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Original Article

Validity and Reliability Assessments of a 16-item Food Frequency Questionnaire as a Probiotic and Prebiotic Consumption Scale in People Aged 20 to 40 Years in Tehran

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ABSTRACT

Background and Objectives: Regarding health effects of probiotics and prebiotics in prevention and control of diseases and the lack of standard questionnaires in this field in Iran, objective of the present study was to assess validity and reliability of questionnaires designed to assess validity and reliability of probiotics and prebiotics in individuals aged 20–40 years in Tehran, Iran.

Materials and Methods: After verifying content validity of the questionnaires by eight experts in fields of nutrition, psychology and psychometrics, face and construct validities (in two levels of exploratory and confirmatory factor analyses) were assessed and then the tool was validated using Cronbach's alpha coefficient (due to the multi-value of the response spectra).

Results: The sampling adequacy index was higher than 0.7, which was the premise of exploratory analysis. For all the items, the standard loading factor was higher than 0.3, and the T-value was higher than 1.96. Furthermore, fit indices in confirmatory analysis were at the desired levels (0/9 and greater), and validity was verified. Cronbach's alpha value was calculated higher than 0.7, and the reliability of the questionnaire was verified.

Conclusions: Results of this study showed that the researcher-prepared food frequency questionnaire developed to assess the level of probiotic and prebiotic consumptions, especially in people aged 20–40 years in Tehran, includes significant validity and reliability.

Keywords: FFQ, Probiotics, Prebiotic, Validity, Reliability

Introduction

Probiotics are beneficial microorganisms for health if consumed in sufficient desirable quantities (1). Probiotics include coordinating roles in intestinal functions as well as clinical medicine, endocrinology, immunology and nutrition (2, 3). Probiotics prevent spread of pathogenic bacteria in intestines, leading to stimulation of host immune response, balances in expression of genes in guts (4, 5) and decreases in risks of cancer, hypertension, osteoporosis and menopausal symptoms. These microorganisms are involved in improving functions of the cardiovascular, digestive and urinary systems, emergence of anti-inflammatory, antimicrobial, and antiviral reactions, prevention of weight gain, cognitive function and bone strength (6) as well as improving symptoms of depression, anxiety and stress (7–10). Usually, sources of probiotics in foods originated from yeasts, fermented products (e.g., milk, cheese, yogurt, kefir and fermented vegetables), fermented fruits, mint, olives, kimchi and fermented cereal drinks can strengthen the beneficial bacteria in gut microbiome (11, 12). It is noteworthy that probiotics should be originated from human natural flora to include potencies to produce biogenic compounds (11–13). Furthermore, probiotics should be stable against stomach acid, digestive enzymes and bile salts to survive until they reach the intestines (14) and bind to the intestinal epithelia (15).

Probiotics feed on prebiotics. In other words, prebiotics provide a bedrock for the growth of probiotics by helping the growth of beneficial bacteria and thereby improving inflammatory statuses and supporting the intestine epithelial barriers (16). The most common prebiotics such as inulin are found in leeks, asparagus, artichokes, garlics and onions (17). Fructooligosaccharides are detected in wheats, honey, onions, garlics and bananas (18). Bacteria

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in the intestinal epithelia are affected by physiology and nutrition and are vital to human health (19). The imbalance in intestinal microbiota or dysbiosis leads to gastrointestinal, immunological and neurological disorders (20). It occurs under the effects of various causes such as genetics, diets, infections, drug uses, ages and genders (21–23). Clinical and preclinical studies have assessed positive nutritional effects of probiotics and prebiotics in clinical trials. For example, results of a study by Seyedi et al. Showed that addition of *Lactobacillus casei* and *L. paracasei* as probiotics and raftilose as prebiotics increased the levels of liver serum enzymes in mice (24). In recent years, several studies have been carried out on prebiotic compounds and dietary supplements and their roles in improving health of aquatic animals and poultries (25–27). In general, diets are addressed as potential options in regulation of people health (28). Awareness of this fact can motivate people to consume probiotic products because values of the products are linked to their health effects; therefore, probiotic products are categorized as healthy and targeted products (1). For example, enrichment of beverages with beneficial components such as inulin prebiotics is a recent development (29). Therefore, there are needs of tools to assess use of probiotics and prebiotics in people diets, which is an essential issue in nutrition epidemiological assessment studies (30). The FFQ questionnaire (as a model in this study) is a tool that participants can use to remember and record frequencies and quantities of the foods consumed at a specific time (31). Although various food consumption questionnaires have been studied in Iran regarding their validity and reliability, no questionnaires that measure probiotic and prebiotic consumption levels are available in Iran. Therefore, this study was carried out to assess validity and reliability of a researcher-prepared probiotics and prebiotic consumption questionnaire.

Materials and Methods

This questionnaire was designed based on the available resources for probiotic and prebiotic consumption in Iran (32–34). In designing the questionnaire, foods containing probiotics and prebiotics were used. These included pasta, rice, traditional bread (35), curd, green tea (36), pickles (37), cabbage, spinach, onion, garlic (38), beans (39), Banana, apple, peach, plum (40), traditional yogurt, milk, cheese (11), dark chocolate (41) and honey (42). Regarding aims of the study, samples were selected using convenience sampling method from men and women aged 20–40 years, who were residents of Tehran with at least a diploma. Draft questionnaire of this study included 25 questions, which were linked to the adequate consumption of foods containing probiotics and prebiotics in the people diets. After assessing content validity, five items were removed and necessary changes were used to the items of the questionnaire according to the experts. No questions were removed from the questionnaire due to the lack of face validity. Four items were neglected in the study of construct validity using exploratory factor analysis. The final scale consisted of 16 items. Items were presented on five-point Likert scale. Each question was scored on a 5-point scale from 0 to 4 (never = 0, rarely = 1, sometimes = 2, usually = 3, always = 4). Since the final questionnaire included 16 items, the score ranged 0–64. To assess use of probiotics and prebiotics, three score categories with equal intervals were used. Classification was carried out as the consumption of low (score of 0–21), moderate (score of 22–42) and desirable (score of 43–64). The highest score on the scale was 60, while the lowest possible score was 0. Achieving high scores indicated high levels of consumption. Participants in the first quarter of 2020 answered the online questionnaire due to the first prevalence peak of Covid 19. After uploading the questionnaire on internet, invitations of participation were sent to eligible people through internet. Eligible individuals participated in the study by filling out the questionnaire. The sample size (300 people) included 10–20 participants per item according to Costello, 2005 (43).

Questionnaire validity using content validity, face validity and structural validity

Since the questionnaire was researcher-prepared, its validity and reliability were essential for the assessment. Similar questionnaires such as FFQ questions were designed based on the objectives of the study and according to several experts on the subject (one expert and two experienced nutritionists, two experienced faculty members and a professor of psychology, a specialist and a psychometric consultant). Content validity index was used to assess the content coordination of measuring instruments for probiotic and prebiotic uses in qualitative and quantitative methods. In the face validity study, ten participants were asked to determine the importance of each item of the questionnaire on 5-point Likert scale from 0 to 4 points (never = 0, rarely = 1, sometimes = 2, usually = 3, always = 4). The effect score of each item was higher than 1.5 as acceptable; thus, the face validity was verified. To assess the construct validity, data were divided into two parts. In one part, exploratory factor analysis (to assess the internal relationships between the items to discover those that were most linked to each other), and in the other part, confirmatory factor analysis (CFA) was used to verify the construct based on the Iranian culture.

Questionnaire reliability using Cronbach's alpha coefficient

Reliability of the questionnaire was calculated using Cronbach's alpha coefficient due to the multi-value of the response spectra. The SPSS Software v.26 and Lisrel v.8.80 were used for the statistical analysis. Figure 1 shows order of the questionnaire development.

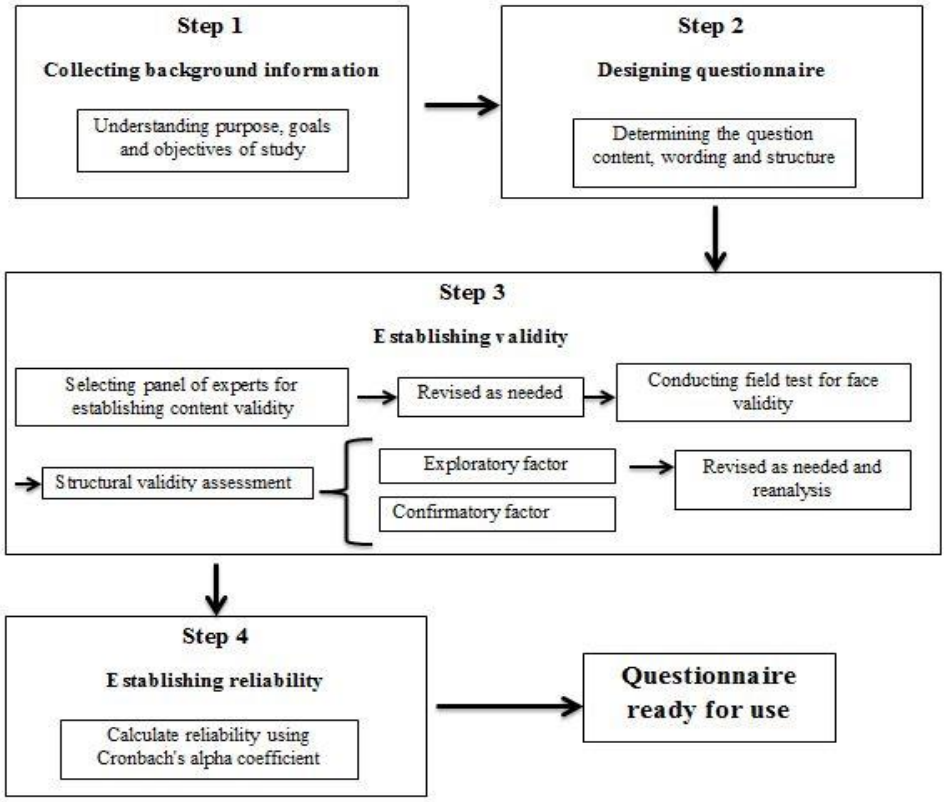


Figure 1. Order of the questionnaire developement

Results

For the statistical analysis of data, data descriptions were used based on the current methods in descriptive statistics, interpretation of the test results and interpretation of the research hypotheses based on Pearson correlation and regression. Total, 21 questionnaires were removed from the study due to incomplete completion, and analyses were carried out on 279 samples. Of these, 76 were males and 203 were females. Of the males, the highest number of participants was in the age group of 35–37 years (28.57%) and the lowest number was in the age group of 32–34 years (8.57%), while 41 people did not declare their age. Of the females, the highest number of participants was in the age group of 20–22 (20%) and the lowest number was in the age group of 35–37 (10%), while 113 people did not declare their age. The highest frequency of educational level distribution in participants was at the bachelor level (38.92% females and 38.16% males). The lowest frequency of educational level distribution in participants was at the master degree (25% males and 26.11% females). Regarding the participants' marital status, 65.79% of men and 64.04% of women were single and the rest were married. Most participants (74.9%) were in level two of probiotic consumption (moderate) and a small proportion (3.6%) were in the third level of probiotic consumption (good). Most participants (74.9%) were in the medium level of probiotics, a small proportion (3.6%) in the level of good consumption and the rest (21.5%) in the low level of consumption. In monthly family income distribution, most participants were in the middle-income level, while 27 men and 107 women did not declare their family monthly income. Table 1 shows demographic and socioeconomic information of the participants.

Assessment of the content validity of the questionnaire using content validity ratio and content validity index

Experts judged the qualitative assessment of content validity using logical analysis of the questionnaire contents in using appropriate understandable words, appropriate scoring and appropriateness of the selected items. Necessary feedbacks were provided by the experts and the items were corrected accordingly. In a quantitative review of the content validity index, questions were provided to the experts to check the necessity and relevance. Content validity ratio (CVR) index was used and the experts were asked to rate each item on a three-point scale (necessary, useful but not necessary and not necessary) and report if the questions measured the scale or not. The index value was higher than 0.75 (that was 1). In assessment of the content validity index (CVI), experts identified each item (based on relevance, clarity and simplicity) on 4-point Likert scale (1 = not relevant, 2 = relatively relevant, 3 = relevant, 4 = highly relevant). The CVI score for each item was higher than 0.79 as appropriate. Therefore, an agreement was reached on content validity of the questionnaire.

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Table 1. Demographic characteristics of the participants

	Men		Women	
	N	(%)	N	(%)
Age				
20-22	7	20/00	18	20/00
23-25	5	14/29	16	17/78
26-28	2	5/71	13	14/44
29-31	4	11/43	12	13/33
32-34	3	8/57	9	10/00
35-37	4	11/43	9	10/00
38-40	10	28/57	13	13/33
Total	41	-	113	-
Education				
Diploma	28	36/84	70	34/48
Bachelor	29	38/16	79	38/92
Masters	19	25/00	53	26/11
Total	76	-	203	-
Marital status				
Single	50	65/79	130	64/84
Married	26	34/21	73	35/96
Total	76	-	203	-
Monthly family income				
Less than 20000000 Rials	17	34/69	34	35/41
Between 200,000,000 Rials and 60,000,000 Rials	23	46/93	46	47/91
Above 60,000,000 Rials	9	18/36	16	16/66
Total	49	-	96	-

Assessment of the construct validity using exploratory factor analysis

Carrying out statistical analyses is linked to the following criteria of default of exploratory factor analysis includes Kaiser-Meyer-Olkin measure of sampling adequacy (KMO) values of 0.7 or higher. Based on Table 2, sampling adequacy index was higher than 0.7; therefore, exploratory analysis could be carried out.

Table 2. Sampling adequacy indices

Index	Value
Kaiser-Meyer-Olkin	0/735
Split Bartlett / Chi Square	1011/23
Degrees of freedom	190
Significance level	0/001

In Table 3, the first exploratory analysis was carried out without fixing the factors. Six factors were identified with an initial eigenvalue of higher than 1. Four items and five factors were removed from the study during six stages of the analysis and one factor was used, which explained 24.66% of the total variance. Reasons for removing factors and items included loading factors of less than 0.3, placing less than three items in one factor and alpha values of less than 0.7. Since six factors out of the six assessed factors included eigenvalues of higher than 1, one factor included all the necessary conditions for a significant factor, describing 20.66% of the total variance as verified by the Pebble diagram (Fig. 2). Loading factors were calculated separately by fixing the loads to 0.3 (Table 4).

Table 3. Findings from several stages of the exploratory analysis

Stage	KMO	Number of factors	Percentage of variance	Finding	Result
1	0/735	6	53/46%	Factor 6 (alpha value less than 0.7)	Remove6 factors Reanalysis
2	0/735	5	47/77%	Factor 5 (alpha value less than 0.7)	Remove 5 factors Reanalysis
2	0/735	4	41/93%	Factor 3 (alpha value less than 0.7)	Remove3 factors Reanalysis
3	0/735	3	35/48%	Factor 3 (alpha value less than 0.7)	Remove3 factorsReanalysis
4	0/735	2	28/88%	Factor 2 (alpha value less than 0.7)	Remove 2 factorsReanalysis
5	0/735	1	24/44%	Loading factor less than 0.3 for items 1, 2, 4 and 20	Delete these itemsReanalysis
6	0/776	1	24/66%	All desirable	Preservation of factors and items

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Table 4. Loading factors of each item

Item		Loading factor
1.	Consumption rice twice a week	delete
2.	Pasta twice a week	delete
3.	Beans 1.5 cups per week (including beans, peas, lentils, chickpeas, barley, mung bean and soy)	0/391
4.	Daily consumption of traditional bread (1 to 3 palms of Barbari or Sangak or Taftoon bread or 4 to 8 palms of Lavash bread)	delete
5.	Daily consumption of traditional yogurt (such as goat and cow milk yogurt)	0/433
6.	Daily consumption of yogurts containing probiotics that are written on their packaging	0/394
7.	Daily consumption of milk (both plain and flavored milk)	0/413
8.	Daily consumption of curd	0/590
9.	Daily consumption of fermented cheeses (such as gouda, cheddar, mozzarella and lactic cheeses)	0/425
10.	Salty products such as pickles, salted cabbage and salted olives 1 to 2 times a week	0/566
11.	Pickled products 1 to 2 times a week	0/581
12.	Daily consumption of fruits such as apples, cucumbers, bananas, plums and peaches	0/494
13.	Cabbage 1 to 2 cups a week (including white cabbage, red cabbage, kale, broccoli and cauliflower)	0/611
14.	Daily consumption of onions	0/440
15.	Consumption 1.5 cups of spinach a week	0/600
16.	Daily consumption of garlic	0/504
17.	Daily consumption of dark chocolate	0/536
18.	Green tea at least once a day	0/381
19.	Natural honey 2 to 3 tablespoons per week	0/484
20.	Taking probiotic capsules as a dietary supplement	delete

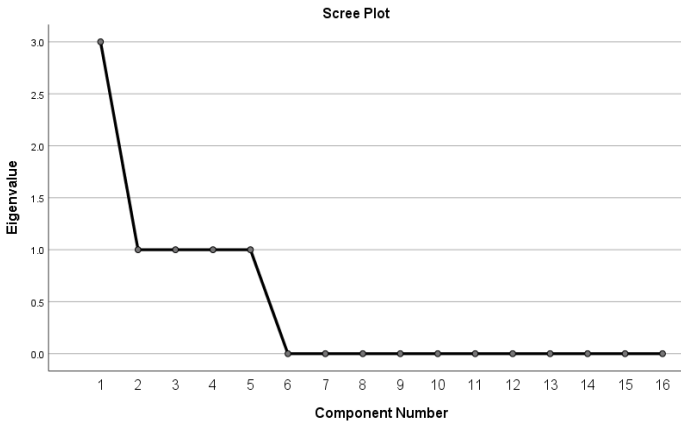


Figure 2. The pebble diagram

Assessment of construct validity using confirmatory factor analysis

Model fit was used for the assessment in CFA. Adjusted fit index, softened fit index, adaptive fit index and the second root of estimating the variance of approximation error are from the most essential and widely used indicators (44). As previously stated, data were divided into two parts. In the first part, exploratory factor analysis was assessed. In the second part, Lisrel Software v.8.80 was used to verify the identified factors as results of Varimax rotation in the software. As shown in Figs. 3 and 4 and in the model, all items clearly included anticipated T-values and loading factors linked to their respective factors. For all the items, standard loading factors were higher than 0.3 and T-values were higher than 1.96. Based on Table 5, the fit indices included the anticipated levels (0.9 and above) and therefore it can be concluded that the proposed model was approved.

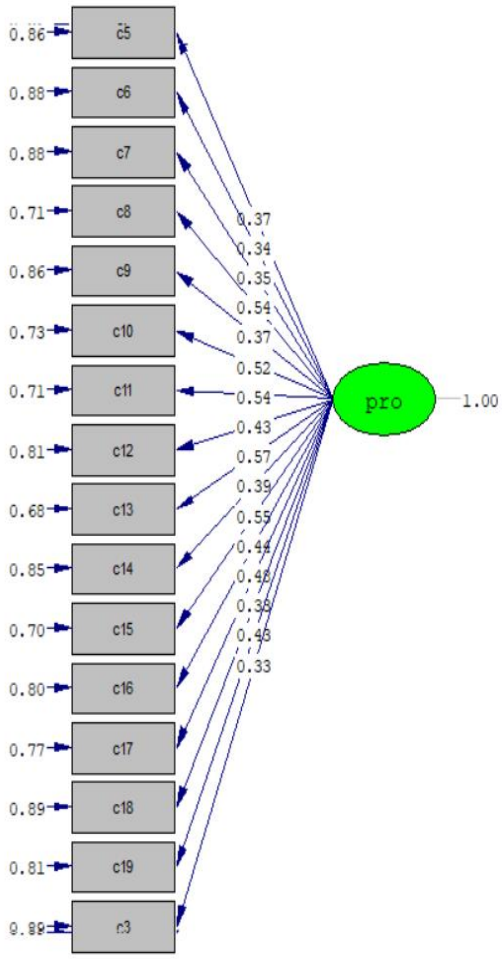


Figure 3. The confirmatory factor structural model based on the standard loads

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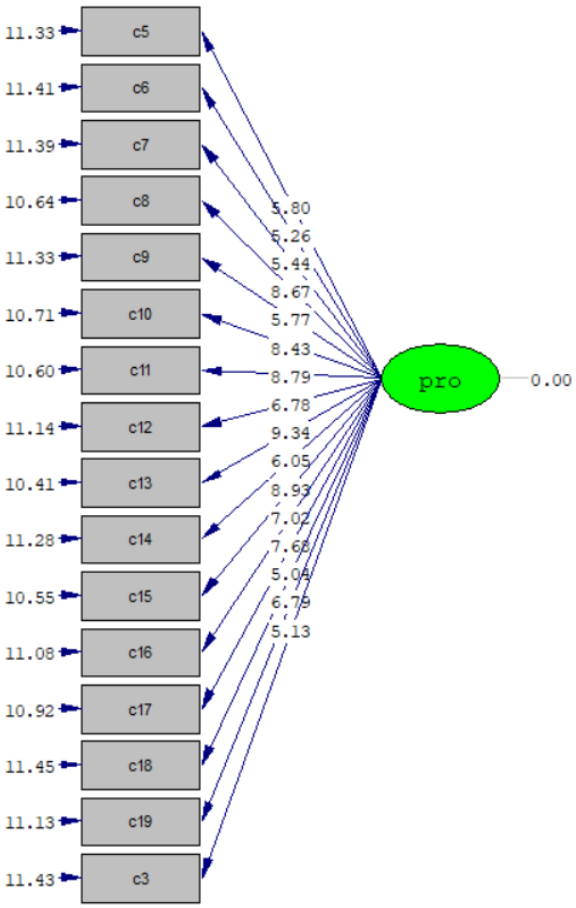


Figure 4. The confirmatory factor structural model based on the T-values

Assessing reliability of the questionnaire using Cronbach's alpha coefficient

Cronbach's alpha method was used to assess reliability of the questionnaire due to the multi-value of the response spectra. Four items with low alpha coefficients were removed from the study. Cronbach's alpha reliability coefficient of the questionnaire was calculated as 0.781. The value was reported as higher than 0.7 if each item was deleted; thus, reliability of the researcher-prepared questionnaire was verified (Table 6).

Table 5. The model fit indices

Index	NFI	CFI	GFI	IFI	RMSEA	SRMR
Values obtained	0/89	0/85	0/88	0/85	0/08	0/07
Round to one decimal place	0/9	0/9	0/9	0/9		

Discussion

Currently, no food frequency questionnaires (FFQ) are available to assess use of probiotics and prebiotics in Iranian people. For the first time in this study, a 16-item questionnaire was designed to assess levels of probiotic and prebiotic consumption in people aged 20–40 years in Tehran, Iran, using probiotic items and sources containing prebiotics.

Table 6. Cronbach's alpha value for each item

Probiotic consumption questionnaire items: Cronbach's alpha total 0.781	Cronbach's alpha if the item is deleted
1. Consumption rice twice a week	delete
2. Pasta twice a week	delete
3. Beans 1.5 cups per week (including beans, peas, lentils, chickpeas, barley, mung bean and soy)	0/0/775
4. Daily consumption of traditional bread (1 to 3 palms of Barbari or Sangak or Taftoon bread or 4 to 8 palms of Lavash bread)	delete
5. Daily consumption of traditional yogurt (such as goat and cow milk yogurt)	0/0/773
6. Daily consumption of yogurts containing probiotics that are written on their packaging	0/776
7. Daily consumption of milk (both plain and flavored milk)	0/776
8. Daily consumption of curd	0/766
9. Daily consumption of fermented cheeses (such as gouda, cheddar, mozzarella and lactic cheeses)	0/774
10. Salty products such as pickles, salted cabbage and salted olives 1 to 2 times a week	0/765
11. Pickled products 1 to 2 times a week	0/765
12. Daily consumption of fruits such as apples, cucumbers, bananas, plums and peaches	0/768
13. Cabbage 1 to 2 cups a week (including white cabbage, red cabbage, kale, broccoli and cauliflower)	0/762
14. Daily consumption of onions	0/776
15. Consumption 1.5 cups of spinach a week	0/764
16. Daily consumption of garlic	0/768
17. Daily consumption of dark chocolate	0/767
18. Green tea at least once a day	0/776
19. Natural honey 2 to 3 tablespoons per week	0/772
20. Taking probiotic capsules as a dietary supplement	delete

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The questionnaire was designed based on literature reviews, interviews and consultations with experts. To assess validity of the questionnaire, content validity methods, face validity and construct validity were used. Awareness of dietary probiotics in societies is critical for the nutritionists and physicians. There is a little research on this topic. For example, the aim of a study by Kim and Shin was to assess relationships between probiotic food consumption and depression status in South Korean population using cross-sectional data analysis. Study population included 26,118 19–64 year-old people from the Korean National Health and Nutrition Examination Survey (KNHANES V, KNHANES VI-1). In this study, a FFQ was used to assess probiotic food consumption, including 112 foods Such as fermented vegetables (kimchi), fermented milk products and other probiotic foods. Classification was based on consumption of foods containing probiotics at three levels of low, medium and high (45). Another study was carried out by Bayaga et al. to develop a culture-sensitive FFQ to assess probiotic and prebiotic intakes of 18–55 year-old low to middle-income urban women in the Philippines. The aim of the study was to introduce FFQ tools with emphases on foods containing inulin and oligosaccharides for the qualitative assessments of probiotic and prebiotic uses. To design this questionnaire, items such as a complete review of eating habits, single 24-h food recalls, assessment of available foods in the market and literature reviews were used to ensure the questionnaire sensitivity and cultural comprehensiveness (35). Questionnaires used in these studies needed redesign due to their incompliance with Iranian culture and food patterns.

In the present study, probiotic consumption of 279 20–40 year-old men and women was assessed in Tehran using a researcher-prepared questionnaire. This assessment was based on the consumption of food sources containing probiotics and prebiotics. Based on the results, participants were classified into three levels in terms of probiotic consumption. The most spread level of probiotic consumption included the medium level with 209 people. Lack of literatures and limited research records of the probiotic use in Iranian people were serious limitations of this study. In this study, probiotics were analyzed as general variables. Furthermore, questionnaires should be filled out online at the first prevalence peak of COVID-19 due to the time constraints. In this study, the only tool of data collection was a questionnaire. Issues such as lack of in-depth interviews and respondent various understandings of the ambiguous term probiotics and their beliefs might lead to directional responses in some cases. Overall, results of this study showed that the FFQ developed to assess levels of probiotic consumption in Tehrani 20–40 year-old people included significant validity and reliability. Collection and assessment of the validity and reliability of similar questionnaires may further be interested by the Iranian researchers to help experts of the associated fields.

Financial disclosure

The authors declared no financial interest.

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