of AFM_1 in demineralized whey powder is not set in the global standards like, EC 1881/2006 or by Codex Alimentarius Commission, but according to EC 1881/2006 the maximum limit of AFM_1 in milk, should not be exceeded than 0.05 ppb [4-5]. The maximum limit of AFM_1 in whey powder according to Iranian national standard [6] is less than 1 ppb. Heavy metals such as arsenic (As), cadmium (Cd), mercury (Hg), lead (Pb) and Aluminum (Al) are widely dispersed in the environment. These elements have no beneficial effects in human health, and there is no known homeostasis mechanism for them. Although toxicity is a function of concentration, it is well-known that chronic exposure to As, Cd, Hg, Pb and Al at relatively low levels can cause adverse effects on human health. Although some individuals are primarily exposed to these contaminants in the workplace, for most people the main route of exposure to these toxic elements is through the diet and this is more critical for medical and infant milk products [7]. PCBs can cause adverse effects on the nervous, immune and endocrine systems, impair reproductive function and may cause cancer. The maximum limit of heavy metals and PCBs has not been defined for DWP by International standards. Since there is few reports on the mentioned contaminants in whey powder especially demineralized form, this study aimed to detect AFM_1, heavy metals (As, Pb, Hg, Cd, Al) and PCBs in Demi90 produced from non UF sweet whey.

MATERIAL AND METHODS

Samples

Twelve DWP-90 samples (400g/sample) collected from Nutricia-MMP factory during 6 months from June to December 2012. The protein content of the samples was between 12.5-14.5% and the fat and ash content was less than 1.3 and 1% respectively.

AFM_1 detection

The HPLC method with fluorescence detection was applied to determine AFM_1 content. The sample was reconstituted and defatted, cleaned-up by immuno-affinity column chromatography. Acetonitrile (HPLC grade) of Sigma-Aldrich (Steinheim, Germany) was used for AFM_1 analysis and for sample extraction, 20g of DWP dissolved in 200mL of deionized

water (40°C for dissolved DWP) and immune-affinity columns (IAC) Afla M_1 (capacity 150 ng; stored at 4°C until use) was acquired from Vicam (Watertown, MA). The eluate was cleaned up using either IAC. With IAC, disk eluate was concentrated to 0.5-1.0mL at 45°C with a Thermo Savant Speed Vac SPD 1010, and then deionized water was added to bring the final volume to 15mL. The solution was gravity fed through the column, and an additional 2mL of deionized water was used to wash the column. After forcing extra water out of the column with a syringe, the IAC was eluted twice with 2mL of acetonitrile/methanol (3:2, v/v) and the eluate was concentrated to 300µL using the Speed Vac. The LOQ of the method was 0.002-0.01µg/kg.

Heavy metals determination

Pb and Cd

Determination of lead and Cadmium was performed by a Zeeman atomic absorption spectroscopy (ZAA). For detection these heavy metals, the sample was directly introduced in the AAS system with a solid sampling graphite-furnace atomic absorption spectrophotometry (GFAAS), dried, ashed and then measured. The LOQ for Pb and Cd was respectively 5 and 1µg/kg.

As, Hg and Al

Determination of Arsenic, Mercury and Aluminum was done by atomic absorption spectrometry (AAS), cold vapour technique (flow-injection-cold-vapour-technique FIAS). As a pre-treatment, the sample was digested with nitric acid in a high pressure asher (HPA instrument, Anto-Paar Gmbh) at 300°C and 100 bar. For pre-reduction of As (V) to As(III) ascorbic acid/potassium iodine was added. The LOQ for As, Hg and Al was respectively 20, 1 and 100µg/kg.

PCBs

Deutsche forschungsge einschaft (DFG) Multi residue method-S19 (MRM) was applied to determine PCBs [8]. The model of high-speed homogenizer was IKA ultra turrax. The entire method consists of four stages: extraction and partition, gel permeation chromatography (GPC), mini silica gel column chromatography, and gas chromatographic determination [9]. The sample was extracted with acetone, after addition of water (depending on the natural water content of the material, in order to ensure an acetone/water ratio of 2/1 (v/v) during the extraction). The extract solution was cleaned up by GPC (Malvern instrument) using the polystyrene gel Bio-Beads S-X3 and elution with a mixture of cyclohexane and ethyl acetate. The GPC eluate was cleaned up further on a small silica gel column. The concentrated GPC eluate was transferred onto a small silica gel column and the column eluted with solvents or solvent mixtures of increasing polarity. For detection of analytes, the gas chromatography with a mass spectrometric detector (MS) was used. Analyzed components included Ballschmiter-Congeners: PCB 28, PCB 52, PCB 101, PCB 138, PCB 153 and PCB 180. The LOQ was 1µg/kg for single substance and 6µg/kg for sum of PCBs.

RESULTS

AFM_1 analysis data

Analytical results showed that the concentrations of AFM_1 in all of the samples were in acceptable range (1000 ppb, maximum limit for sweet whey powder) based on Iranian standard organization [6]. The maximum, minimum and average concentrations of AFM_1 in the samples were 99, 11 and 45.7ppt respectively (Table 1).

Heavy metals analysis data

Heavy metals are potentially toxic substances, especially for the susceptible infants. Table 2 shows the incidence of heavy metals (Cd, Hg, Pb, As and Al) in the DWP-90 samples. Since in international standards, the maximum level for heavy metals had not been defined for DWP, data was compared with maximum

Table 1: AFM1concentration (ppt) in DWP-90 samples analyzed by HPLC technique

Samples	AFM_1con. (ppt)	Samples	AFM_1con. (ppt)
1	**22**	7	**24**
2	**40**	8	**72**
3	**50**	9	**19**
4	**76**	10	**99**
5	**11**	11	**40**
6	**28**	12	**69**
Minimum		**11**	
Maximum		**99**	
Standard deviation		**26.5**	
Average		**45.7**	

The LOD and LOQ of the method were respectively 8 and 10ppt

Table 2: Heavy metals concentration (ppb) in DWP-90 samples

Heavy metal	No Samples	Minimum	Maximum	Mean	LOQ	SD
Pb	12	<LOQ	55	18.1	5	14
Cd	6	<LOQ	3	<LOQ	1	—
Al	12	910	5290	2679	100	1284
As	12	<LOQ	<LOQ	<LOQ	20	—
Hg	12	<LOQ	25	10.5	3	8

Table 3: Heavy metal content of samples (ppb)

Item (ppb)	1	2	3	4	5	6	7	8	9	10	11	12	Min	Max	Ave	LOQ
Pb	5	5	27	55	5	15	18	21	13	20	29	8	5	55	**18.1**	5
Cd	<LOQ	<LOQ	<LOQ	1	<LOQ	3							**<LOQ**	3	**1**	1
Al	4080	1450	3200	910	5290	1230	1770	2880	3035	4000	1550	2750	**910**	5290	**2679**	100
As	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	---	---	**<LOQ**	20
Hg	25	10	2	7	25	6	1	18	14	10	6	2	**1**	25	**10.5**	3

Table 4: Heavy metals concentration (ppb) of DWP-90 samples compared to national and international standards

Standard Type:	CODEX[a]	Average Con.	
Type of Product:	Natural mineral waters	DWP-90	Reconstituted DWP-90 [b]
Cd	3	1	0.1
Hg	1	10.5	1.05
Pb	10	18.1	1.81
As	10	<20	<0.2
Al [c]	1000 (bw/PTWI)	2679	268

(a) Defined limits in CODEX standard, refers to natural mineral water because no limitation was defined for heavy metals in DWP or Milk
(b) It should be considered even if DWP consists 100% of a recipe, it should be diluted to 10-15%v/v to be as a ready to use product. So the values for DWP-90 should be divided by 10 to calculate the actual amounts in wet weight.
(c) The maximum limit for Al in CODEX (Based on the Joint FAO/WHO Expert Committee on Food Additives (JECFA) is 1000 (bw/PTWI) which based on RDI for infants, it should be less than 3000 ppb in the powder. (bw/PTWI: body weight per Provisional Tolerable Weekly Intake)
ND: Not defined
bw/PTWI: body weight per Provisional Tolerable Weekly Intake

Table 5: PCBs residues (ppb) in DWP-90 samples

Parameter	No of samples	Minimum	Maximum	Mean	LOQ[a]	SD
PCBs	**12**	**<LOQ**	**<LOQ**	**<LOQ**	**<1**	**----**

(a) single substances <1 ppb, sum of PCBs (PCB 28, PCB 52, PCB 101, PCB 138, PCB 153 and PCB 180). including 100 % LOQ <6 ppb

limit for residues of metals in natural mineral waters.

PCBs

Table 5 shows PCBs residues in the samples. The maximum level for sum of dioxins and dioxin-like PCBs, based on EC 1881/2006 is 6 ppb. The concentration of PCBs for all of 12 samples, was less than maximum level defined in EC 1881/2006 (PCBs <6 ppb).

DISCUSSION

Aflatoxin M1

In four samples (33% of samples) the AFM_1 content was greater than the maximum tolerance limit (50ng/L) accepted by European Union and Codex Alimentarius Commission for liquid milk but this rate of contamination decreases in recipes containing DWP such as infant milk powder products. In the case of 10 fold dilution rate, the AFM_1 content will decrease to 0.1 and reaches to about 4.6ppt. So AFM_1 contamination is not a risk in the samples studied in this research.

Heavy metals

As it was noticed, in international standards, the maximum level for heavy metals had not been defined for DWP, so data was compared with maximum limit for residues of metals in natural mineral waters. The maximum level of Aluminum for natural mineral waters had not been defined in Codex and results compared with internal standard of Nutricia Co. for DWP-90 (3000 ppb).

The average concentration of Pb was 18ppb in the samples, which was less than defined maximum level in CODEX [10] and EC [4] for milk (20ppb) and milk powder (200ppb). The concentration of Cd was in acceptable range in all of 6 tested samples (average content=1 ppb) according to acceptable limit of Codex for natural mineral waters (3 ppb). The concentration of Arsenic in DWP 90 was less than 20 ppb (less than 2ppb in wet weight) in the samples (LOQ<20 ppb) which was in acceptable range according to Codex standard for natural mineral waters (10 ppb). The average concentration of Hg was 10.5ppb (1.05ppb in wet weight) in DWP 90 samples which is approximately equal to the maximum limit for Hg, defined in Codex for natural mineral waters (1 ppb), however in 30% of the samples, Hg exceeded the maximum limit according to Codex standard. The average amount of Aluminum was determined 2679 ppb (Max=5290, Min=910 ppb).

Aluminum content in 40% of the samples exceeded the maximum limit according to internal standards of Nutricia-MMP Company (3000 ppb**).** According to Table 3, Aluminum contamination is more common in the samples. Aluminum intake more than recommended value may cause adverse effects on human health including dysuria, discomfort, cataract and neurotoxicity. This high amount of Al content could be due to bad practices during manufacturing or packaging. The concentration of Al was evaluated in breast milk, whole milk and infant milk powder (50, 400 and 2346 mcg/L respectively) in a study by Pietrzak-Fiećko [11]. The infant milk powder samples had the highest Al content, which is due to different factors. Soybean as an ingredient in infant formula, is a source of Al contamination, also additives such as calcium and phosphorus, manufacturing processes and storage containers are another factors [12]. Based on a research in Saudi Arabia, the amount of Al, Cd, Hg and Pb was evaluated in infant milk powder and cereals [1]. Results showed that Al content in 19 infant milk powder samples was acceptable (1944±104 ppb) based on EC 1881/2006 and CODEX but in the cereal samples it was more than acceptable limit (9880 ppb). Since infant milk powders contain about 20-25% DWP as a major ingredient, concentration of heavy metals in DWP is very important and it can be effective on amount of these elements in final product.

In a research in Australia, concentration of Hg and Pb evaluated in breast milk which showed low contamination, Hg: 1590 ppb, *n*=116 and Pb: 1630 ppb, *n*=138 [13]. In Saudi Arabia, the average content of Pb in 25 samples of infant milk powder for infants less than 12 months was determined about 34ppb [14]. The concentration of Pb and Cd was determined in Egypt in 16 samples of infant milk powder for age group less than 2 years old respectively about 156 and 1.2ppb. The estimated weekly intake (EWI) of Pb for one year olds infants was between 6.5422.3 ppb and it was less than maximum level (25 ppb bw/PTWI) defined by WHO expert group [15]. In Philippines, concentration of Arsenic, Lead and Mercury in Infant milk powder, for infants aged 6 to 12 months evaluated. As and Pb was negative in all samples and Hg concentration was 633 and 833 ppb in two samples which was more than defined PTWI (5 ppb) by WHO [16].

PDBs

Based on a study in Poland, the PCB level was evaluated in human milk (174 samples, average content 218 ppb), UHT cow's milk (16 samples, average content 35 ppb) and infant formulas (6 samples, average content 13 ppb) [11].

CONCLUSION

From the presented study, we can conclude that quality of evaluated DWP samples is acceptable in terms of AFM 1, Heavy metals and PCBs to be used in Infant milk powder. Average result of AFM 1 in DWP samples was according to mentioned standards. The concentration of heavy metals in reconstituted DWP was less than maximum limit in CODEX for mineral water which is more strict than Infant milk powder. The PCB content in DWP samples in our study was <6ppb which is less than defined range for infant milk formula in different standards.

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