Fried Carbohydrate-rich Food as a Potential Source of Malondialdehyde and Acrylamide: a Consumption Pattern to Evaluate the Risk of Cancer in Population

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Research

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Abstract

Background: Tah-Dig (A food rich in carbohydrate that is highly fried) is one of the most popular and tasty Persian food. However, toxic compounds such as malondialdehyde (MDA) and acrylamide are vastly formed in Tah-Dig. The purpose of this study was to investigate the cooking and consumption pattern, intake frequency, acrylamide and malondialdehyde content of Tah-Dig in Iranian population.

Methods. The content of acrylamide consumption in population was measured by LC-MS/MS and the MDA in cooking oils used to prepare Tah-Dig was also quantified. Data mining was used to investigate the relationship between the influential factors and Incremental Lifetime Cancer Risk (ILCR).

Results. The most consumed type of Tah-Dig among Iranians is Tah-Dig of bread. The highest amount of acrylamide consumed by population was found in potatoes Tah-Dig. The highest MDA content was detected in canola frying oil.

Conclusions. Considering data mining analysis, it was shown that the most influential factors related to ILCR are a cumulative factors of Tah-Dig color, portion size, Tah-Dig type, oil type, and the level of education.

1. Introduction

The food contains macronutrients, micronutrients, minerals, and vitamins that dietary habits such as methods of cooking can affect these nutrients. Cuisines refer to the traditional methods of cooking and consumption common in each country and are one of the factors that can be beneficial or harmful to health [1]. Rice is the chief staple food of the Iranian diet [2]. Tah-Dig is typically made with rice, which is one of the most popular and delicious cuisines of the Iranian people [3]. Tah-Dig is a thin crunchy and slightly browned rice or bread or potato which is formed in the process of cooking rice at the bottom of the pot (Tah = Bottom + Dig = Pot). This discoloration is due to the Maillard reaction, which is a non-enzymatic reaction between free amines and carbonyl groups of carbohydrates exposed to high heat by cooking methods (fried, cooked, and roasted foods) [4]. Tah-Dig can be included potatoes, rice, bread, lettuce, sesame, baguettes, etc. However, Tah-Dig of bread, potato and rice are more common than the others.

In the cooking process, acrylamide is one of the by-products produced by high-temperature cooking of the foods. Acrylamide is a water-soluble white crystal that is mostly used in industry as a material for dam construction, tunneling, paper industry, gel electrophoresis, and water treatment [5]. According to a report by the International Agency for Research on Cancer in 1994, it has been identified as a potential carcinogen for humans [6]. Further experiments [7] confirmed the possibility of carcinogenicity in humans [8]. Before 2002, acrylamide was thought to accumulate in the human body, mostly through occupational exposure to cigarette smoke, and rarely through drinking water and cosmetics, but in a preliminary study in Sweden on food Rich in starch exposed to high temperatures, high levels of acrylamide were observed. Further studies in other sources have shown that acrylamide is found in fats [9] and proteins, but is most

commonly found in carbohydrate-rich foods (150-4000 µg /g). Other research has been done to measure acrylamide level in various foods. Various methods such as HPLC [10], LC-MS/MS, and GC /MS can be used to measure acrylamide levels, but LC-MS/MS method is one of the most widely applied techniques due to its high sensitivity and accuracy [11].

Other ingredients used in cooking rice are solid and liquid oils. Oxidation, resulting from high temperature, is a notable feature of oils. Long-time exposure of oil to a high temperature for producing a crunchy layer of rice at the bottom of the pot intensifies the oil oxidation process leading to MDA production, a carcinogenesis compound that is bound to DNA and changing the signaling pathways [12]. The degree of lipid oxidation depends on the percentage of saturated and unsaturated hydrocarbons[13]. MDA is the best indicator for detecting lipid oxidation [14].

Genetic predisposition is responsible for only 5-10% of cancers, whereas 90-95% of cancers have their roots in the lifestyle. 30-35% of cancer incidence may be related to negative dietary habits [15]. Cancer is the second leading cause of death in the United States [16] and the third leading cause of death in Iran [17]. Due to the high economic, psychological, and social burden of cancer treatment, in recent years, significant attention has been paid to identifying patterns for cancer prediction and prevention. Cancer-related predictive indicators include incremental lifetime cancer risk index (ILCR) [18] as well as predictive data mining equations that can predict cancer-related patterns by considering the existing risk factors [19].

As cooked rice is one of the most popular dishes in Iran and Tah-Dig, is an integral part of this popular food, the following questions arise; How much is the consumption of Tah-Dig among Iranian people? According to the ILCR index and data mining equations, is there any relationship between Tah-Dig consumption and risk of cancer incidence among children and adults?

2. Materials And Methods

2.1. Questionnaire

To investigate the consumption and cooking patterns of Tah-Dig among the community, a questionnaire containing 34 questions was designed taking into account the cooking methods, consumables, and also the types of interests of the people. For content validity, CVR = Content validity ratio and CVI = Content validity Index were calculated. Five nutritionists, three food industry specialists, a statistician, and an epidemiologist familiar with Iranian cuisines were consulted. Questions that did not meet the quorum of CVR = 0.062 and CVI = 0.79, and had less than 5% response were eliminated. To evaluate the reliability, Test-Retest was used in a pilot population equivalent to 10% of the prototype volume at 15-day intervals. Subjects with a special diet were excluded from this study. The final questionnaire consisting of 25 questions was prepared. KUPPUSWAMY's SES scale was used for socio-economic ranking with three criteria of the job, education, and monthly income. Individuals' monthly income was measured indirectly based on the level of the urban area. Three levels of SES were considered high, medium, and low.

2.2. Determine the sample and size of the population

The cross-sectional, descriptive-analytical study was conducted on the population of Kermanshah (Western Iran) from 2019 to 2020. To identify possible differences in cooking and consumption methods among Iranian population, questionnaires were randomly completed in four other cities besides Kermanshah. The sample size considering the confidence level of $P = 0.095$ which is proportional to the consumption of Tah-Dig in the community and due to the lack of similar studies, 600 people for the city of Kermanshah (with eight urban areas) as the study center and the cities of Sari (north), Isfahan (center), Bandar Abbas (south) and Hamedan (west) a total of 150 people were identified. The sample size was proportional to the total population of that area. 48 and 270 Tah-Dig in pieces were required to measure the levels of the acrylamide and malondialdehyde content, respectively.

2.3. Sample collection and categorization

Participants were asked to provide us Tah-Dig in one piece cooked in the same way as usual in the time allotted for collecting the sample, and because of the stability of acrylamide and oxidized fatty acids we stored them in a -20°C freezer until delivery. A total of 343 samples were collected. Due to the non-uniform cooking conditions such as temperature, time, and type of oil in households, unlike laboratory conditions, the color was considered as a criterion, and samples with similar colors were placed in a group due to having the same amount of acrylamide. The samples were compared using Adobe Photoshop CC20.0.1 (Figure 1), and the samples with the same RAL and mean $\text{RBG} \pm 15$ were grouped (Table 1). The classification was based on the spectrum of colors in gold, light brown, and dark brown for each type of Tah-Dig. Four control samples of high-consumption flat breads in Iran (Lavash, Taftoon) used in baking Tah-Digs were also prepared. Oils were classified based on the three main groups (solid, liquid, and combined) and nine subgroups (Kermanshahi oil, vegetable solid, canola cooking oil, sunflower cooking oil, oil Sunflower frying and combination of Kermanshahi oil with other oils). Kermanshahi oil is prepared by animal butter especially sheep butter, with a pleasant taste and smell.

Table 1

Classification of Tah-Dig samples based on color analysis (RBG).

Type of Tah-Dig	Color grade	Red	Green	Blue
Rice	R1	194.2 ± 12.93	175.4 ± 12.9	97.36 ± 16.36
	R2	188.36 ± 14.5	166.45 ± 8.9	60.36 ± 15.22
	R3	147.20 ± 35.7	109.00 ± 42.6	34.00 ± 12.36
Bread	B1	215.40 ± 15.23	187.70 ± 27.166	82.90 ± 24.97
	B2	182.10 ± 12.81	134.00 ± 16.06	14.00 ± 7.3
	B3	163.00 ± 28.26	109.00 ± 32.42	15.20 ± 7.16
Potato	P1	232.70 ± 3.16	196.00 ± 17.98	80.80 ± 26.39
	P2	204.90 ± 5.01	176.10 ± 13.81	48.5 ± 15.50
	P3	208.50 ± 8.700	110.90 ± 15.98	6.900 ± 3.695

* The numbers represent the pixels counted by color and are reported as Mean ± SD.

- The samples had a significant difference ($P < 0.0001$) within the group at the color level, which represents three separate color spectrums.

- R = rice, B = bread, P = potato. Grades 1, 2, and 3 indicate an increase in color.

2.4. Statistical analyses

In the present study, central tendency indicators such as mean and standard deviation or percentage for continuous and classified variables were used to describe the data. Due to the method and nature of the research, analysis of variance (ANOVA) was used to compare continuous demographic variables and the Chi-square test was used for variables classified into different amounts of consumption. Data were analyzed using SPSS 24.0 software package. The data were collected precisely according to hypothesis and the methods were planned in-line with major purpose of the study.

2.5. Standard preparation for acrylamide

Stock solutions of acrylamide and acrylamide-d₃ (Sigma-Aldrich, USA) were diluted to 1 mg/ml in deionized water. A standard diluted solution of 10 µg/ml in deionized water (18 Ω) was then prepared. Tah-Dig samples were evaluated within the dynamic standard range of acrylamide 20-3000 ng/ml. To prevent light exposure, all standard solutions were placed in a volumetric flask and kept at 4 °C for 4 hours. Carrez I solution was prepared by dissolving 1.5 g of potassium hexacyanoferrate in 10 ml of water, and Carrez II solution was prepared by dissolving 3 g of zinc sulfate in 10 ml of water to make diluted standard solutions.

2.6. Extraction of acrylamide

Tah-Dig samples were homogenized at 100 mg/ml in deionized water (18Ω). Then, 50 ng/ml of acrylamide-d3 as internal standard (Sigma-Aldrich, Germany) was added to the samples and incubated for 20 min at 4 ° C in the dark to allow for equilibration. It was then centrifuged at 6000 × g for 20 minutes. One milliliter from the aqueous phase of the supernatant was removed and 650 µl of supernatant was purified by Oasis HLB 6 cc Vac Cartridge, 200 mg Sorbent (Waters Corp, USA). The samples were then stored at -20 °C until analysis.

2.7. LC-MS/MS analysis

Samples were analyzed by LC-Tandem Mass (Quattro Micro API micro mass Waters 2695, USA) [1]. EC HPLC column (analytical), NUCLEODUR C18 Gravity, 5 µm, 250x2 mm (MACHERY-NAGEL, GERMANY) was used for chromatography with isocratic conditions: Methanol: Formic acid (94: 5: 1) was used at a flow rate of 1 ml/min. The LC-MS/MS parameters were as follows: Source temperature 500 ° C; Separation Gas Collision (CAD) 5; Nebulizer gas (GS1) and auxiliary gas (GS2) below 40 psi and 50 psi, respectively, and curtain gas (CUR) below 10 psi. Declining potential (DP) and collision energy (CE). Finally, a general scan was performed to determine the standard m/z ratio of acrylamide and acrylamide-d3 to select the most appropriate mass spectrum [20]. Dynamic range was considered 15-1000 ng/ml and LOQ was considered 15 ng/gram of Tah-dig that the device could detect.

2.8. MDA Extraction and Evaluation

Tah-Digs samples were homogenized at 100 mg/ml in 50% acetic acid. It was then centrifuged at 6000 × g for 20 minutes and the supernatant was stored at -80 °C until the time of the experiment.

The commercial lipid peroxidation kit (Kiazist Life Sciences, IRAN) was used to measure MDA. 200 µl of samples, standards, and blanks were poured into the microtubes, then 600 µl of TBA solution in the kit was added and the lids of the microtubes were tightly sealed with parafilm to prevent evaporation. It was then incubated for one hour at 95 °C. After incubation, the samples were cooled to room temperature and 200 µl of samples were transferred into a 96-well plate of the kit and their absorption was read at 560 nm by plate reader.

2.9. Measurement of triglycerides

Due to the difference in the amount of oil consumed for making Tah-Digs, MDA samples were evaluated based on grams of oil consumed. The triglyceride content of the samples was measured by the commercial kit (Pars Azmoon, Iran) to normalize the MDA values. Triglyceride in the samples was evaluated by the glycerol phosphate dehydrogenase (GPO) method. Homogenized samples were exposed to Working Solution for 10 minutes at room temperature to measure MDA. Afterwards, the absorption of TG at 500 nm was measured by a plate reader (BioTek Instruments' USA) and the TG value of the samples was calculated based on the standard absorption diagram.

2.10. Risk assessment

The incremental lifetime cancer risk equation (ILCR) was used to assess the risk of Tah-Dig consumption:

$$ILCR = CDI \times CSF$$

The ILCR is an estimate of chronic daily intake (CDI) of the chronic daily intake of acrylamide (mg / kg/day), the values of which are based on the average intake based on questionnaire data and measurement of LC-MS/MS acrylamide. The equation is shown below. CSF equivalent to cancer slope factor -1 (mg / kg-day), which was considered equal to 0.5 based on 2009 Oehha [20].

$$CDI = \frac{C * IRi * EFi * EDi}{BW * AT}$$

The CDI is derived from the above-mentioned equation in which C: the amount of acrylamide per gram Tah-Dig, IRi: the amount of Tah-Dig received based on the defined serving (the amount of Tah-Dig consumed per person according to the usual cooking utensils on the market (sizes 18 cm, 24 cm, 26 cm and above, with an estimated amount of 300, 200 and 100 g of Tah-Dig). Then, the amount of Tah-Dig (gram) was divided by the number of family members to get the consumption (per person (g) / 365), BW: Individual weights (45, 65, and 70 kg), which were defined for people aged 18, 20 years and those over 70 years, respectively. EDi: years of consumption (due to not consuming Tah-Dig in childhood, the age minus four was considered), and EFi: exposure time per year (repeated per week × 56).

2.11. Data mining analysis of Tah-Dig cooking patterns related to cancer risk

Data mining analysis is a process to extract implicit patterns based on the factors leading to the prediction of an event. This study aimed at developing a predictive model to categorize the participants into two groups: those at high risk of cancer and those with no known risks based on the answers to the questions in the questionnaire and the effect they had on ILCR; therefore, the decision tree algorithm was used [21]. According to this classification, items such as high flame temperature, MUFA, and PUFA lipids, and fried Tah-Dig texture were found to be associated with high levels of acrylamide, while low flame temperature, SFA oils, and soft tissue of Tah-Dig were found to be associated with low levels of acrylamide. Other questionnaire responses were also included in the program. The ILCR index was defined as a dependent variable and other questionnaire questions were defined as an independent variable. Finally, the accuracy and performance of the model for use in similar conditions were evaluated.

3. Results

3.1. Consumption pattern evaluation

Figure 2 provides information on age, education, occupation, number of family members, and SES. About 40% of households consumed rice seven times per week (Table A as supplementary for details). 82.5% of

households always had rice with Tah-Dig. Households consuming different types of bread, rice, and potato Tah-Digs were 41.4%, 37.2%, and 21.4%, respectively (Figure 2A). 89.2% of the participants tended to eat fried Tah-Dig. About half of the families reported cooking time of 15-30 minutes (Figure 1B) and 34% of the participants chose the option of using a medium flame. 12.3%, 62%, and 25.7% of households consumed a quarter, half, and the total amount of rice Tah-Dig, respectively. Participants showed their tendency towards cooking and eating Tah-Digs as follows: golden brown (16%), light brown (57.8%) and dark brown (26.2%), respectively. The most requested Tah-Dig among households was golden-brown layer with potatoes (26%) and then the bread with dark brown color (19.3%). 98.2% of the cases stated that if the color of the bottom of the pot becomes darker, they will use it again. There was no significant difference between SES at all three levels and the type of oil selected by households. Moreover, there was no significant difference between the choice of the type of Tah-Dig consumption and the social level of households.

3.2. LC-MS/MS of acrylamide

94.4% of Tah-Dig samples were in the range of LOQ. It was also seen that samples of Taftoon and Lavash bread were below the LOQ before baking. The overall mean of acrylamide of bread Tah-Dig ($n=15$) was 124.5 ± 68.5 ng / g, 75, and 125 ng/g for golden, light brown, and dark brown, respectively. The acrylamide content of rice Tah-Dig was 576 ± 136 , which was 128, 143, and 203 ng/g for golden, light brown, and dark brown, respectively, and for potato Tah-Dig was 1097 ± 637.1 , which was 499, 868, and 1926 ng / g for golden, light brown, and dark brown, respectively. The most temperature-dependent changes measured by color were observed in Tah-Digs made with potato, bread and rice (Figure 2D). These results were generalized to all respondents based on the colors of Tah-Dig [22].

There was also a significant difference between the acrylamide content of Tah-Dig samples of potato, rice, and bread in three different colors of golden, light brown, and dark brown and in groups (Intragroup assay) ($P < 0.005$) (Figure 3). There was a statistically significant difference between potato and flatbread Tah-Digs in the acrylamide levels ($P < 0.05$). Moreover, a statistically significant difference was observed in the acrylamide levels between rice and potato Tah-Digs ($P < 0.001$). In addition, the findings showed a statistically significant difference between bread and rice Tah-Digs ($P < 0.001$).

3.3. MDA content of samples

Liquid cooking oil (23.6%), Kermanshahi oil (16.9%), and Kermanshahi oil in combination with other oils (15.5%) were among the most edible oils used in the process of cooking rice (23.6%) (Table B as supplementary). The highest MDA values measured before cooking were for canola oil at 354 nmol / g of TG and the lowest was for the combination of solid vegetable oil and frying oil at 29.9 nmol / g of TG (Figure 4). Measurements were made at 20, 25, 30, 35, 40, 45, 50, 60, and 65 minutes based on the answers given by the participants in the questionnaire. The highest MDA production after cooking was related to canola cooking oil with an average of 1527.89 nmol / g of TG, and the lowest value was related to the combination of Kermanshahi oil and frying oil with an average of 546.812 nmol / g of TG. The most changes were related to Kermanshahi oil at 45 °C, canola oil at 30 °C, and sunflower cooking oil at

40 °C. There was a significant relationship between the type of oil consumed and the SES of households ($P<0.001$). The frequency of Kermanshahi oil among SES1, SES2, and SES3 households was 18.8%, 16.7%, and 14.5%, respectively. The most consumed oils for cooking rice in households with SES1, SES2, and SES3 were olive and sesame oil (23.4%), cooking oil (28.2%), and solid vegetable oil (25.8%), respectively. Furthermore, the percentage of combined oils used in SES1, SES2, and SES3 was 19.3%, 12.8%, and 14.5%, respectively.

3.4. Prediction of cancer risk

According to the US-EPA definition, ILCR values of 1×10^{-5} were in the safe range for cancer and $ILCR \geq 1 \times 10^{-5}$ were in the cancer risk range [20]. In this study, it was found that 24.6% of people have no risk of cancer. 76.4% of people had a ILCR with the concept of cancer risk. The relationship between ILCR values and type of Tah-Dig was not significant ($P=0.317$). There was a significant relationship between ILCR and age ($P=0.005$) in this study.

Moreover, a strong significant relationship was found between the portion size in each household with ILCR values and the risk of cancer ($P<0.001$). This correlation between flame intensity and cancer risk was statistically positive and significant ($P<0.001$). However, the relationship between rice cooking time and at-risk ILCR values was not statistically significant and no correlation was found.

The study showed that there is no significant difference between the level of education and the color of Tah-Digs consumed by households. But, the results showed that there was a relationship between the number of family members and cancer risk prediction ($P<0.001$).

3.5. Evaluation of data mining of cooking and consumption patterns.

Tree diagram displays the application of the decision tree algorithm on the collected data (Figure 5). The nodes in the diagram represent the absence of cancer risk with zero code. The risk of cancers was coded as one and predictive patterns were extracted. From 165 patterns, two-thirds of them were associated with cancer risk, and one-third was associated with non-cancer risk.

About two-thirds of the predicted patterns were related to cancer risk and involved 491 of 611 respondents. The color of Tah-Dig is the most important influential factor related to the risk of cancer. Other variables influence the risk of cancer include SES, level of education, number of times per week, type of Tah-dig, type of oil consumed, and portion size (Table 2). The total number of participants ($n=120$) were associated with no cancer risk patterns. The accuracy of the models for use in similar patterns was 85.15%.

Table 2 Frequency of effective features in predicting ILCR

Features	Color Tah-	Oil	Education	SES	Serving	Type of Tah-	Frequency
	Dig	Type			Number	Dig	(week)
ILCR0*	43	31	40	24		13	18
(frequency/pattern)							
ILCR1**	119	24	38	91	21	30	80
(frequency/pattern)							

* No risk of cancer

** With the risk of cancer

4. Discussion

One of the most useful tools for assessing the relationship between the amount and manner of eating with a wide range of chronic diseases and types of cancer is to study the dietary patterns of people in the community. The geometry of nutrition is based on the effect of nutritional patterns on a set of conditions on health and disease, not nutrients alone. Foods are a collection of micronutrients, macronutrients, and toxins, but the point to be considered about their impact on health is the reproducibility of patterns in the society.

Acrylamide is a food contaminant formed in thermal processes, which can cause cancer. Tolerable daily intake (TDI) for acrylamide was estimated to be 2.6 µg/kg [23] in previous studies. In the United States and Europe, acrylamide contains about 30% of calories [24]. However, since the frequency of rice consumption is high in Iran, there is a possibility of receiving higher amounts of acrylamide through Tah-Dig. In this study, tolerable daily intake (TDI) for acrylamide in 60.9% of participants was higher than expected values just for consumption of rice Tah-Dig.

In this study, the highest levels of acrylamide were found in Tah-Digs with potato, rice, and bread, respectively as previous studies [3, 20]. Potato Tah-Digs with higher content of asparagine compared to bread and rice resulted in higher production of acrylamide [25]. Moreover, bread fermentation leads to a decrease in the level of asparagine and conversion of reducing sugars to ethanol lowers the pH values and production of acrylamide [26]. The amount of acrylamide obtained from the present study was higher than laboratory samples, which indicates that domestic cooking methods will produce higher amounts. Contrary to other studies in which FFQ questionnaire was used to estimate the intake level of acrylamide [27], this study designed a specific questionnaire to identify cooking methods, consuming materials, tendency of people even children as high-risk groups and the amount of consumption in the Iranian society.

Temperature and time are the most important factors in the formation of acrylamide [28]. Most acrylamide is formed in the first 8 to 10 minutes of cooking time [29] and with increasing cooking time, its production decreases [30]. But, it was also observed that the production of acrylamide increases with increasing cooking time [31]. In our study, there was no significant relationship between the cooking time and the amount of acrylamide, but this relationship was significant with the flame intensity, which may indicate that the cooking temperature is a more important parameter. Temperature and time can be in equilibrium, but the color appears to be a far more accurate indicator of acrylamide levels [22]. It can be said that the formation of acrylamide is in equilibrium with a set of cooking factors that ultimately leads to the formation of acrylamide at the level of a particular color.

In this study, there was no significant difference between the parameters that show people's attitudes towards food contamination and the harmfulness of a particular type of Tah-Dig and Tah-Dig oil with the level of education and different classes of SES. High tendency of people towards eating crunchy rice or Tah-Dig may be affected by some properties such as texture, appearance and taste [32].

In the analysis of oils, two main factors were obtained: **A)** The amount of MDA before cooking and the slope of changes: This measurement was made to find out which oil contains the maximum amount of MDA before cooking and which oil has the most slope of change. MDA production increased with cooking time [33]. The highest amount of MDA and the slope of changes were related to vegetable oils with polyunsaturated chains such as canola and sunflower for cooking. The higher the ratio of PUFA and MUFA fatty acids in oils, the higher the MDA production [34]. It was also found that the lowest amount of MDA before cooking and the slope of changes over a period of time are related to Kermanshahi oil and the combination of Kermanshahi oil with frying oil, which indicates that the higher the SFA in oils, the lower the amount of MDA [35]. **B)** Combination of Kermanshahi oil with other oils: The composition of Kermanshahi oil is such that it contains short-chain fatty acids (60%), medium-chain fatty acids (31.4%), and long-chain fatty acids (4%) [36]. When combined with other oils, MDA production decreased due to changes in the composition of fatty acids. It was also observed that the composition of Kermanshahi oil reduced the slope point of changes at lower temperatures, which contradicts the fact that the resistance of saturated oils to high temperatures is high. Regarding oils, it was seen that there was no significant difference between Kermanshahi oil consumption and SES. The special flavor of Kermanshahi oil is the most important factor affecting the choice of cooking oil.

In the higher social classes, the use of sesame and olive oils, which contains more PUFA and MUFA acids, leads to a reduction in cardiovascular diseases [37]. If the cooking time for the olive oil to be used in cooking becomes short [38]. The maximum slope of MDA production changes in the first 25-30 minutes of cooking. The concern about the consumption of these oils is the lack of knowledge about their cooking time, which is associated with the risk of cancer if high levels of aldehyde metabolites are produced [39]. Rice is regarded as a cooked food in Iranian culture, but Tah-Dig, which is placed in the bottom of the dish and exposed to heat and oil for at least 15-30 minutes, has the characteristics of fried foods that produce high content of MDA in saturated fatty acids. It seems reasonable to use Kermanshahi oil with other oils at the end of cooking time (less than 25 min) due to the reduction of MDA. If people consume laboratory

levels of Tah-Dig samples, a high percentage of people are at risk of cancer [20]. In the present study, it was also seen that three-quarters of people are at risk of cancer by consuming different types of Tah-Dig, with the difference that these findings were obtained from community. A significant relationship observed between cancer risk and the number of family members per household was related to cooking rice in the traditional way. In this method, more heat and longer time are required for cooking rice in large quantity, resulting in high levels of acrylamide. Furthermore, the positive correlation between portion size and ILCR values indicates that portion size as an independent factor of color and type of Tah-Dig can predict cancers.

Predictive models can largely estimate the factors influencing acrylamide intake and the risk of cancer [21]. In this study, a set of factors, including the color of Tah-Dig [29], the type of oil consumed [3], the portion size [40], the type of Tah-Dig consumed [3, 20], social class and education have the most power to predict cancer.

5. Conclusion

In this study, a large percentage of participants consumed Tah-Dig per week, which led to the formation of high levels of acrylamide and oxidized fatty acids. Data mining revealed that factors such as lifestyle and cooking methods might be involved in increasing risk of cancer. No significant difference was observed between the amount and manner of Tah-Dig consumption at different socio-economic classes. A large percentage of families will be affected by cancer if present cooking patterns and amount of consumption continue and no modification occurs. Nevertheless, it is important to consider that cooking carbohydrates with oils promotes the formation of toxic substances such as acrylamide and malondialdehyde at the bottom of the pot.

Declarations

Ethics approval and consent to participate

The study was performed in accordance with Research Ethics Committee of Kermanshah university of medical sciences that is in-line with NIH guidelines (Ethics code: IR.KUMS.REC.1398.207).

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

SK: Conceptualization, Methodology, Writing - Original Draft. FG: Methodology, Supervision, Writing-Original draft preparation, Resources. MP: Data Curation. FH: Methodology, Formal analysis. BM: Formal analysis. SMN: Reviewing and Editing, Project administration.

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Supplemental Data

Supplemental Tables A & B are not available with this version.

Figures

Figure 1

Tah-Dig Samples in three color ranges. Each number represents the increase in color intensity. No. 1= golden, No. 2= light brown, No. 3=dark brown. R = rice, B = bread, P = potato. Grades 1, 2, and 3 indicate an increase in color.

Figure 2

Data frequency distribution. (A) Frequency distribution of participants' education at five levels: elementary, secondary, bachelor's, and postgraduate. (B) Frequency distribution related to the job of the participants in the four levels of housewife, self-employed, employee and special occupations. (C) Frequency distribution related to the amount of consumption per week in four levels <3, 4-5, 5-6, 6-7 times a week. (D) Frequency distribution related to consumption of three types of Tah-Dig bread, potatoes and rice. (E) Frequency distribution related to cooking times in four levels of 15-30, 30-45, 45-60 and > 60 minutes.

Acrylamid Content

Figure 3

The amount of acrylamide in all three samples of the Tah-Dig of bread, potatoes and rice in three colors: golden, light brown and dark brown. Acrylamide levels are significantly different in intragroup and intergroup comparisons. **** = $P < 0.0001$. B= Bread, P= Potato, R= Rice.

MDA (nmol/gram of Triglyceride)

Figure 4

Malondialdehyde formation in types of oils used for Tah-Dig preparation over time.

Figure 5

Decision tree. The Frequency of the features (as rules) used to predict the risk of cancer by data mining.

Supplementary Files

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- [GraphicalAbstract.jpg](#)