Authentication of Whey Protein Supplements with Their Labeling

Neveen F. M. Agamy, Mennatallah K. S. & Samar M. Abo-Rhyem
Department of Food Analysis, High Institute of Public Health, Alexandria University.

Received: 24 May, 2023 *Revised: 19 June, 2023* *Accepted: 29 June, 2023*

ABSTRACT

The consumption of supplements by active individuals has been increasing over the last few years. Whey protein is one of the highly consumed supplements. Hence, this study aims to analyze the proximate and heavy metal content of different brands of whey protein. This cross-sectional study was conducted on 6 brands (5 international and 1 local), The results showed that brand VI had the most protein content percent change of - 18.65%, brand V showed the highest fat percent change of -83.12%, brand II showed the highest carbohydrate percent change of +867.52%. Regarding the heavy metals, brands land IV-VI were free from Pb. The highest arsenic concentration was in brand III (8.69 mg/kg). The highest cadmium concentration was found in brand III (1.3 mg/kg), followed by 0.58 mg/kg and 0.6 mg/kg in brands II and VI, respectively. Aluminium concentration was the highest in Brand VI. The examined brands of whey protein supplements sometimes lack the macros that are written on the package, thus they fail to produce the planned results from having whey protein supplements in the diet. Furthermore, they may have a risk of heavy metal contamination, the higher the supplementation dose (acute), the higher the contamination and the risks.

Keywords: authentication, whey protein supplements, heavy metals, proximate analysis, protein content.

INTRODUCTION

Consumption of whey protein supplements has increased but is poorly regulated, posing unknown health risks to consumers (Binns *et al.*, 2018). Fresh cow's milk contains 3.5% total protein, of which 80 % is casein, and 20% is whey protein. The dairy industry usually treated the whey liquid as waste for decades, but since the whey liquid is a protein source and contains biologically active ingredients, the industry began using it as a supplement (Golinelli *et al.*, 2011, Lollo *et al.,* 2011, Josse & Phillips, 20l3). There are three main types of whey protein (WP) supplements. The WP concentrate has protein concentrations ranging from 25 to 89% (Mostly 80%), and it's made up of some fat, lactose, and minerals (as the protein concentration increases, fat, lactose and mineral content decreases), WP isolate contains protein concentrations that range from 90 to 95% and it has almost no lactose content.

Hydrolyzed WP has different protein concentrations since it is created by the breakdown of large proteins into smaller peptides. The hydrolyzed WP reduces the potential for allergies compared to non-hydrolyzed ones (Marshall, 2004, Jager *et al.*, 2017).

For physically active individuals, the recommended daily protein intake is 1.4-1.6 g/kg/day. To manage and adhere to this recommendation, they purchase and use whey protein supplements to meet their daily protein requirements (Jäger *et al.*, 2017). There are some concerns about the safety of using whey protein powder supplements because the products analyzed contained heavy metals such as Cadmium [Cd], Arsenic [As], Lead [Pb], and Aluminium [Al] (Bandara *et al.*, 2020). Skin lesions and carcinogenic effects are associated with chronic arsenic exposure, while kidney disease, thyroid diseases, and weakened bones are associated with chronic cadmium exposure (Zhou & Xi, 2018, Wallace *et al.*, 2020). Aluminium ions play no physiological role in metabolic processes. However, when humans and animals are heavily exposed to Al metal, it becomes a metal poison after natural or unnatural exposure. Aluminium was considered dangerous to humans, as elevated aluminium concentrations were detected in the brain tissue of encephalopathies patients. The develop-

ment of aluminium toxicity in mammalian tissues is thought to be involved in several pathological diseases, reproductive toxicity, lung lesions, impact on the breast tissue, bone abnormalities, immunotoxicity, and neurological disorders (Igbokwe *et al.*, 2020).

In Egypt, milk can be contaminated with aluminium, since it is obtained from manufacturers who use low- grade material for boiling, like aluminium tanks. Another problem is that the freshly collected milk is added to the milk stored in the milk cooler the next day. This may increase the risk of milk contamination. Accidental leakage of aluminium from the tank into the milk is affected by the condition of the aluminium tank and the pH of the milk (Al Juhaiman, 2010). In addition, water may be added to the milk, which may also contain aluminium residues. This could be another potential source of contamination (Amer *et al.*, 2021). Lead is a major toxic substance that is widely used in industry. This could in turn lead to increased lead contamination and exposure in the environment. When lead is ingested in large amounts, lead competes with calcium in the body. This impairs heme synthesis and neurotransmitter release, with adverse effects on nerves, blood, reproduction, and kidneys. Furthermore, exposure to lead (Pb) is also intricately associated with changes in RNA expression (Wallace *et al.*, 2020).

The global dietary supplement markets expected to reach USD 278.02 billion by 2024. However, the dietary supplement (DS) market is still growing in the Middle East and Africa. South Africa remains the Middle East's largest market. However, DS sales have increased in Egypt since 2017. This is likely due to a strong desire to correct nutritional imbalances (caused by their poor eating habits) and strengthen the immune system. Despite the growing popularity of DS use, there is a lack of documentation on DS use in Egypt (Hegazy *et al.*, 2020). Gym- goers are more likely to use whey protein supplements to meet their daily protein requirements, which are otherwise difficult to meet from their daily diet (Hegazy *et al.*, 2020). Since not all whey protein supplements contain the macronutrient percentages that are written on their labeling, whey protein supplements regularly may not supplement gym users with what is promised, and some whey protein supplements have been found to contain heavy metal compounds. The regular intake of whey protein supplements may increase the risk of toxicity (Bandara *et al.*, 2020, Wallace *et al.*, 2020). Therefore, the purpose of this study was to analyze the content of different brands of whey protein supplements. It authenticates products by comparing protein, fat, moisture, ash, and carbohydrates against labeled data and analyzes whether these brands are contaminated with heavy metal compounds. Arsenic, Aluminum, Cadmium and Lead.

MATERIALS AND METHODS

Materials

Six brands of the most consumed whey protein supplements were purchased in four replicates of each brand from different retailers, coded as brands I, II, III, IV, V, and VI. Brands I-V are made in USA (international), while brand VI is made in Egypt (local). Brands I, IV, V, and VI are Whey protein concentrates, while brands II and III are whey protein isolates.

Methods:

Proximate Analysis

Gross chemical composition

Moisture, crude protein (N×6.25), crude fat and ash content were determined, according to AOAC (2000) while total carbohydrate content was calculated by difference.

Determination of Heavy Metals

Determination of heavy metals (cadmium, lead, aluminium, and arsenic) was performed by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) in the Environmental Pollutant Analysis Laboratory at The High Institute of Public Health, Alexandria, Egypt. For the digestion of the sample, 1 gm of the sample, 9 ml HNO_3 and 1 ml H_2O_2 were mixed. The sample was then digested in Ethos advanced microwave digestion system.

Statistical Analysis

Data collected were expressed as Mean ± S.D. The normality of samples was checked and one-way ANOVA was used for comparing the studied groups. In the case of significant difference, Post Hoc Test (Tukey) was adopted for Pairwise comparison between the groups. Statistically significant was adjusted at $P<0.05$.

RESULTS AND DISCUSSION

Moisture Contents

The moisture content of six brands of whey protein supplements was tested using the oven method. The results revealed the following (a percent change was not calculated because the whey protein supplements had no claimed moisture content on their labeling).

Figure (1) displayed the moisture content % in the tested samples. One-way ANOVA results indicated a significant (P<0.05) difference among the tested groups. Brands I-VI had moisture equal 7.13, 6.63, 7.18, 10.10, 6.46 and 5.92%, respectively. No significant differences were observed between the brands I, II, and III. However, brand IV showed significantly (P< 0.05) higher moisture than all other brands I-III, V and VI.

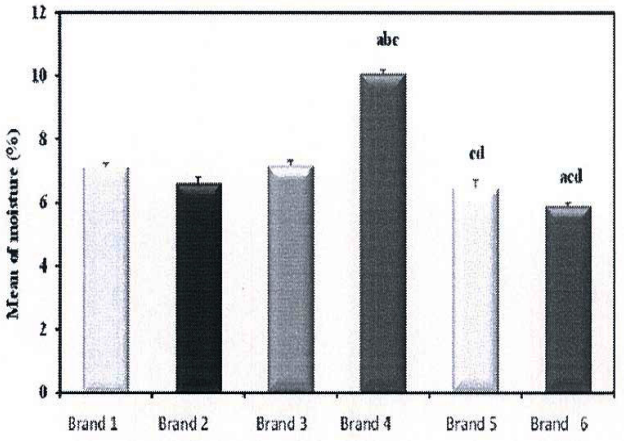

Fig. 1: Comparison between the different studied brands of whey protein supplements according to moisture percent

Protein contents

Recently, the popularity and consumption of sports supplements, especially whey protein, has skyrocketed. However, to prevent possible risks associated with consuming these sports supplements and to obtain the maximum benefit from their consumption, it is important to analyze and test the quality aspects of these sports supplements from a label perspective, to test and check the authenticity of the supplement (Binns *et al.*, 2018). In this study of label authentication of whey protein supplements, six brands of whey protein supplements (four replicates each) were analyzed for protein content. It was obvious that none of the six brands were authentic to what the brand had claimed on their labeling.

The brand 1 exhibited 69.52% protein content which was more than the value (62.5%) stated on the label with higher change +11.2%. Whereas the rest of brands 11-V1 had lower protein contents than those reported on the labels. In brief, brands II and III had the highest mean protein content 80.49% and 80.22%, with lower percent change –5.31% and –5.62% and –5.94%, respectively then brands IV and V had 71.25%, and 67.63%, respectively. Band VI had the lowest protein content 59.63% with higher percent change –18.66% (Fig. 2). These findings are consistent with those of Saxton & McDougal (2021) who looked at the five protein powder supplement labels to see how much protein is found compared with that claimed. Only 21.5 g of protein per serving, which shows a 28.3% discrepancy between the reported protein and actual protein found. To our knowledge, this is the first study to evaluate individual ingredients in whey protein supplements, comparing them to their label data.

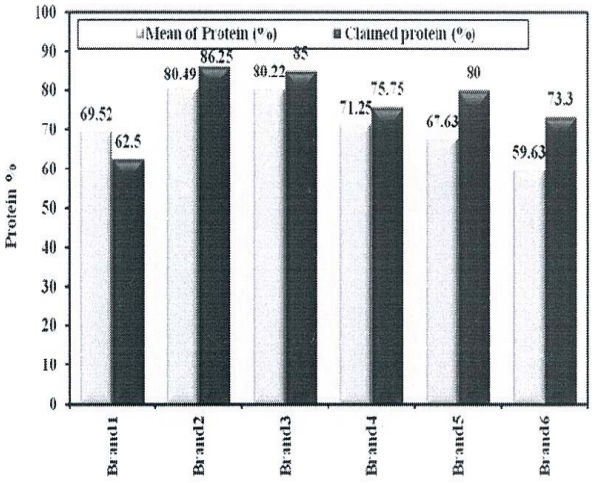

Fig. 2: Comparison between the determined means and the claimed protein content in the different brands

Fat Contents

Figure (3) shows the fat percent in all the tested products. All brands exhibited less fat content than that stated on the labels. The claimed fat percent was 5.5%, 1.5%, 2.5%, 4.2%, 7.2%, and 2.0% for brands I, II, III, IV, V, and VI, respectively. Brand II had the lowest fat mean content of 0.68%, followed by brands V and III having 1.22% and 1.25%, respectively, then brands VI and IV having 1.34% and 1.44 respectively. The highest mean fat content was recorded in Brand I with 2.79%. Regarding the percentage of changes of fat contents, the results showed that brands I, II, III, and IV had a com-

mon percent of changes –49.27, –54.5, –49.9, and –65.83%, respectively. Nevertheless, brand V had the greater percent of change equal -83.12 % from the claim on the product. The least brand was brand VI (local) having a percent change equal -32.12%.

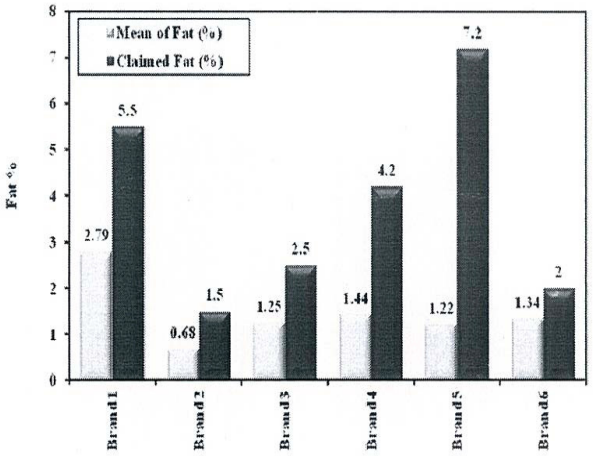

Fig. 3: Comparison between the determined means and claimed fat content in the different brands of whey protein supplements

Ash Contents

Figure (4) represented a comparison between studied groups according to the Ash%. Brands I-VI had 2.67, 2.53, 2.79, 2.58, 4.38, and 6.56%, respectively. Statistical analysis demonstrated that ash percent differ ($P< 0.05$) among the brand tested. In details, brands V and VI had significantly ($P< 0.05$) higher ash contents than those of the rest of the brands (a percent change is not calculated because the whey protein supplements had no claimed ash content on their labeling).

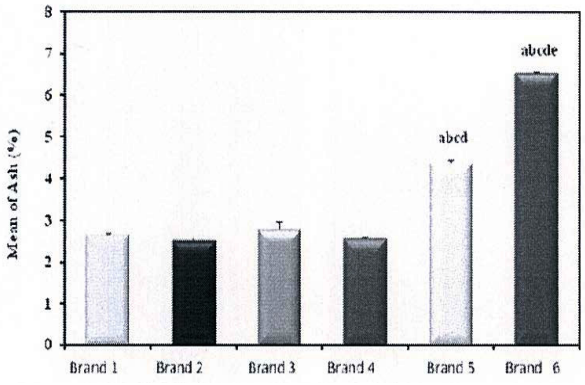

Fig. 4: Comparison between the different studied brands according to Ash percent

Carbohydrate Contents

Figure (5) represented the carbohydrate percentage in the selected samples. The claimed carbohydrate % in the selected brands were reported as 8.8%, 1.0%, 2.0%, 14.32%, 6.1 %, and 13.33% for the brands I-VI, respectively. All brands had higher percentages of carbohydrates than those labeled. The highest carbohydrate % was found in brand VI (26.56%), followed by brand V (20.39%). Brands I and IV had intermediate contents of 17.9% and 14.63%, respectively. The lowest mean carbohydrate contents were found in brand III (8.55%) and brand II (9.68%).

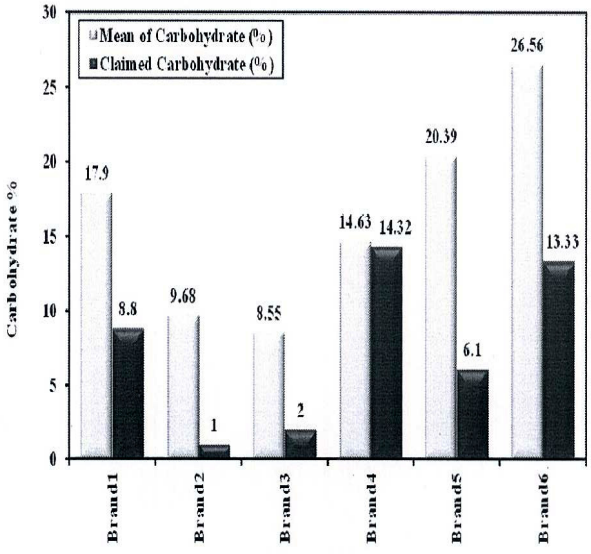

Fig. 5: Comparison between the determined mean and the claimed carbohydrate content in the different brands of whey protein supplements

Heavy Metal Contents

The studied heavy metals included arsenic [As], aluminium [Al], cadmium [Cd], and lead [Pb], are shown in Figure (6). No significant differences could be traced among the brands regarding Cd concentration (Table 1). However, the detected Cd concentrations were recorded in brand III with 0.66 mg/kg, brand II with 0.58 mg/kg, and brand VI with 0.34 mg/kg, while brands I, IV, and V had no detected values. Considering Pb, Al, and As, one-way ANOVA demonstrated significant: differences among brands (Table 1). Brand III recorded the highest Pb concentration ($P<0.05$) level of 43.9 mg/kg among other studied brands. It's also show that the highest concentration level of Al was found in brand VI with 2459 mg/kg. For (As), the highest concentration was observed in brand III with 8.69 mg/kg, followed by brand VI with 1.08 mg/kg.

In the present study, it was found that the whey protein supplements were contaminated with one

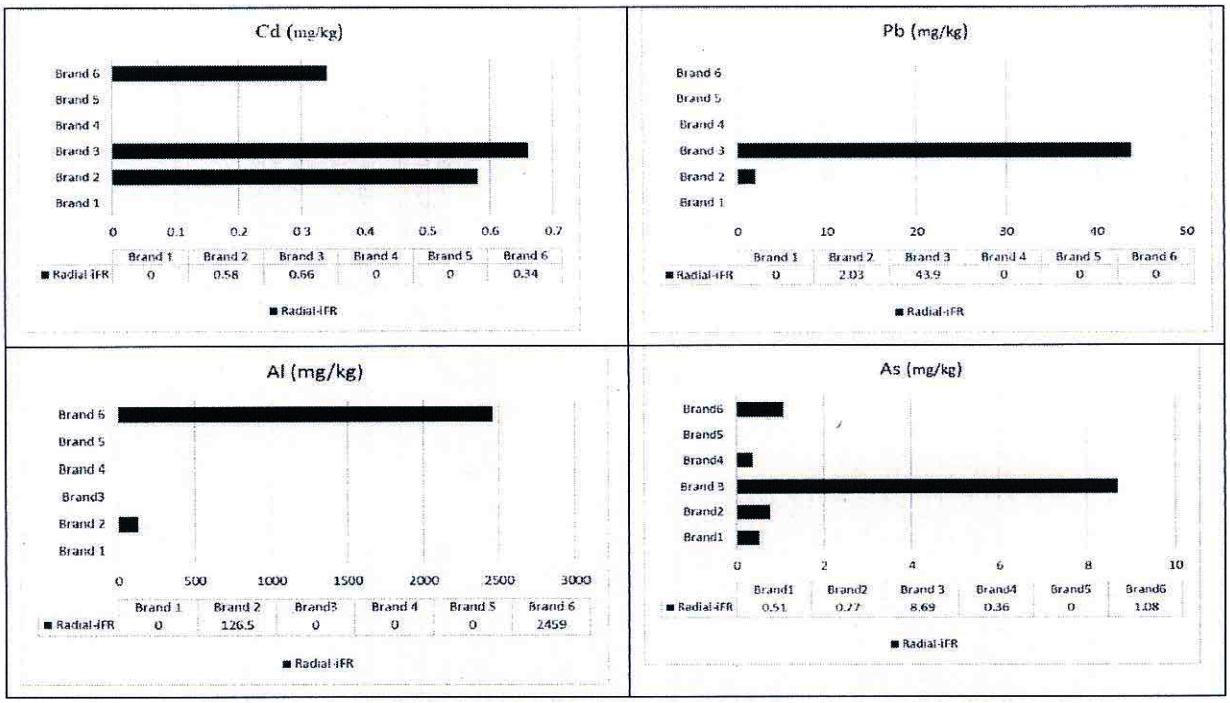

Fig. 6: Heavy metal contents (Cd, Pb, Al, As) in different whey protein supplements

Table 1: Heavy metals (mg/kg) detected in whey protein supplements of different brands

Brands	Cadmium	Lead	Aluminum	Arsenic
I	ND	ND	ND	0.51 ±0.73
II	0.58+ 1.37	2.03 ± 2.44	126.5 + 6.5	0.78 + 2.84
III	0.66+ 1.35	43.92^{b}± 50.64	ND	8.69^{ab} ± 7.06
IV	ND	ND	ND	0.36^{c} ± 0.75
V	ND	ND	ND	ND
VI	0.34+ 1.84	ND	2459.09^{abcde} ± 1333.97	1.08^{c} ± 6.91
p-value	0.788	0.004*	<0.001*	0.009’
FDA: recommended daily maximum limits (Igbokwe *et al.*, 2020, Wong *et al.*, 2022).	0.83 µg/kg/day	0.16 µg/kg/day	6 to 14 µg/kg/day	0.3 µg/kg/day

Data were expressed by using Mean ± SD. n=6 replicas in each group. ND refers to a non-detected value, p: p-value for One-way ANOVA test for comparing among the studied groups and using Post Hoc Test (Tukey) for Pairwise comparison between every two groups. *: Statistically significant at $P < 0.05$.

a: Significant compared with Brand I, b: Significant compared with Brand II, c: Significant compared with Brand III, d: Significant compared with Brand IV, e: Significant compared with Brand V, f: Significant compared with Brand VI

or more heavy metal compounds. The brand I had 0.51mg/kg arsenic only. Four heavy metals were detected in brand II: Cd (0.58 mg/kg), Pb (2.03 mg/kg), A1 (126.5 mg/kg), and As (0.78 mg/kg). Brand III showed contamination of three contaminants, Cd (0.66 mg/kg), Pb (43.92 mg/kg) and As (8.69 mg/kg). Brand IV showed contamination with As (0.36 mg/kg) only. Brand VI showed contamination with Cd (0.34 mg/kg), A1 (2459.09 mg/kg), and As (1.08mg/kg), while brand V showed no detected contamination with any of the four heavy metals analyzed. These results are in agreement with the published findings on heavy metal contamination of powdered protein being (As) 0.009 µg/g, (Cd) 0.001 µg/g, and (Pb) 0.003 µg/g (Skibola *et al.*, 2017). Similarly, Guefai *et al.* (2022), reported an average result of (Al) 8 mg/kg whey, (Pb) 10.8 µg/kg, (Cd) 12.9 µg/kg and (As) 14.9 µg/kg for whey protein supplements.

In the current study, brand VI contained the highest amount of aluminium (2459 mg/kg), while brand III had the highest concentration of lead

(43.9 mg/kg). Moreover, brand I showed the presence of arsenic (0.51 mg/kg). These results are in agreement with the findings of Skibola *et al.* (2017) and Bandara *et al.* (2020). The health hazard of heavy metal ingestion was measured among regulars of whey protein supplements, and it was found that the recommended servings calculated range for heavy metals in 1–3 servings of protein powder supplements were: 0.2–16.9 µg/day for As, 0–5.6 µg/day for Cd, and 0–13.5 µg/day for Pb. Assessing the heavy metal contamination in different brands of whey protein has a top priority in terms of quality. The present study revealed that five out of six studied whey protein supplements contained one or more heavy metals. Similarly, Maughan (2013) tested 15 protein powders for the existence of arsenic, cadmium, lead, and mercury. Three products were found to contain excess levels of heavy metals in regard to safety levels. Elgammal *et al.* (2019) also studied heavy metal content in whey protein samples from markets in Giza, Egypt and they found similar findings of cadmium in 23 whey protein samples ranging 0.0022–0.335 mg/kg, aluminium <5–6.26 mg/kg and lead 0.036 to 0.096 mg/kg. other support came from the work of Filipiak-Szok *et al.* (2015) who determined the toxic metals in dietary supplements using ICP-MS and they found lead (0.24-0.7) in the complex dietary supplements, cadmium (0.01-0.10), aluminium (11.98–62.13) and arsenic (0.06–0.21) µg/tablet. Van Der Voet *et al.* (2008) studied the clinical and analytical toxicology of dietary supplements and found arsenic < DL (50 ppm) and lead was found to be 11 ng/g in the whey samples.

The FDA recommended daily allowances for cadmium 0.83 µg/kg/day, lead 0.16 µg/kg/day, aluminium 6-14 µg/kg/day, and arsenic 0.3 µg/kg/day. None of the tested whey protein samples exceeded the FDA-recommended daily allowance for cadmium, however, brands 11 and III exceeded that limit of lead 2.03 and 43.9 ppm, respectively. Brands II and VI exceeded the limit of aluminium 126.5 and 2459.09 ppm, respectively. Regarding arsenic, the results showed that Bands, I-III and VI exceeded these limits. These results are consistent with the previous investigations (Zhou & Xi, 2018, Bandara *et al.*, 2020, Wallace *et al.*, 2020, Wong *et al.*, 2022). So, there is a great concern for public health when the intake of heavy metal-contaminated dietary supplements is a daily routine, or supplements not containing what is claimed on the labeling is usual. Heavy metals can build up in the body over time and cause irreversible damage in humans, particularly in highly vulnerable residents such as pregnant women and children, teenagers, the ill, and old. This could be happening because these supplements are sold as healthy food that supplements people with a diet with protein. Many believe they are natural and safe and won't generate harmful health effects in the long run and that is why they buy and consume whey protein products (Binns *et al.*, 2018). Another factor that may increase this risk is the globalization and lack of clarity and integrity from the dietary supplements industries about the origins and the quantities of their used ingredients. There is no way for the buyer to tell whether the supplements they are consuming are authentic or not, and if they are of the quality they claim to be (Binns *et al.*, 2018).

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مصادقة مكملات بروتين اللبن مع الملصق الغذائي الخاص بها

نيفين فهمي محمد عجمي، منةالله خالد صابر، سمر أبو رحيم

قسم تحليل الأغذية، المعهد العالي للصحة العامة - جامعة الإسكندرية

يتزايد استهلاك مكملات بروتين مصل اللبن من قبل الجمهور النشاط بدنيًا، الذين يذهبون إلى صالات الألعاب الرياضية بشكل متكرر أكثر من أقرانهم غير النشطين، على أمل أن يصلوا إلى الإضافات اليومية الموصى بها من البروتين ٤ , ١-٨ , ١جم/ كجم/ يوم، والتي قد يكون من الصعب الحصول عليها من الطعام وحده. قد يمثل هذا النمو في الاستهلاك والاستخدام مشكلة تتعلق بمصداقية مكملات بروتين مصل اللبن من خلال البيانات المدونة على العبوات عليها وجودتها/ تلوثها بالمعادن الثقيلة. لذلك كان الهدف من هذه الدراسة هو تحليل العلامات التجارية المختلفة لمكملات بروتين مصل اللبن لاختبار ما إذا كانت تلتزم وتتوافق مع البطاقات الغذائية الخاصة بها أم لا. وقد أجريت دراسة مقطعية على ٢٤ عينة من بروتين مصل اللبن: ٦ علامات تجارية (٥ دولي و١ محلي) تم شراؤها من بعض المتاجر المحلية ومن على الإنترنت (٤ نسخ طبق الأصل لكل منها). وتم تقييم محتوى مكملات بروتين مصل اللبن من البروتين، الدهون، الرطوبة، نسب الرماد، الكربوهيدرات وكذلك للتعرف على المعادن الموجودة فيها ومحتوى المعادن الثقيلة بها (الكاديوم والرصاص والألمنيوم والزرنيخ) باستخدام (ICP-OES). وكشفت الدراسة أن العلامة التجارية VI بها نقص في البروتين بنسبة -١٨ , ٦٥ وأظهرت العلامة التجارية V أعلى نقص في نسبة الدهون بلغت -١٢ , ٨٣. كما أظهرت العلامة التجارية II أعلى زيادة في نسبة الكربوهيدرات +٥٢ , ٨٦٧٪، تليها العلامة التجارية III والعلامة التجارية V، +٦٣ , ٣٢٧، +٢٦ , ٢٣٤٪ بالمقارنة مع البيانات الخاصة بها على الترتيب ولقد أظهرت النتائج خلو العلامات التجارية I وIV-VI أما الألمنيوم فقد كان هو الأعلى في العلامة التجارية VI، وبناءً عل هذه النتائج فإنه يمكن القول أن العلامات التجارية التي تم فحصها لمكملات بروتين مصل اللبن في هذه الدراسة تفتقر في بعض الأحيان إلى المغذيات الكبرى المدونة على العبوة، ومن ثم فإنه لا يحقق النتائج المرجوه من تناول مكملات بروتين مصل اللبن في النظام الغذائي. أضف إلى ذلك احتمال وجود خطر من التلوث بالمعادن الثقيلة، فكلما زادت جرعة المكملات، زاد التلوث والمخاطر المحتملة.