Validation and application of a total dietary fiber determination method to meat products

A Bajic1, R B Petronijevic1, D Vranic1, D Trbovic1, N Betic1, A Nikolic1 and L Milojevic1

1 Institute of Meat Hygiene and Technology, Kacanskog 13, 11000 Belgrade, Serbia

E-mail: radivoj.petronijevic@inmes.rs

Abstract. This paper presents a modification of the reference method for the determination of dietary fiber in food, its validation using two quality control materials and application to meat products. Dietary fiber is a very important food ingredient with multiple positive effects in human nutrition. In recent decades, efforts have been made to enrich with fiber some foods that do not naturally contain fiber to a significant extent, such as meat products. Fiber content must be declared in accordance with legal regulations, and it is necessary to have reliable methods for determining their amount in food. The results obtained show the described modified and optimized method can be applied to meat products, with significant savings in the preparation time and consumption of reagents.

1. Introduction

Dietary fiber is, generally, carbohydrates that are indigestible by the human population. There are several ways to classify this fiber, but the most widely used classification is according to their solubility in water. Insoluble fiber is poorly fermented, while soluble fiber is more easily fermented [1].

More recently, the addition of dietary fiber to meat products is gaining in importance with novel understanding of fiber’s role in nutrition and human health aspects. Consumption of dietary fiber has a preventive role in the onset of several diseases. Fiber can act as a protective agent against cardiovascular diseases. It reduces the concentration of LDL in the blood and thus acts as reducing agent for hypercholesterolemia and hyperlipidemia [2-5]. Fiber shows affinity for bile acids and cholesterol metabolites, binding them to the small intestine during digestion and preventing their absorption, resulting in a reduction in blood cholesterol [6]. The fiber binds to water, which has a beneficial effect in the gastrointestinal tract, as the volume of the contents increases, reducing the time food is unnecessarily retained in the colon. Thus, the release of toxins and the emergence of cancer are prevented [7]. One of the most popular dietary fiber roles is the ability to regulate overweight and prevent obesity. Fiber consumption slows down the emptying of the stomach by decreasing the absorption of nutrients [8, 9], and thus, the feeling of satiety is prolonged [10-12].

On the other hand, from the aspect of the food industry, the use of dietary fiber has multiple positive effects. Effects that will be manifested in meat products depend on the type and quantity of fiber or the mixture of added fiber. Some of fiber’s properties, for example, to bind water, have a positive effect on food consistency, and since fiber is neutral, it will not change the sensory properties of the product. Fiber also has the ability to bind oils, which is essential for the stabilization of
emulsions [13-17]. However, in the food industry, economic profitability is also important. As the sources of dietary fiber are predominantly agricultural by-products which are relatively cheap, their use is very cost-effective [1].

Dietary fiber in the meat industry is mostly used in boiled sausages, fermented sausages, and minced meat products [18-21]. The recommended daily intake of dietary fiber is regulated and is not the same in all countries. It is believed the daily amount of fiber load should not exceed 28-36 g for adults, with 70-80% being insoluble fiber [1]. The main negative effect of excessive fiber intake in humans is the appearance of diarrhea.

For determination of total dietary fiber (TDF) content in food, the reference AOAC 985.29 [22] method is most often used. The aim of this paper was to examine possible application of this method using the FibreBag system and adequate optimization of this analytical process to meat products.

2. Materials and methods
All chemicals were purchased from Sigma-Aldrich (Merck, Darmstadt, Germany). The total dietary fiber assay kit was also from Sigma. All other chemicals and solvents were analytical grade. FibreBags S were from Gerhardt (Koenigswinter, Germany). Quality control (QC) materials T2477QC porridge oats and T2479QC bread crumbs were from Fera Science FAPAS (Sand Hutton, York, UK).

2.1. Meat products
TDF in 37 meat products from retail were analyzed by the proposed method. Samples were from different brands, manufacturers and retailers. The group consisted of 15 frankfurters, 10 Parisian sausages and 12 chicken burgers. All products had a declared TDF content. Meat products were defatted and dried prior to analysis. Fat content was utilized in calculating the final result % TDF in the meat products.

2.2. TDF determination
Reference method AOAC 985.29 “Total Dietary Fiber in Foods Enzymatic-Gravimetric Method” was optimized and applied to meat product samples [22]. The Gerhardt manual fiber analysis FibreBag system FBS6 was used for digestion.

According to the method procedure [22], TDF is determined on duplicate samples of dried and defatted (if fat content is >10%) material. Foods are cooked with heat stable α-amylase to induce gelatinization, hydrolysis and depolymerization of starch; incubated at 60°C with protease (to solubilize and depolymerize proteins) and amyloglucosidase (to hydrolyze starch fragments to glucose); and treated with four volumes of ethanol to precipitate soluble fiber and remove depolymerized protein and glucose (from starch). The residue is filtered; washed with 78% ethanol, 95% ethanol, and acetone; dried; and weighed. One duplicate is analyzed for protein and the other used to determine ash. The TDF is the weight of the filtered and dried residue less the weight of the protein and ash.

Taking advantage of FibreBag utilization, further investigations were also performed to optimize the process to digest multiple meat samples simultaneously.

2.3. Statistical analysis
Statistical evaluation of validation results was performed in MS Office Excel with Data Analysis ToolPack add-in.

3. Results and discussion
3.1. Method optimization and validation using QC materials
According to application notes from the manufacturer [23], FibreBags are used to determine TDF in the method’s filtration step, before determination of ash and proteins in the residue. The FibreBag method was optimized to digest six simultaneous probes (three samples, each in duplicate), and
considering savings of time and material, was evaluated using two QC materials. Six FibreBags with sample portions approximately 1g were placed in the holder and then subsequently digested in accordance with method procedure [22]. After rinsing with ethanol and acetone, FibreBags with samples were dried for 3 h in an oven and consecutively subjected to ash and protein content determination. This procedure provided six times lower consumption of reagents for digestion, and it reduced the analysis runtime.

Validation results of this method procedure are presented in Table 1. The TDF contents determined were inside the declared limits. Interday repeatability was calculated from three replicas of the same sample materials on three different days. TDF determination in QC material T2477QC showed better matching and lower dispersion than results for material T2479QC.

Table 1. Validation results of optimized method (* results are in %)

| Material      | Assigned value* | Low* | High* | Determined* | Low* | High* | Repeatability |
|---------------|-----------------|------|-------|-------------|------|-------|--------------|
| T2477QC       | 9.50            | 6.92 | 12.09 | 9.52        | 9.23 | 9.71  | 0.25         |
| T2479QC       | 6.58            | 4.79 | 8.37  | 5.42        | 4.82 | 7.01  | 0.71         |

3.2. Determination of TDF in meat products

Results of determination of TDF in meat products from retail are shown in Table 2. The TDF contents in the examined meat products were relatively uniform. Slightly greater amounts of TDF were observed in chicken burgers, due to their higher content of vegetables. Uncorrected TDF analysis results showed the fiber content in dried, defatted meat samples was from 2.5 to almost 5 percent.

Table 2. TDF in meat products

| Meat products | Number of samples | TDF range (%) | Average TDF (%) | Uncorrected range (%) |
|---------------|-------------------|---------------|-----------------|-----------------------|
| Frankfurters  | 15                | 0.50-0.89     | 0.69            | 1.35-3.66             |
| Sausages      | 10                | 0.61-0.83     | 0.71            | 1.43-3.86             |
| Chicken burgers| 12               | 0.68-1.07     | 0.88            | 1.67-4.71             |

4. Conclusion

The proposed modified procedure of the reference AOAC 985.29 method for determination of TDF in food using FibreBags can be satisfactorily employed in analysis of both meat products and fiber-rich, vegetable origin food.

The optimization results showed the time required for analysis is significantly reduced, and the consumption of the digestion reagent is six times lower than in the procedure given by the reference method and method recommended by the manufacturer. The consequences are a cost effective method and a larger number of analyzes completed in less time.

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