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ОРИГИНАЛЬНАЯ СТАТЬЯ

**MASS BALANCE STUDIES OF IRON  
WITHOUT THE NEED OF SUBSAMPLING  
USING LARGE SAMPLE NEUTRON ACTIVATION ANALYSIS**

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**ABSTRACT.** Accurate assessments of the iron (Fe) intake from food is mandatory for mass balance studies. The reliability of such assessments is strongly dependent on the representativeness of the analytical test portion and, as such, the quality of the homogenization of the double portions collected. Large sample Instrumental Neutron Activation Analysis (INAA) circumvents these problems as the entire double portions can be analysed without homogenization.

Fe was measured both in approximately 1 kg freeze-dried food as well as in moist products. A (commercially available) porridge fine wheat grain was used as a reference sample (assumed to be homogeneous in the Fe content). The amount of iron in the fine wheat grain was also measured using small sample INAA. The moisture content of the fresh food did not cause any problem during the irradiation such radiolysis and excessive gas formation due to low radiation dose during the irradiation. The results obtained for the moist sample were statistically equivalent to those found for the dried sample ( $73.1 \pm 4$ ,  $74 \pm 3$  mg/kg respectively, zeta ( $\zeta$ ) score = 0.18). The applicability of LS-NAA was further illustrated by measurement of Fe in commercially available microwave meals which was found to be  $30 \pm 2$  mg/kg. Large Sample INAA is a novel and attractive approach for measurement of element content of the dietary intake by the double portion technique collected during 5–7 day in mass balance experiments. Similarly, it can be directly applied without sample preparation for the analysis of faeces collected in such studies.

**KEYWORDS:** neutron activation analysis, large samples, iron, dietary intake, double portion.

**INTRODUCTION**

Mass balance studies are used to get information on the actual bioavailability of major and trace elements present in food. In such studies it is essential to measure the amounts of an element both at the site of intake and at the sites of excretion, such as in urine and faeces. A mass balance study can easily cover a period of 5–7 days in which 8–10 kg of food and 10–14 L of drinking solutions are consumed. A double portion technique, one portion consumed by the test person, an identical other portion used for analysis, is usually used to quantify the intake of an element. The intake of an element is calculated by either adding up the amounts of that element present in each component of the food intake or by homogenizing the entire intake and analysing a representative sub-sample of e.g. 1 g or less. The latter will only give reliable data in case the element of interest is distributed homogeneously and the sub-sample is truly representative. The quality of the homogenization has to be checked by analysis of e.g. 15 small test portions.

Dietary intake collection by the double portion technique will result in a highly inhomogeneous mixture of food ingredients, including liquid ones. From experiments with food using the traditional (small sample) instrumental neutron activation analysis (INAA) technique we faced difficulties in preparing representative sub-samples. The common approach implies freeze drying of the food and very careful homogenization after which small samples (200 mg) are available for measurement. However, if e.g. pork liver is freeze dried the resulting product is quite a hard piece of dried liver that cannot easily be crushed in usable small parts. In another case freeze-dried peaches, could be easily crushed, but the material, still containing sugar, gets very sticky once liquid nitrogen is poured on it (Figure 1).

It would therefore be highly attractive if the dietary intake collected over several days, could be analysed as received without attempting to remove the moisture fraction by drying it to constant weight.

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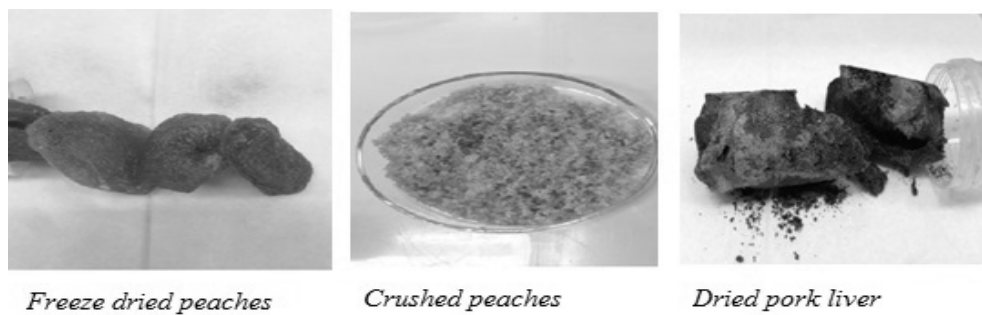


Fig. 1. Peaches and pork liver after freeze drying

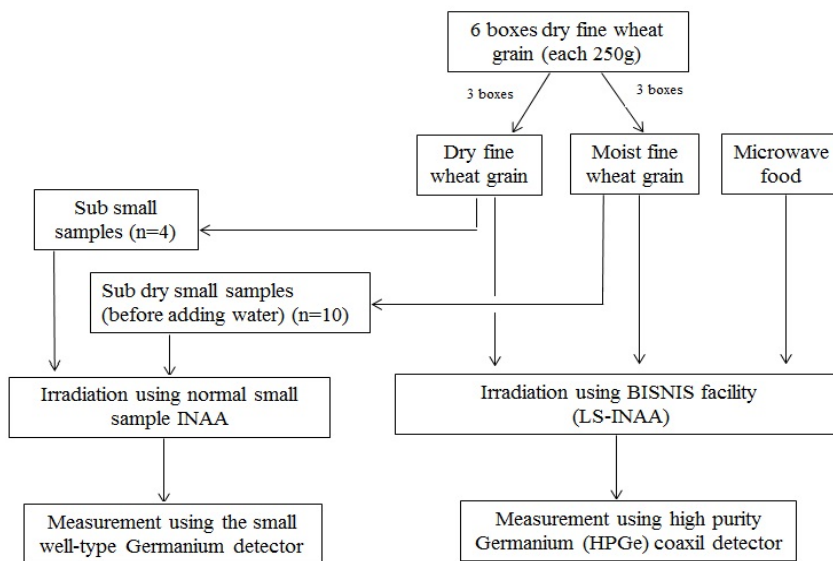


Fig. 2. The design of the study

These problems can be overcome by using large sample instrumental neutron activation analysis (LS-INAA) since this technique has nowadays the capability of measuring the content of an element in the entire collected amount of food (multi-kilogram size) without sub-sampling and homogenization.

Neutron irradiation of moist samples results into enhanced neutron self-absorption and neutron self-thermalization of epithermal and fast neutrons. The latter effect is not opportune for the large sample INAA at the facilities in Delft due to the high ratio of thermal to epithermal and fast neutrons in the thermal column facility (a factor of 3000). The first effect is accounted for by the calculus of the thermal neutron flux distribution –and thus also the average thermal neutron flux on basis of the neutron flux depression outside the sample, and comparison thereof with a reference condition (Overwater, 1994).

One of the characteristics of large sample analysis is that there are no quality control (‘trueness control’) materials available at such a large scale.

We therefore verified the validity of this calculus for the analysis of a large moist food sample has by using a powdered wheat flour, assumed to be homogeneous in trace element composition. The design of the study is shown in Figure 2.

The material was analysed as a small-dry- sample by ‘normal’ INAA to obtain a reference value for the Fe mass fraction. The same powdered wheat grain material was converted into a moist porridge to test the validity of the LS-INAA software accounting for the neutron self –absorption by the moisture in food. As such, an indication for the degree of trueness of large, moist, sample analysis can be obtained.

The feasibility of real dietary intake analyses was tested by the analysis of commercially available microwave food products –inhomogeneous in composition- after freeze drying.

## MATERIAL AND METHODS

### Validity assessment

These experiments were performed with commercially available porridge fine wheat grain pow-

der purchased from Nestlé (Figure 3). About 750 g dry material was used for the large sample analyses; another 750 g was prepared as a real porridge by adding 1.3 L of Millipore water. The completed porridge was then transferred to a 2 L polyethylene bottle for irradiation. A 2 L bottle only filled with Millipore water was used as a blank.

Four subsamples from the dry porridge were used for the first experiment and 10 subsamples from the finished porridge of the second experiment were prepared for analyses by traditional small sample INAA along with the Standard Reference Material NIST-1547 (Peach leaves) as a control sample.

#### *Feasibility of real meal analyses*

**Materials.** Five different microwave meals and bread were purchased from a supermarket in Delft, The Netherlands. These meals contained different types of food like chicken, beef, liver, rice, salad, pasta, and different vegetables like potato and pea. All types of meats contain some fats and muscles which make it difficult to prepare a complete freeze dried and homogeneous powder. On the other hand

the plant origin foods such as salad and vegetables are easy to be dried.

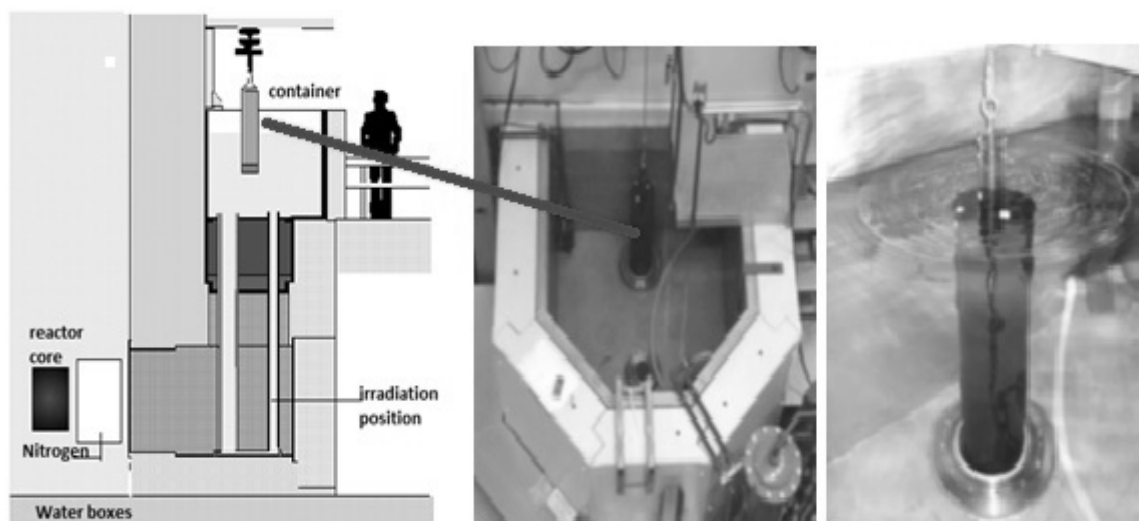
**Sample preparation.** Before freeze drying, these products were kept in a freezer at  $-50\text{ }^{\circ}\text{C}$  for about 24 hours. As such, it was assumed that all moisture present such as sauces were frozen. The meals were freeze dried in an EZ-dry freeze drier (MNL-036-A) from FTS System Inc., Stone Ridge, New York, mortared and transferred to a polyethylene bottle of 2 L volume and shaken for an even distribution of the materials inside the bottle. The total mass of the 5 meals was 738 g.

#### *Neutron irradiation*

Neutron irradiation was performed in the Big Sample Neutron Irradiation System (BISNIS) in the thermal column at the Hoger Onderwijs Reactor of the Reactor Institute Delft, Delft University of Technology, The Netherlands (Figure 4). The samples are positioned inside a graphite cylinder insert in the irradiation container. Each sample was surrounded by eighty neutron flux monitors (zinc foils) positioned in a fixed grid in the walls of this graphite cylinder.



*Fig. 3. Porridge fine wheat grain from Nestlé*



*Fig. 4. A cross-section of the thermal column with the irradiation container*

The dry and moist porridge as well as the real meal samples were irradiated for 6 days at a thermal neutron flux of  $\sim 3.0 \cdot 10^8 \text{ cm}^{-2} \text{ s}^{-1}$ .

Fe fractions in the small samples from the dry porridge fine wheat grain were measured using normal INAA, they were irradiated for 10 hours at a thermal neutron flux of  $\sim 4.5 \cdot 10^{12} \text{ cm}^{-2} \text{ s}^{-1}$ . Zn was used as a flux monitor for both LS-INAA and normal small sample INAA (Blaauw, 1993).

#### Measurement

All large samples were measured during four days, starting ca. 15 days after irradiation using a high purity Germanium (HPGe) coaxial detector, relative efficiency 96 %, from ORTEC, Oak Ridge, USA. The measurement facility is shown in Figure 5 and described before (Overwater, 1994; Lakmaker, van Aller, 1997; Overwater et al., 1996).



*Fig. 5. The large food sample being counted after neutron activation*

The distance of the sample (bottle) vertical center axis to the detector endcap is 20 cm, which is large as compared to normal NAA counting geometries. The obtained spectra were corrected taking into consideration the corresponding gamma ray background spectra and the sample's natural radioactivity (Overwater, 1994; Lakmaker, van Aller, 1997). Since in the large sample irradiation facility the ratio of thermal to epithermal neutrons is very high ( $\sim 3000$ ) only  $^{59}\text{Fe}$  can be measured as an indicator for total iron. The zinc flux monitors (0.937 mg each) were measured with a well-type Germanium detector (active volume ca.  $250 \text{ cm}^3$ ) from ORTEC, USA, with an absolute photopeak efficiency of 13% for the 1099 keV photopeak of  $^{59}\text{Fe}$ .

The small samples from the dry porridge fine wheat grain were measured during 3 hours using the

same well-type Ge detector. The gamma-ray spectra were analyzed using the APOLLO software [2].

#### Data processing

Spectrum analysis and interpretation was done on basis of the  $k_1$  method (Blaauw, Bode, 1993) which is related to the  $k_0$  method (Simonits et al., 1975).

Neutron and  $\gamma$  self-attenuation corrections were performed on the basis of the measured values of the neutron flux depression outside the sample, from which the neutron diffusion coefficient and the neutron diffusion length of the sample could be derived (Overwater, 1994) resulting in the neutron density distribution in the sample. The  $\gamma$  ray transmission coefficients were measured separately (Lakmaker, van Aller, 1997). For more information about the facility see ref. (Overwater, 1994; Lakmaker, van Aller, 1997; Overwater et al., 1996; Bode, et al., 1997).

#### Validity assessment

The Zeta ( $\zeta$ ) score has been used to compare the mass fractions measured in the Standard Reference Material NIST-1547 (Peach leaves) used for control in normal NAA with its certified reference values, as well for comparing the mass fractions measured in the small samples NAA (as a reference values to the large samples) with those from the large sample analysis. The score is calculated as follows:

$$\zeta = \frac{|x_m - x_{ref}|}{\sqrt{u_m^2 + u_{ref}^2}}$$

In which  $x_{m,ref}$  is the mass fraction from the measured samples ( $m$ ) and the reference value ( $ref$ ), respectively and  $u_{m,ref}$  is the combined standard uncertainty<sup>1</sup> of the mass fraction from the measured sample and the reference value, respectively. Our acceptance criterion for degree of equivalence of the results was  $|\zeta| < 3$ .

The  $\zeta$  scores between the large sample results and the reference values from the small sample results are considered acceptable if  $|\zeta| < 3$ , in agreement with the quality control criterion of the laboratory for INAA in Delft (Statistical Methods..., 2005).

<sup>1</sup> Please note that throughout this document  $u_{m,ref}$  and all other values given after the  $\pm$  sign are not reflecting standard deviations of replicates but represent the combined standard uncertainty of measurement (Greenberg, et al., 2011), evaluated and quantified following the guidance of the Guide for Expression of Uncertainty in Measurement (Evaluation of measurement data..., 2008).

## RESULTS

The results of the dry porridge fine wheat grain analysis by large and small sample NAA ('reference' value) are shown in Table 1.

*Table 1. Fe mass fraction in dry porridge sample by large sample and normal INAA*

Parameter	Fe and combined standard uncertainty (1 SD), mg/kg	Irradiation time	Decay time	Measurement time	Detection Limit, mg/kg
LS-NAA	74±3	6.0 (d)	14 (d)	4.0 d	6.2
Normal INAA (n=4)	67.1±1.3	10 (h)	14 (d)	3 (h)	2.0

The result of the prepared porridge (porridge +water) measured and the derived small sample by large and small sample INAA are shown in Table 2.

*Table 2. Fe mass fraction in prepared porridge (porridge +water) by large sample and normal INAA*

Parameter	Fe and combined standard uncertainty (1 SD), mg/kg	Irradiation time	Decay time	Measurement time	Detection Limit, mg/kg
LSNAA	73±4	6.0 (d)	14 (d)	4.0 (d)	3.7
Normal NAA (n=10)	66.8±1.3	10 (h)	14 (d)	3 (h)	2.0

*Table 3. Results of SRM NIST-1547 and zeta score for comparison with certified value*

Parameter	Experiment	Certified value with combined standard uncertainty (1 SD), mg/kg	INAA result with combined standard uncertainty (1 SD), mg/kg	$\zeta$
Normal INAA	Dried samples	218±7	217.4±3.5	0.08
	Prepared porridge samples		218.3±3.2	0.04

The results of the (small sample) analysis of the Standard Reference Material NIST-1547 (Peach leaves) used for trueness control in normal NAA for the dried samples, and for the prepared porridge samples are given in Table 3.

The Fe mass fractions in the large sample analysis of the large dry powder fine wheat grain and the derived moist porridge material are mutually in excellent agreement (74±3 mg/kg, and 73±4 mg/kg respectively,  $\zeta = 0.18$ ); the same mutual agreement is for the small sample analyses (67.1±1.3 mg/kg and 66.8 ± 1.8 mg/kg,  $\zeta = 0.14$ ) However, the results in Tables 1 and 2 indicate a systematic difference with the small sample results of the same material, resulting in a bias for the dry material of 10.3 % and zeta score of 2.1, and for the moist material a bias of 9.3 % and  $\zeta = 1.5$ .

The approximate 10% bias could, in retrospect, be traced back towards an unexpected difference in the neutron flux distribution in the thermal column irradiation facility between the date of calibration and the date of the experiments described in this paper. The measurement procedure has meanwhile been adapted to accommodate such variations.

### *Feasibility of real meal analyses*

The measured Fe mass fraction as found after analysis of the freeze dried (combined) 5 meals was (30±2) mg/kg with a detection limit of 6 mg/kg.

## DISCUSSION AND CONCLUSION

This study shows that LS-INAA is a useful method for non-destructive multi-element analysis of bulky food samples, up to several kilogram with adequate accuracy (Blaauw, 1993). It is an attractive alternative for the standard approach, that has to rely on careful and laborious homogenization and the representativeness of small samples. LS-INAA can be directly applied in mass balance studies both for element analysis of food supplied for several days and faeces collected during such a test. The water content of the fresh food does not cause any problem during the irradiation such as sample swelling and exploding. Moreover, the moisture sample can give a result statistically comparable to the dried sample. These observations are also valuable in view of eventual large sample analysis of faeces, as might be collected during a multi-day mass balance study. Another advantage, as shown in this study, is that a

sample can be analysed again after a reasonable decay time of 2-3 weeks.

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## МАССОВЫЕ БАЛАНСОВЫЕ ИССЛЕДОВАНИЯ ОБМЕНА ЖЕЛЕЗА БЕЗ НЕОБХОДИМОСТИ СУБДИСКРЕТИЗАЦИИ С ИСПОЛЬЗОВАНИЕМ НЕЙТРОННО-АКТИВАЦИОННОГО АНАЛИЗА БОЛЬШОЙ ПРОБЫ

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**РЕЗЮМЕ.** Точная оценка алиментарного поступления железа (Fe) является обязательной в массовых балансовых исследованиях. Надежность таких оценок сильно зависит от репрезентативности тестовой порции и качества гомогенизации собранных дубликатных проб. Инструментальный нейтронно-активационный анализ (ИНАА) большой пробы обходит эти проблемы, так как дубликатные пробы могут быть проанализированы целиком без гомогенизации. Содержание железа определяли в ~1 кг лиофилизированной пищи и во влажных продуктах. В качестве эталонного образца (предположительно гомогенного по содержанию железа) использовали коммерчески доступную пшеничную крупу. Содержание железа в пшеничной крупе также измерялось с использованием ИНАА. Наличие влаги в свежей пище не вызывало каких-либо проблем при облучении (таких, например, как радиолиз или избыточное образование газа) благодаря низкой дозе облучения. Результаты, полученные для влажного образца, были статистически эквивалентны результатам, полученным для высушенного образца ( $73,1 \pm 4$ ;  $74 \pm 3$  мг/кг соответственно,  $Z$ -score ( $\zeta$ ) = 0,18). Применимость нейтронно-активационного анализа большой пробы была дополнительно проиллюстрирована определением железа в коммерчески доступных готовых блюдах, где содержание оказалось равным  $30 \pm 2$  мг/кг. Применение ИНАА большой пробы представляет собой новый и привлекательный подход для измерения содержания элементов в пищевом рационе при использовании дубликатных проб, собранных в течение 5-7 дней, в массовых балансовых исследованиях. Аналогичным образом он может быть непосредственно применен без подготовки образца для анализа фекалий, собранных в таких исследованиях.

**КЛЮЧЕВЫЕ СЛОВА:** нейтронно-активационный анализ, большие пробы, железо, поступление с пищей, дубликатный метод.