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Protein supplements between consumer's opinion and quality control: an applied study in jordan

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ABSTRACT

The consumption of dietary supplements has nowadays become popular, especially in Jordanian sports clubs and gyms. In fact, there is a widespread idea, among consumers, that these proteins contain hormones in order to increase their efficiency. The objective of this study is to develop a better understanding of customer opinion in an era that increased growth in Jordan and improves a chromatographic method to detect the testosterone in protein supplements. The method of this study, six popular types of proteins in the Jordan market have been chosen after conducting a primary study of the proteins' users by questionnaires to identify their opinions about these proteins. These proteins have been analyzed by reverse-phase high-performance liquid chromatography by developing an easy and fast method to detect testosterone signal between 8-9 minutes of the chromatogram. The results of the study showed that 61% of the users believe that sport proteins contain hormones and other substances that are not mentioned in the list of ingredients. While 39% believe otherwise. On the other side, HPLC results of six proteins showed no signs for testosterone hormone. The main reason that drives them to take sport proteins is for building muscles in spite of they believe it could be harmful due to containing hormones and other substances. So in future investigations, it might be possible to use different brands and investigate them by using the same method.

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INTRODUCTION

Protein supplements are the most frequently consumed dietary supplement by Jordanian youth, especially those who enroll in the training in sports clubs and centers. They take proteins in as food-supplementary to build muscles faster and to improve their athletic performances, and physical appearances.

Taking proteins, after and/or before the workout is considered as a major step that athletes do in order to increase the average of muscular protein creation and prevent the collapse of the muscular protein. This will allow the accumulation of pure muscu-

lar protein during the refreshment and stimulation period after practising (from 1 – 3 hours) (Cribb *et al.*, 2006; Juhn, 2003; Bemben and Lamont, 2005). Moreover, it is very often recommended to take proteins as food-supplementary to increase adaptation (adjustment) response for the muscles (Cermak *et al.*, 2012).

The consumers' knowledge of sport proteins is important to be aware of their future attitudes and trends concerning their purchase decision (Abraham *et al.*, 2018). The opinion is a judgment or decision about some subject or issue formed through experience (Yousif, 2012). The opinion is considered as the verbal explanation for an attitude, where people express their opinions about beliefs (Alsamydai, 2014). This opinion is influenced by the information received from media and reference groups like friends and family. Kotler and Armstrong have mentioned that consumers gain believes and attitudes from experiences and learning (Armstrong *et al.*, 2014).

Aforementioned beliefs and attitudes represent emotions and evaluations towards a certain subject. These evaluations have an important role in forming consumers' opinions and their purchasing decisions (Al-Samydai *et al.*, 2019; Alsamydai, 2016; Jasim *et al.*, 2015).

The trend of using sport proteins encourage companies to produce and provide many types. At the same time, the companies which produce and market these products compete to gain consumers by forming positive feelings towards them in large-scale promotion campaigns and providing discounts. Consequently, there are consumers who may feel that companies do not publish true and real information about these proteins. Also, they believe that the companies do not tell about all ingredients of these proteins that may contain hormones like testosterone and other substances. Athletes are always looking for the best way to increase their performance. So this could lead many companies to add testosterone to their products since it may increase the building of muscle mass, which improves strength and physical appearance. In addition, testosterone increases tissue oxygenation by its ability in increasing the production of red blood cells, oxygen carriers, the more oxygen the tissues receive, the better they work (Guo *et al.*, 2013). In the same way, testosterone restores muscle tone, elevates mood, increases mental alertness, and improves stamina (Wynne and Khalil, 2003; Bhasin *et al.*, 1996).

The appropriate role of testosterone in sports is a frequent topic of debate. Although anabolic agents are banned from competition by most international sports federations, numerous competitors test positive every year. The resulting challenge is to discriminate illicit exogenous testosterone use from natural variation in endogenous androgen production in men and women (Wood and Stanton, 2012).

Testosterone is both an endogenous hormone and a drug of abuse. Testosterone is the principal androgenic steroid produced by the testes (Bahrke and Yesalis, 2004). It is also a precursor to estrogen synthesis by the ovary in women. Steroids are hormones derived from cholesterol, and androgens promote the development and maintenance of male characteristics, In addition to their androgenic (masculinizing) effects, testosterone, and indeed all androgens, also has anabolic (muscle-building) actions. Hence, they are known collectively as anabolic-androgenic steroids (AAS). Testosterone is normally used as a replacement therapy in the male in conditions associated with symptoms of deficiency or absence of endogenous testosterone including primary hypogondism where testicular failure due to cryptorchidism, bilateral torsion, orchitis, vanishing testis syndrome, or orchidectomy; and hypogonadotropic hypogonadism gonadotropin or LHRH deficiency, or pituitary-hypothalamic injury from tumors, trauma, or radiation (Leet *et al.*, 2011)

The common side effects of Testosterone include enlarged breasts in men, prolonged erections, patterns of baldness , water retention,nausea, vomiting, headache, skin color changes, increased/decreased sexual interest, alterations in liver function, oily skin, hair loss,menstrual irregularities in women, azoospermia (absence of sperm in semen), alterations in cholesterol and other blood lipids, high blood pressure, infertility and acne (Viswanathan and Eugster, 2011).

The JFDA (JORDANIAN FOOD AND DRUG ADMINISTRATION) law defines dietary supplements in part as products taken by mouth that contain a "dietary ingredient." Dietary ingredients include vitamins, minerals, amino acids, and herbs or botanicals, as well as other substances that can be used to supplement the diet. Dietary supplements come in many forms, including tablets, capsules, powders, energy bars, and liquids. These products are available in stores throughout Jordan, as well as on the internet. They are labelled as dietary supplements. Federal law does not require dietary supplements to be proven safe to JFDA's satisfaction before they are marketed. For most claims made in the labelling of dietary supplements, the law does not require the manufacturer or seller to prove to JFDA's satis-

faction that the claim is accurate or truthful before it appears on the product (Dietary Supplements, 2019).

In general, JFDA's role with a dietary supplement product begins after the product enters the marketplace. That is usually the agency's first opportunity to take action against a product that presents a significant or unreasonable risk of illness or injury, or that is otherwise adulterated or misbranded. Once a dietary supplement is on the market, JFDA has certain safety monitoring responsibilities. These include monitoring mandatory reporting of serious adverse events by dietary supplement firms and voluntary adverse event reported by consumers and health care professionals (Dietary Supplements, 2019).

A number of people believe that there are hormones in the proteins that enter the country because of the rapid response of the human body to build muscles once these proteins are taken very quickly. Therefore, the purpose of this study was to develop and optimize a chromatographic method of analysis to detect the testosterone on proteins. Six types of proteins that are mostly used and popular in Jordan market were chosen. A primary study with proteins' users to identify their opinions about these proteins was conducted, and finally, we made a comparison between consumers' opinion and lab results.

MATERIALS AND METHODS

Study hypotheses

Based on the objectives and questions of the study, it is hypothesized that:

H01

There is no consensus between consumers' opinions about sport protein supplements and the results of laboratory analysis.

H02

There is no statistical significance ($\alpha \leq 0.05$) of the (attitude, belief, sports center coach, reference group and risk of use) on the opinion of the consumer to use sport protein supplements.

Study model

The following diagram (Figure 1) represents the study model which is based on the study problem, objectives and hypotheses (Alsamydai and Baqer, 2015; Alsamydai, 2014; AL-Samydai *et al.*, 2018; Alsamydai *et al.*, 2018).

Statistical analysis

Study sample

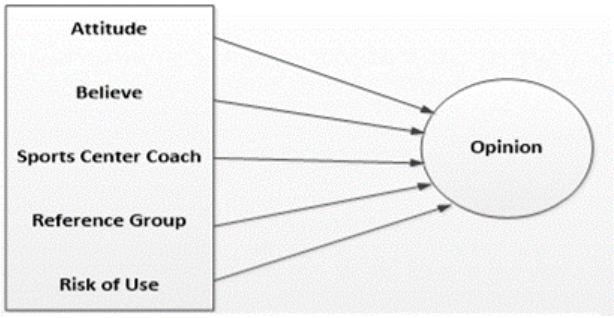

Figure 1: Study Model

The study took place in Jordan, from January 2019 to April 2019. Two strategies of questionnaire distribution were utilized. The first one included the printing and distribution of400 paper surveys to members of a health club we received only 209 fully answered questionnaires from them. The second strategy was using a Google drive questionnaire, made shared and paid post-boot on the Facebook page for two times It reached to more than 10000 people, 811 engaged with the post, and 366 athletes filled the questionnaire.

Chromatographic analysis

Samples and reagents

Testosterone standard was obtained as a gift from the Jordan Food and Drug Administration (Amman, Jordan). Proteins were purchased from local markets. Deionized Water (LABCHEM®, USA), methanol and acetonitrile (FULTIME®, China) HPLC grade were used as solvents.

Instrumentation and chromatographic conditions

An HPLC (DIONEX UltiMate^{TM}3000). (Thermo Fisher Scientific, Waltham, MA, USA). The detector (UV-VIS-PDA Detector). the pump (solvent delivery systems pump) (UltiMate^{TM} 3000) and the autosampler (UltiMate^{TM} 3000).

Computer software used was Chromeleon®. HPLC system was set at a wavelength of 245 nm and coupled with aKromasil®C-18 Column (KNAUER, Germany); (150 mm x 4.6 mm, 5μm) with a flow rate of 1 ml/min using a 20 μl injection volume. The mobile phase was (90% Methanol, 10%Water).

Preparation of standard testosterone solution

Testosterone (2.5 mg) dissolved in5ml ethanol to obtain a final concentration of (0.5mg/ml) stock solution of testosterone.

Preparation of sport protein supplements samples solution

Sports supplements (2.5 mg) dissolved in 4ml acetonitrile to obtain a final concentration (0.6mg/ml)

stock solution of sports supplements.

Table 1: Gender

Total	Female Freq.	Male Freq.	Gender Reason
363	56	307	Muscles builder
148	56	92	General tonic
43	13	30	Weight gainer
21	4	17	Others
575	129	446	Total

Method development

During the development of the procedure, various conditions were optimized to find the most appropriate methods for the determination of testosterone in sports supplements. Many wavelengths have been proceeding, but to achieve high sensitivity, the detection was performed at 202 nm. For determination of testosterone, different mobile phase compositions and ratios were applied. The mobile phase composed of methanol: water (90:10 v/v) at a flow rate of 1 ml/min generated a sharp peak.

RESULTS AND DISCUSSION

Consumer's Opinion

Sports proteins usage

According to the responses of the sample, the main reason that drives them to take sport proteins is for the building of muscles. The highest percentage of 69% was by males, while 43% of women use protein for the building of muscles. And this represented 63% of the entire sample(Table 1).

As shown in (Table 2), the percentage of 77.7% is of 18 and 31 age groups which consume proteins.

Sport proteins contents

The results of the study showed that 352 representing 61% of the sample believe that sport proteins contain hormones and other substances that are not printed on the list of ingredients, while 223 representing 39% of the sample believe that all the information are printed on the list of ingredients (Table 3).

Reliability

The reliability analysis applied to the level of Cronbach Alpha (α) is the criterion of internal consistency, which was at a minimum acceptable level ($\alpha \geq 0.60$) as suggested by (Sekaran, 2003). T is the value of α reached (0.988) for the questionnaire as a whole. This is an excellent value since it is higher than the accepted value (0.60). T is the value of α reached (0.795) for the questionnaire as a whole. This is an excellent value since it is higher than the accepted value (0.60).

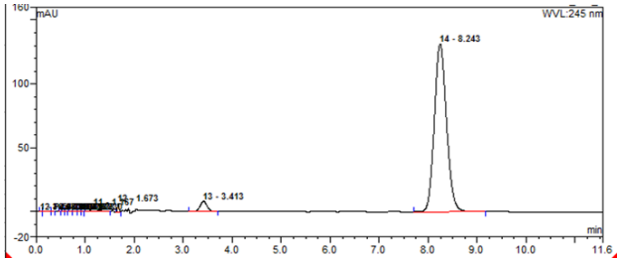

Figure 2: Chromatogram of standard testosterone.

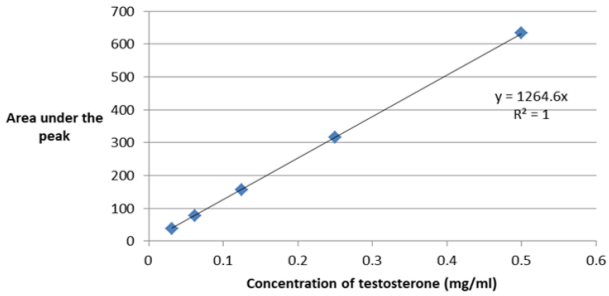

Figure 3: calibration curve of peak area versus concentration for testosterone

Dimensions

As shown in (Table 4), and within the dimension of the attitude: the brand has an impact on the attitude of the members of sample who have a positive image of the brand of protein. In the dimension of the belief, the members of the sample do not find that the use of protein will improve health. In reference to reference groups, the members of the sample point that family member's lack of complete information about proteins so they do not encourage them to consume proteins. In the dimension of the risks of use, a great effort has been made by the individuals to gather information about proteins in order to ensure their safety because they have concerns and fears that they may cause diseases especially when friends warn them against consuming proteins.

As shown in (Table 5) the research dependent variables (attitude, belief, sports center coach, reference group and risk of use) are significant, because F significant is (0.00) which is less than (0.05), and the calculated F value (94.702) is more than an F table (2.89). Therefore, we reject the null hypothesis and accept the alternative one which states that there is statistically significant effect at the level of ($\alpha \leq 0.05$) of the (attitude, sports center coach, reference group and risk of use) on the opinion of

Table 2: Age

		q2				
		Body muscles	General tonic	Weight gain	Others	Total
Age	18-24	187	36	15	11	249
	25-31	121	51	20	6	198
	32-38	49	39	6	2	96
	39-45	6	19	2	0	27
	45- and more	0	3	0	2	5
Total		363	148	43	21	575

Table 3: Gender cross-tabulation

Gender	q3		Total
	Yes	No	
Male	262	184	446
Female	90	39	129
Total	352	223	575

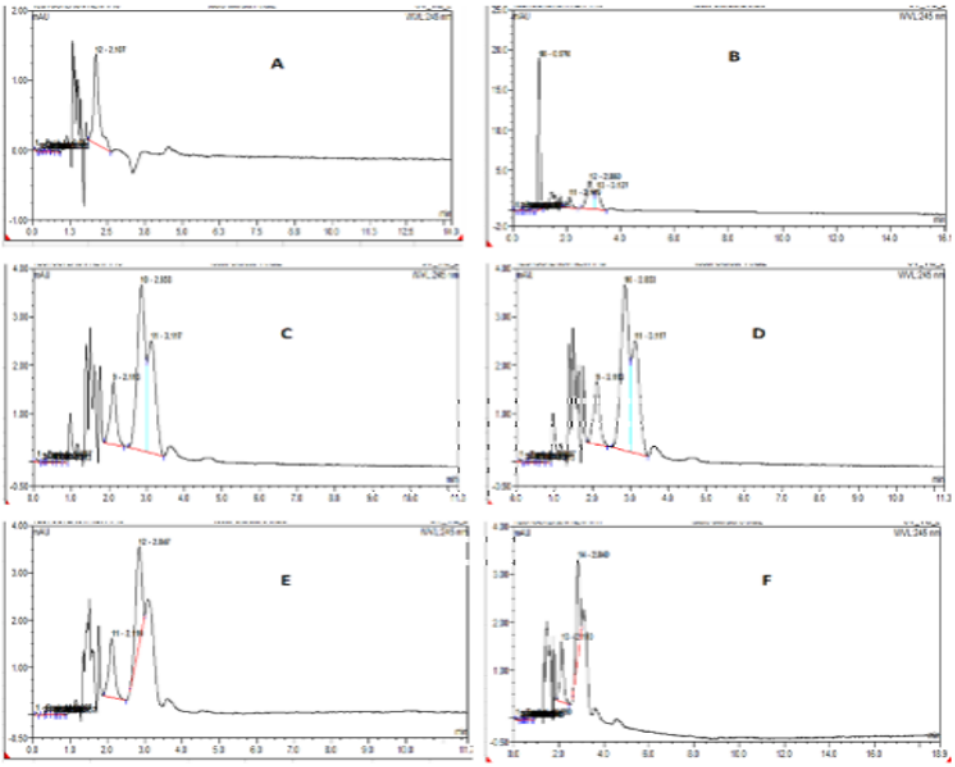

Figure 4: Chromatogram of $^{A.}$Protein 1, $^{B.}$Protein 2,$^{C.}$ Protein 3, $^{D.}$ Protein 4,$^{E.}$Protein 5, and $^{F.}$ Protein 6

Table 4: Descriptive statistics of dimensions

Questions	Mean	Std. Deviation
Dimension One: Attitude		
Taking sport proteins will help in the improvement of your health.	3.9583	.91921
You feel comfortable when you take protein.	3.7583	.89462
You find that the brand of sports protein has an effect on your attitudes.	4.1478	.92435
You have a positive image of protein using, which contributes to the formation of positive attitudes towards protein.	4.0191	.80589
Dimension Two: Believe		
You believe that taking proteins will help in improving your health.	3.5165	1.16988
You believe that the use of proteins is safe.	3.5739	1.03813
You think that using proteins will positively reflect on your health	2.9304	1.10999
Dimension Three: Sports center coach		
Using the experience of the Sports Center's coach to select the type of protein.	3.7722	1.08775
Trust the information you get from the Sports Center coach to choose the type of protein.	3.5617	1.10869
Sports club employees encourage you to take protein.	3.6452	1.08170
Dimension Four: Reference group		
You find that family members have complete protein information.	2.5930	1.11434
Family members encourage you to take protein	2.4870	1.20079
You find that your friends have reliable information about proteins	3.2713	1.07366
Use the opinions of my friends to choose the brand of proteins	3.6522	1.03462
Dimension Five: Risk of use		
A great effort has been made to gather information about proteins in order to ensure their safety and non-harm when using	3.7600	1.12841
Your anxiety is generated when you think about taking proteins	3.0174	1.19218
You are afraid to become ill when you use proteins	3.1635	1.20150
You receive information from a friend who warns you of using proteins	3.8574	1.02095
Dimension six opinion (Depended Variable)		
You have a positive image of the protein brand, which gives you a positive attitude towards it	3.7148	.92509
The coach in the sports club and the staff played a major role in the formation of an appropriate progenitor towards proteins	3.5426	1.12518
Your family and friends influence your view of proteins	3.3965	1.17434

Table 5: Results of multiple regressions

Dependent Variable	R	R^2	F	Sig.	Independent Variable	B	T	Sig.
The opinion of the consumer	0.674[a]	0.454	94.702	0.000	Attitude	0.246	6.651	0.000
					Beliefs	0.280	0.814	0.416
					sports center coach	0.334	9.718	0.000
					Reference group	0.197	5.833	0.000
					Risk of use	0.289	8.886	0.000

Table 6: parameters of system suitability.

No.	Parameters	Testosterone
1	Retention Time	8.31
2	Theoretical plate	5270
3	Area (AUC)	142.29525
4	Asymmetry	1.1475

Table 7: Precision values of testosterone

Sample name	Testosterone AUC
Injection 1	315.809
Injection 2	317.503
Injection 3	313.126
Average	315.479
Standard Deviation (SD)	2.2070
Relative Standard Deviation (% RSD)	0.7

consumer to use proteins,with the exception of the beliefs where the results showed that the level of significance is (0.416), which is more than (0.05).

The relationship between the dependent and independent variables is strong and positive. It is more than (0.5), R= 0.674$^{\alpha}$. Also, the R^2 =0.454, 0.454, which means that the independent variables contribution strongly effect the dependent variables with the percentage of of45.4%.

Since the value of the calculated t value for the variables (Attitude, 6.651, sports center coach, 9.718 Reference groups 5.833, and Risk of use 8.886), are more than the t value table (1.96). This means they have a statistically significant effect on the dependent variables, except the beliefs where the results showed that the t value is 0.814 less than the t value table 1.96 and the level of significance is (0.416), which is more than (0.05). Therefore it has no effect on the opinions of consumers to use the proteins.

Validation

System Suitability Parameters

The stock solution was injected into the chromatographic system, and system suitability parameters were determined (Table 6).

Specificity

The method was found to be specific since there was no interference of the mobile phase in the retention time of the analytical peak (Figure 2).

Linearity

The linearity range was found to 0.03mg /ml to 0.05mg /ml for testosteroneCalibration curves were plotted between the peak area and the concentrations the linear regression coefficients for testosterone found to be 1(Figure 3).

Precision

In the method precision study %, RSD was found to be less than 2 %. This indicates that the method has good repeatability (Table 7).

Detection of testosterone in sport supplements samples

As shown in (Figure 4), there is no peak detected for testosterone at 8.2 min in all sport protein supplements samples.

The present study was designed to determine the variation between Consumer opinion and chromatographic analysis about sport protein supplements. Contrary to consumer expectations about sport protein constituents like hormones, the chromatographic analysis does not detect testosterone content in all the tested sport protein supplements samples.

But according to Australian research, anabolic drugs have been found in 13 out of 67 supplements. It was familiar over a decade ago that some 20% of nutritional supplements sold in Europe and the USA confined anabolic steroids (Geyer *et al.*, 2004; Agency, 2016). A possible explanation for this might be that some products are contaminated with substances that are prohibited in sport (Viswanathan and Eugster, 2011).

This study confirms the association between sport proteins usage and muscle- building. A 2018 analysis of 49 studies support the use of protein supplementation for this purpose. The research suggests that protein supplements significantly

increase muscle size and strength in healthy adults who perform resistance exercise training, such as lifting weights[26].

The relationship between the independent variables (attitude, belief, sports center coach, reference group and, the risk of use) and the opinion is stronger because the value of Coefficient of correlation is0.674 and the calculated F value (94.702) is more than an F table (2.89). Therefore, we reject the null hypothesis and accept the alternative one which states that there is statistically significant effect at the level of ($\alpha \leq 0.05$) of the (attitude, sports center coach, reference group and risk of use) on the opinion of the consumer to use proteins.

The study recommends the importance that promotional campaigns focus on removing doubts and uncertainty of proteins' users and making advertisements include information published in scientific research.

CONCLUSION

The present study may be the first to compare between consumer opinion and chromatographic analysis of sport protein supplements. It has developed an easy and fast method to detect testosterone signal between 8-9 minutes of the chromatogram.

The results of six proteins that have been chosen showed no signs for testosterone hormone in proteins subjected to lab analysis. In future investigations, it might be possible to use different brands of protein supplements and investigate the presence of testosterone derivatives by using this method.

On the other hand, most opinions of the respondents, 61% of the sample, are found to believe that there are undeclared materials on the pack of proteins. Therefore, companies that produce and commercialize proteins must intensify their advertising campaigns to persuade their consumers otherwise.

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