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Short communication

Total dietary fiber analysis in dates and other dry fruits without starch and protein hydrolyzing enzymes

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ABSTRACT

Analysis of total dietary fiber (TDF) is an expensive method due to the use of enzymes. The present study compared the measured TDF content in dry fruits using two methods (enzymatic gravimetric method with and without enzymes α-amylase, protease and amyloglucosidase) to validate the cost-effective non-enzymatic analysis for TDF measurement. The study analyzed 16 samples of dry fruits: 12 date fruit cultivars (ten from UAE and two from Sudan) and two samples of figs and raisins. We found comparable TDF content in the dry fruits with and without the addition of enzymes in our analysis. Our results indicate that the level of agreement between the enzymatic and non-enzymatic methods falls within the acceptable range. Based on these results the non-enzymatic method can be a suitable, cost effective alternative to the enzymatic method for TDF analysis in dry fruits with low starch and protein contents.

1. Introduction

Extensive research over the last few decades has demonstrated the vital role dietary fiber plays in gastrointestinal health and its ability to prevent or modulate symptoms associated with chronic conditions such as obesity (Bozzetto et al., 2018), type-2 diabetes (Reynolds et al., 2020) and cardiovascular diseases (Evans, 2020). Moreover, recent evidence indicates the ability of the dietary fiber to act as a prebiotic to support a healthy gut microbiome (Koç et al., 2020). Due to the multiple health benefits associated with dietary fiber consumption, there is an increasing need for dietary fiber analysis to provide adequate recommendations. Numerous analytical techniques are available for measuring TDF content in foods, including non-enzymatic gravimetric and enzymatic gravimetric methods with colorimetric or GLC/HPLC techniques. The enzymatic gravimetric method (AOAC 985.29, AOAC 991.43) is the commonly adopted analytical technique for determining TDF (Lee et al., 1992). ANKOM Technologies (Macedon, NY, USA) automated the enzymatic gravimetric method of TDF analysis. The total dietary fiber analysis method uses three enzymes, namely α-amylase, amyloglucosidase, and protease (Bolen et al., 2018). However, these enzymes are expensive, which ultimately increases the cost of analysis and limits the number of samples that can be analyzed. Li and Cardozo suggested elimination of the enzymatic hydrolysis step for samples containing <2% starch, and the method was adopted as AOAC 993.21 (Li and Cardozo, 1994). Accordingly, it may be possible to eliminate the enzyme treatment steps in the enzymatic gravimetric method.

The fruits of the date palm (*Phoenix dactylifera* L.) are low in starch and protein content once fully ripe (Ghnimi et al., 2017). Date fruits are typically consumed throughout the year in the Middle East, and their consumption increases exponentially in the month of Ramadan (Assaad Khalil et al., 2021). Dates are characterized by the richness in carbohydrates (60–80 %), including soluble sugars and dietary fiber (El-Sohaimy and Hafez, 2010). Date fruits are usually consumed at three developmental stages: Bisr stage (or Khalal stage), Rutab, and the fully mature Tamr stage (Amira et al., 2011). At the Bisr stage, the fruits contain about 50 % starch, which degrade during further development to the soluble sugars; glucose, fructose and sucrose (Amira et al., 2011). The main differences in the nutritional value of date fruits are mainly attributed to the components of dietary fiber, polyphenols, vitamins, and minerals (Ghnimi et al., 2017). The dietary fiber content in dates is

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highly variable depending on the cultivar. A study by Shahib et al. reported that the total dietary fiber in date fruits varieties range between 6.5%–11.5% (Al-Shahib and Marshall, 2003). Another study on 18 cultivars from the UAE reported a total dietary fiber content between 5.5 %–9.1 % (Habib and Ibrahim, 2011). A recent study by George et al. also showed comparable TDF content in dates (5.3–8.4 %) (George et al., 2020). Previous research indicated relatively low protein content in the Tamr stage ranging between 1.6–3.16 % (Ghnimi et al., 2017; Habib and Ibrahim, 2011). Therefore, it may be possible to eliminate the enzyme treatment steps in the ANKOM Dietary Fiber Analyzer for the enzymatic gravimetric method for TDF analysis. This study compared the total dietary fiber content values (%) in 12 date fruit varieties using the enzymatic-gravimetric method in ANKOM dietary fiber analyzer with and without enzymatic hydrolysis. The TDF content in date fruits vary considerably across different cultivars (Al-Shahib and Marshall, 2002) and this was shown to be related to the fiber content (Kamal-Eldin et al., 2020). Thus, in our analysis we included date varieties of various textures: hard, soft and semi-hard. Moreover, we analyzed two other commonly consumed dry fruits (figs and raisins) to determine the potential application of the non-enzymatic method to other dry fruits.

2. Materials and methods

2.1. Materials

Ten date cultivars (Barhi, Khalas, Fardh, Neghal, Reziz, Dabbas, Bouman, Shishi, Sagae, Lulu) were obtained from Al Foah Emirates Dates Company (Al-Saad, United Arab Emirates) and two cultivates from Sudan (Gundella and Barakawai). These 12 date cultivars were selected to include date fruits with soft, semi-hard, and hard textures and cover date cultivars with a wide range of fiber content. The two samples of figs and raisins were sourced from local supermarkets in Al-Ain, UAE.

2.2. Chemicals and reagents

All chemicals in the study were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless stated otherwise. Heat-stable α-amylase (Cat# TDF80), protease (Cat# TDF82), amyloglucosidase (Cat# TDF84), diatomaceous earth (Cat# DE1/DE2), 2-(N-Morpholino) ethanesulfonic acid (Cat# 4432-31-9), TRIS-Tris(hydroxymethyl) amino methane (Cat# 77-86-1) were obtained from ANKOM Technologies (Macedon, NY, USA).

2.3. Sample preparation

After de-seeding, the flesh of the date fruits was ground to a homogeneous paste in a standard food processor. The date paste was then desugared six times with 85 % ethanol (v/v) as described in AOAC 991.43. Briefly, 30 g of the date samples were mixed with 170 mL of 85 % ethanol using a table-top shaker for 10 min and centrifuged at 4200 rpm for 10 min. The supernatant was discarded, and the step was repeated five more times. The de-sugared samples were dried in a hot air oven at 40 °C before TDF analysis. Figs and raisins were ground to a homogeneous paste and analyzed directly.

2.4. Total dietary fiber analysis

ANKOM Dietary Fiber Analyzer (ANKOM Technologies, NY, USA) was used to analyze TDF content in the 16 samples in our study. This automated instrument analyzes TDF based on AOAC 991.43 method (Bolen et al., 2018). Briefly, 0.5 g of the sample is mixed with MES-TRIS buffer (0.05 M, pH 8.2) for the enzymatic digestion step. The enzymatic digestion was performed with three enzymes from Megazymeα-amylase (TDF80/TDF81) at 95 °C for 35 min, followed by protease (TDF82/TDF83) at 60 °C for 30 min, and amyloglucosidase (TDF84/TDF85) at 60 °C and pH between 4.0–4.5 for 30 min. Following enzymatic digestion of the sample, the fiber components in the samples are precipitated with aqueous ethanol. For the non-enzymatic method, all three enzyme vials (α-amylase, protease and amyloglucosidase) in the instrument were filled with distilled water instead of enzymes. The TDF filter bags with the samples were collected following the instrument run and dried before determining their ash and protein contents. The ash content in the sample was determined by burning the samples in a standard furnace oven at 550 °C for 4 h, and the total protein was determined by the Kjeldahl method (Lynch and Barbano, 1999) using the general factor (6.25) for the conversion of nitrogen to protein. The TDF (%) in the sample was then calculated using the formula,

$$\%TDF = \left[\frac{[(R_1 + R_2)/2] - P - A - B}{(M_1 + M_2)/2}\right] \times 100$$

$$R_1 = f_{F1} - f_{S1} - D_1$$

$$R_2 = f_{F2} - f_{S2} - D_2$$

where M1, M2 is the original weight for duplicate samples adjusted for pre-treatment fat and sugar losses (g); R1, R2 is the residue of the duplicate samples (g); f_F is the final weight of the filter bag (g); f_S is the initial weight of the filter bag (g); D is the original weight of the diatomaceous earth (g); P is the protein value of the residue and bag (g); A is the ash of the residue and bag (g); B is the blank value (g). We also analyzed the TDF in other dry fruits such as raisins and figs to test the applicability of the non-enzymatic methods to other fruits with low starch and protein content. The instrument conditions for both enzymatic and non-enzymatic analysis were similar.

2.5. Statistical analysis

TDF analysis was replicated three times for each dry fruit sample. Statistical analysis for the experiments was performed using GraphPad Prism software version 9.1.0 (Graphpad Software Corporation, CA, USA). The data model assumptions were checked by the D'Agostino & Pearson test for normality. The linear relationship between the two methods was checked with Pearson's correlation analysis. Bland -Altman plot was used to visually examine the agreement between the total dietary fiber values obtained from enzymatic and non-enzymatic methods. A p-value of <0.05 was considered statistically significant.

3. Results and discussion

The measured TDF content in the dry fruit samples analyzed is

Table 1
Total dietary fiber content in dry fruits analyzed by enzymatic gravimetric method with and without enzymatic digestion[a].

Fruit	Enzymatic Method	Non-Enzymatic Method
Barakawai	9.88 ± 0.49	10.05 ± 0.16
Barhi	5.43 ± 0.14	5.46 ± 0.08
Bouman	8.18 ± 0.21	8.34 ± 0.13
Dabbas	9.89 ± 0.01	9.26 ± 0.14
Fardh	10.61 ± 0.14	12.23 ± 0.22
Gundella	13.59 ± 0.15	12.43 ± 0.16
Khalas	8.87 ± 0.22	8.59 ± 0.28
Lulu	7.95 ± 0.08	7.21 ± 0.45
Neghal	9.03 ± 0.55	9.03 ± 1.70
Reziz	8.31 ± 0.10	7.79 ± 0.23
Segae	8.73 ± 0.12	8.96 ± 0.04
Shishi	7.42 ± 0.06	8.97 ± 0.13
Fig. 1	9.53 ± 0.15	10.43 ± 0.25
Fig. 2	9.47 ± 0.06	10.70 ± 0.26
Raisin 1	1.60 ± 0.20	2.57 ± 0.12
Raisin 2	1.67 ± 0.15	2.43 ± 0.25

[a] TDF content expressed as %g/100 g fruit. Data is presented as mean ± s.d.; analysis for each sample was performed in triplicates.

presented in Table 1. In the 12 date samples analyzed by the enzymatic method, the mean TDF content ranged from 5.4 ± 0.1 %g/100 g fruit in Barhi to 13.6 ± 0.2 %g/100 g fruit in Gundella. Similarly, the measured TDF content in dates by the non-enzymatic method ranged from 5.5 ± 0.1 %g/100 g fruit in Barhi to 12.4 ± 0.2 %g/100 g fruit in Gundella. The measured TDF content by the two methods was significantly different in 3 date cultivars: Fardh, Gundella and Shishi. On the other hand, the two samples each of figs and raisins measured comparable TDF content in the enzymatic and non-enzymatic method and no differences were observed between the two analytical methods. The calculated Pearson's correlation coefficient between the enzymatic and non-enzymatic methods was 0.9643 with a highly significant p-value (<0.0001) (Fig. 1). The Bland Altman plots analysis indicates high agreement between the two methods for all 16 samples; thus, highlighting the excellent agreement between the two methods (Fig. 2). As easily abstracted from the plot, the average difference between the two methods is very low, measuring only 0.0419. The limits of agreement were small (upper 2.273 and lower -2.189), and all samples in the study measured within these limits of agreement.

The present study compared the TDF content in 12 date fruit samples using the AOAC 991.43 enzymatic gravimetric method, with and without enzymes, in the assumption that due to the negligible starch and low protein content in fully ripe dates, the results will be comparable. Moreover, to evaluate the potential application of the non-enzymatic method to other dry fruits, we analyzed two samples each, of figs and raisins. The results of these analyses also showed comparable results between the two methods.

Among the 12 date cultivars in the study, the measured TDF contents were similar to those reported in previous studies (Al-Shahib and Marshall, 2002; Habib and Ibrahim, 2011). The same 10 date fruit samples studied here were previously analyzed for dietary fiber components using the Uppsala method and the comparison of results is given in Fig. 3 (George et al., 2020). This comparison shows that the analysis of total dietary fiber in date fruits with the gravimetric method (with or without enzymes) gave higher values than the Uppsala method, which is based on the determination of the actual fiber components. The difference can be explained by the fact that date fruits have high levels (1–5 %) of insoluble phenolic compounds, which are tightly associated with the fiber (Alam et al., 2021). According to the detailed fiber analysis, date fruit fibers consist of fructan, pectin, galactomannan, arabinoxylan, cellulose/β-glucan, and lignin, which is the major component and determinant of the fiber content in the fruit (George et al., 2020). The TDF content between the cultivars varied significantly, and it could be related to the date growing conditions as it is critical to the ripening process. Another observation of our results is that the trend of measured TDF content between the two methods was not even. In Gundella, the TDF content measured by the enzymatic method was higher than in Shishi and Fardh varieties, which gave higher TDF content than the

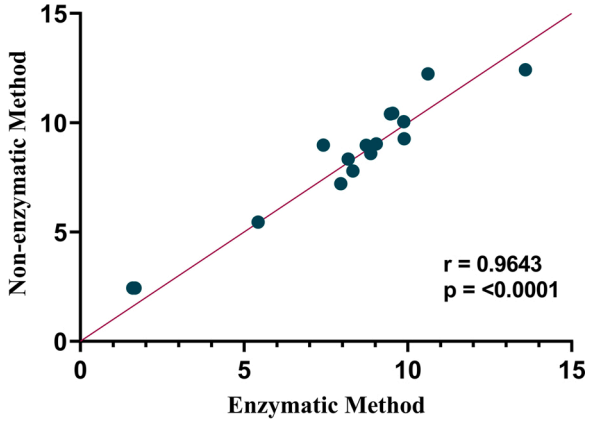

Fig. 1. Correlation Plot of TDF data from 16 dry fruit samples.

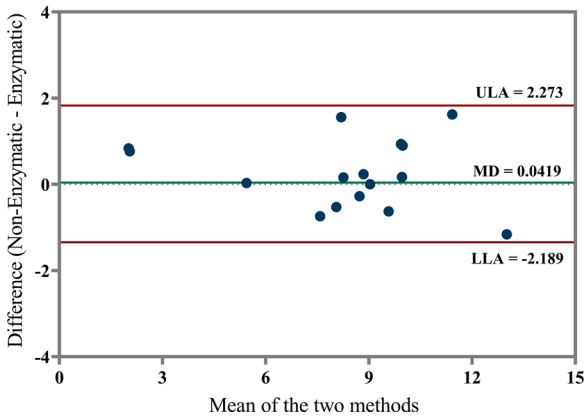

Fig. 2. Bland-Altman plot of the relation between the enzymatic and non-enzymatic methods for total dietary fiber analysis in 16 samples of dry fruits. ULA- Upper limit of agreement, MD- mean of the difference, LLA- Lower limit of agreement.

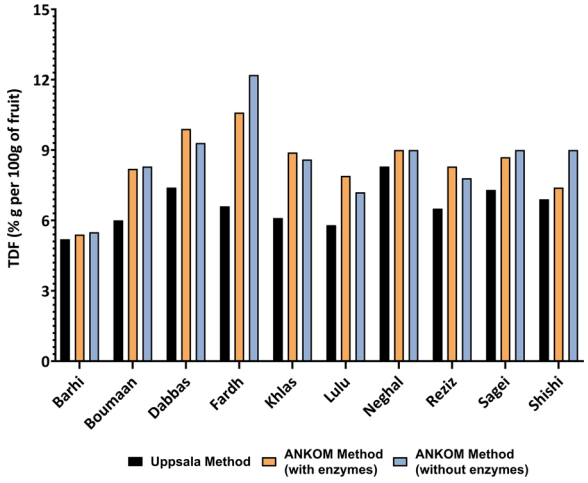

Fig. 3. Comparison of the results on total dietary fiber contents in 10 date varieties by three different methods.

non-enzymatic method. This might be due to the lignin, as a previous analysis of the same date samples found that lignin is the primary dietary fiber component attributed to varying textural characteristics between the date varieties (George et al., 2020). Accordingly, we observe higher TDF content in the enzymatic method in Gundella, which is a texturally harder cultivar than Shishi and Fardh. In addition to lignin, date fruits vary widely in their content of phenolic compounds and their association with the fibers (Alam et al., 2021), which might have some influence on the determination of total dietary fiber in these samples. Presuming the variability in the trend of the TDF content between the two methods results from the differences between the fiber component within the sample. Therefore, future work can address the gap by analyzing the soluble and insoluble fiber fractions in these samples separately using the same two methods. The observed variability in the trend between the enzymatic and non-enzymatic processes can also occur due to the variability in the analysis between two consecutive experiments. One strategy to minimize this variability in the experiment is to increase the number of analyses per sample. Since the current work adopted a single sample of each dry fruit with three replications in experiments, this limits the scope of our study to account for such variability. Nevertheless, our results demonstrate that the non-enzymatic method can be a suitable alternative to the enzymatic method of total dietary fiber determination in dry fruits.

Although we did not analyze the protein levels in the samples of

raisins and figs used in this study, according to the United States Department of Agriculture (USDA) National Nutrient Database for Standard Reference (SR-28) the protein content in raisins and dry figs are 2.5–3.2 % and 3.3 %, respectively. These values are within the same range as in date fruit varieties (USDA, 2019).

Future experiments can be designed to test the precision of the method to address these observations. To the best of our knowledge this is the first study to compare TDF measurement using enzymatic and non-enzymatic methods and demonstrating its application in dry fruits such as dates, figs and raisins. A significant benefit of our novel non-enzymatic method for TDF analysis is that it is far less costly than the standard enzymatic method and can help laboratories analyze more samples at a lower cost.

4. Conclusions

The results from our study conclude that the TDF analyzed using AOAC 991.43 without the enzymes gave comparable readings to the enzymatic method. This makes the non-enzymatic method an economical alternative for analyzing TDF in date fruits and other similar dry fruits such as raisins and figs. However, further studies with more sample replications are warranted to confirm the precision and accuracy of the non-enzymatic method in measuring the TDF content in dry fruits. Furthermore, studies conducted in different laboratories are also warranted to confirm that the non-enzymatic method of TDF in dry fruits is comparable to the official enzymatic gravimetric method of TDF determination.

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CRediT authorship contribution statement

Habiba I. Ali: Conceptualization, Formal analysis, Investigation, Writing - original draft. **Salma Alhebshi:** Formal analysis, Investigation. **Serene Hilary:** Formal analysis, Writing - original draft. **Usama Souka:** Formal analysis. **Fatima Al-Meqbaali:** Formal analysis. **Lily Stojanovska:** Investigation, Writing - review & editing. **Afaf Kamal Eldin:** Conceptualization, Formal analysis, Investigation, Writing - review & editing.

Declaration of Competing Interest

The authors report no declarations of interest.

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