Revisión

Chemical composition, antioxidant capacity and content of phenolic compounds in meals collected in hospitals in Bolivia and Sweden

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Abstract

The objective of this study was to evaluate the proximal composition, as well as Total Antioxidant Capacity (TAC) and Total Phenols (TPH) in meals that represent a complex food matrix, from different hospitals in Bolivia and Sweden. Protein, fat, ash, dietary fiber and carbohydrate contents were measured in 29 samples: 20 from two Bolivian hospitals and 9 from the university hospital in Lund, Sweden. The antioxidant capacity was measured by three spectrophotometric methods: the ferric reducing antioxidant power (FRAP) method, the 2, 2’- azinobis-3-ethylbenzotiazoline-6-sulfonic acid (ABTS) method and Total Phenolic Compounds (TPH) using the Folin-Ciocalteu reagent.

The results show that fat, protein, carbohydrate and dietary fiber in Bolivian and Swedish hospital meals are following internationally established recommendations. Regarding the main courses, TPH contents in both countries were in the same range. However, TAC and dietary fiber content were higher in Swedish meals than in Bolivian meals and the TAC was far lower, in both cases, in comparison with the value obtained from individual food items reported from literature. The results show that antioxidant levels can be easily overestimated by considering only individual uncooked ingredients. An interesting consideration is, the fiber content in the meals, which can be an important source of antioxidants and non-extractable phenolic compounds.


Key words: Total antioxidant capacity. Phenolic compounds. Bolivian meal. Swedish meal.

Resumen

El objetivo de este estudio fue evaluar la composición proximal, así como la capacidad antioxidante total (CAT) y los fenoles totales (FT) en alimentos que representan una matriz compleja en diferentes hospitales de Bolivia y Suecia. Se midieron las proteínas, las grasas, la ceniza, la fibra dietética y el contenido en hidratos de carbono en 29 muestras: 20 de dos hospitales bolivianos y 9 del hospital universitario de Lund, Suecia. La capacidad antioxidante se midió mediante tres métodos espectrofotométricos: el método del poder antioxidante reductor férrico (PARF), el método del 2, 2’- azinobis-3-etilbenzotiazolina-6-acido sulfónico (ABTS) y el de Compuestos fenólicos totales (TPH) empleando el reactivo Folin-Ciocalteu.

Los resultados muestran que las comidas de los hospitales bolivianos y sueco siguen las recomendaciones internacionales con respecto al contenido de grasa, proteínas, hidratos de carbono y fibra dietética. En cuanto a las comidas principales, el contenido de FT estaba en el mismo rango en ambos países. Sin embargo, la CAT y el contenido de fibra dietética fue superior en las comidas suecas que en las bolivianas y la CAT estaba muy por debajo, en ambos casos, en comparación con el valor obtenido para los alimentos individuales reportado en la bibliografía. Estos resultados muestran que los niveles de antioxidantes pueden sobrestimarse fácilmente si sólo se considera los ingredientes no cocinados. Una consideración interesante es el contenido de fibra en las comidas, que puede ser una fuente importante de antioxidantes y compuestos fenólicos no extraíbles.


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Introduction

In recent years the study of food quality through the measurement of active compounds has evolved to be an important area. There is great scientific interest in the nutritional importance of antioxidants and other compounds e.g. vitamins C and E, polyphenols and carotenoids. Both epidemiological and clinical studies have indicated that increased consumption of phenolic antioxidants present in cereals, fruits, and vegetables may be linked to reduced incidences of chronic and degenerative diseases.1-6

In contrast, literature data on the content and composition of polyphenols in food is limited and often insufficient to determine the total dietary intake. Certain studies have provided data concerning the intake of some types of polyphenols such as flavonols, flavanones, catechins, phenolic acids and flavan-3-ols, but there is a lack of comprehensive data on total polyphenol intake.7,8 Mostly the data found in the literature regarding antioxidant content in food are referred to uncooked food and plant material9-12 while information regarding cooked meals is scarce. It is, however, important to investigate the antioxidant content in whole diets.

A drawback of studies on individual foods is that they may overestimate their relative contributions to the antioxidant capacity within a whole diet. Individual foods known to have a high antioxidant capacity may contribute very little to the antioxidant capacity of whole diets.7 Furthermore, when foods are consumed together in a diet, the total antioxidant capacity may be influenced via synergistic, additive, or antagonistic interactions among the components, which may in turn alter their physiological impacts.13-16

Hospital meals would be a good indicator of macro-nutrients and antioxidant levels. Furthermore, the aim was to illustrate the importance of determining antioxidant content in whole prepared meals as compared to individual food stuffs.

Materials and methods

Chemicals

Folin-Ciocalteu reagent, gallic acid, sodium carbonate, acetone (p.a.) were purchased from Merck (Darmstadt, Germany), ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)], potassium persulphate, Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid, 97%), TPTZ (2,4,6-tripyridyl-s-triazine) were obtained from Sigma-Aldrich (St. Louis, MO, USA), ferric chloride from ICN Biomedicals (Costa Mesa, CA, USA), glacial acetic acid (p.a.) and sodium acetate from BDH Chemicals (Poole, UK) and methanol HPLC grade from Laboratory Supplies (Poole, UK).

Sulfuric acid (95-97%), reagent grade ISO, was obtained from Scharlan (Barcelona, Spain. Sodium hydroxide (p.a.) was purchased from Biopack (Buenos Aires, Argentina).

Collection of meals

The description of the ingredients for the whole meals collected at the different hospitals is reported in table I. Lunch meals were collected from three hospitals. The hospitals were Lund University Hospital (LUH, Lund, Sweden), Hospital of the Seguro Social Universitario in La Paz (SSU) and Hospital Obreño in La Paz (HO). The hospital meals varied regarding the type of diet served to the patients, which include standard diets (LUH, HO) as well as soft diet, diabetic diet, low sodium diet and staff diet (SSU).

For the collection of fresh lunch meals from SSU a packing cooler for each meal (soup and main course)
was used. The meals were kept at -20 °C before the start of the analysis. The collection was made every lunch time during 5 days. A main course and soup were picked up, and on each day a different kind of meal was collected, namely: soft diet, diabetic diet, low sodium diet, standard diet and staff diet. The collection of fresh lunch meals from HO followed the same procedure as for the SSU but all meals were standard diets.

Fresh lunch meals were collected from LUH on five different days (two meals per day during four days and one meal the fifth day) and kept at -20 °C. These meals were standard diets for patients.

Sample preparation

The collected meals were thawed and homogenized with 200-400 mL of sodium acetate buffer 0.1 mol/L (pH 5.0) for 5 min (the amount of added buffer varied depending on the viscosity of the homogenate). Two aliquots (50 g) of each homogenate were lyophilized. The lyophilized samples were extracted with methanol: water (9:1, by volume) in a liquid: sample proportion of 10:1 by vortexing and then sonicating the sample in an ice-water bath (0 °C, 15 min). The mixture was centrifuged at 20,000 G for 30 min at 4 °C, and the aspi-
rated supernatant was stored at -80 °C. The extraction was performed in duplicates during three different days.

**Chemical composition**

Protein content (N 6.25) was determined by Kjeldahl digestion technique followed by volumetric analysis of the result of ammonia. Fat content was determined by exhaustively extracting samples in a Soxhlet apparatus with petroleum ether. The dietary fiber content was determined after preceding separation of apolar compounds after which the sample was subjected to acid and alkaline digestion and the dietary fiber content was determined gravimetrically. Ash was determined based on the content of inorganic matter after the incineration. Moisture content was determined by Bolivian norm. Total carbohydrates were calculated by difference.

**Measurement of TAC**

The ABTS method

To oxidize the colorless ABTS to the blue-green ABTS+ radical cation, 5 mL of ABTS solution (7 mmol/L) was mixed with 88 µL of potassium persulfate (140 mmol/L) and stored at room temperature in the dark overnight. On the day of analysis the ABTS+ radical cation solution was diluted with acetate buffer to an absorbance of 0.70 (± 0.02) at 734 nm. A Trolox standard stock solution, 5 mmol/L in ethanol, was diluted with acetate buffer to concentrations of 20-200 µmol/L. Different standards or samples (100 µL) were added to 1 mL of ABTS+ solution, mixed for 30 s, after which the absorbance reading was started after another 30 s and maintained during 6 min at 734 nm and 25 °C. The concentration was plotted against percent inhibition which was used for the calculation. The results were expressed as Trolox equivalents.

The FRAP method

The FRAP method was performed as described by Benzie at al. (1996). A solution of TPTZ (10 mmol/L) was made in HCl (40 mmol/L). The FRAP reagent solution was prepared on the day of analysis by mixing 25 mL of 0.1 M acetate buffer (pH 3.6), 2.5 mL of TPTZ, and 2.5 mL of ferric chloride (20 mmol/L). A Trolox standard stock solution (5 mmol/L), prepared in ethanol, was diluted with acetate buffer to a concentration range of 100-1,000 µmol/L. Each standard and sample (30 µL) were mixed with 900 µL of FRAP solution and 90 µL of water. A blank sample was prepared by mixing 900 µL of FRAP solution with 120 µL of water. The mixtures were measured after 10 min in a spectrophotometer at 593 nm. The results were expressed as Trolox equivalents.

**Measurement of total phenolic compounds**

Total Phenolic Compounds (TPH) was determined by the Folin-Ciocalteau reagent which oxidizes the phenolic compounds to phenolates at alkaline pH in a saturated solution of sodium carbonate, resulting in a blue molybdenum-tungsten complex. The Folin-Ciocalteau reagent was diluted with water (1:10 by volume) prior to analysis. A gallic acid stock solution was prepared in 80% aqueous acetone (1:1 by volume), and the gallic acid standard solution was diluted with water to concentrations of 235-1,180 µmol/L. From each standard solution and sample 50 µL was mixed with 2.5 mL of Folin-Ciocalteau reagent and 2.0 mL of sodium carbonate solution. The samples were mixed and incubated at 45 °C for 30 min. The absorbance was read at 765 nm after cooling the sample to room temperature. The results were expressed as gallic acid equivalents (GAE).

**Statistical analysis**

The data is reported as mean and standard deviation SD of two extractions each measured in triplicates for ABTS, FRAP and TPH.

**Results**

The results of the proximal analysis for the whole meals are reported in table II and III. Comparing the results with the recommendation by FAO/WHO, the meals prepared at HO, showed higher protein content representing 17% of the total sample while soups showed lower fat content (4%). The main courses in SSU were prepared for different types of special diets and the protein content was higher, approximately 30%, (the highest in comparison with the other hospitals) as well the carbohydrate content in soups (84%).

On the other hand the main dishes prepared by LUH were standard diets showed somewhat higher protein content (19%).

Comparing the standard diets from HO and LUH the macronutrient content was similar.

Regarding the dietary fiber content in the foods, the Swedish national food administration recommends a consumption of approximately 30 g of dietary fiber per day, which could be around 6 g per meal.

Comparing the main dishes from the three hospitals, the meals prepared in LUH were richer in dietary fiber (2.3 g), followed by SSU (1.45 g). The dietary fiber content in soups, from SSU (0.9 g) had higher dietary fiber content than from HO (0.6 g). The dietary fiber...
content in main courses and soups did not fulfill the quantity of dietary fiber recommended in any investigated case.

Analysis of meals from the three hospitals showed a similar range of total antioxidant capacity and total phenolic compounds (table IV). However, Bolivian lunches apart from main courses, include soups and for this reason the TAC value content is expressed in separated items. The results obtained by the ABTS and FRAP methods are expressed as median (range) table IV.

In the Bolivian meals the TAC values obtained by the ABTS and FRAP were 0.16 (0.11-0.39) and 0.22 (0.10-0.38) Trolox eq./g fw respectively for SSU samples, and 0.23 (0.15-0.33) and 0.22 (0.12-0.47) for HO.

The LUH menu showed TAC values somewhat higher than in Bolivian hospitals. For instance, 0.36 (0.12-0.43) and 0.41 (0.14-0.62) Trolox eq./g fw were obtained respectively by the ABTS and FRAP method.

The TPH values measured in Bolivian soups showed lower amount of total phenols than main courses, 0.4 (0.22-0.68) and 0.39 (0.24-0.49) Trolox eq./g fw respectively. The TPH found in main Bolivian and Swedish courses are in the same range 1.11 (0.38-1.34), 0.98 (0.62-1.03) and 0.92 (0.38-1.28) Trolox eq./g fw respectively in SSU, HO and LUH samples.

Discussion

In general, the proximal analysis showed similar patterns for the hospitals. However HO showed higher amount of fat and carbohydrates in comparison with the other parameters which is also reflected when energy content is calculated.

In general, fat, carbohydrate and protein contents of the three hospitals were within the nutrition recommendation by FAO/WHO.28

The consumption of dietary fiber is 30 g per day according to the Swedish National Food Administration, but on the chart is considered one of the five meals per day.
Table IV
The total antioxidant capacity (TAC) and total phenols content (TPH) in the investigated meals.
The number in brackets is the standard deviation

<table>
<thead>
<tr>
<th>Sample code</th>
<th>ABTS (TAC) [µmol Trolox/g fw]</th>
<th>FRAP (TAC) [µmol Trolox/g fw]</th>
<th>TPH [µmol GAE/g fw]</th>
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<tr>
<td><strong>Seguro Social Universitario (SSU)</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>SSU_1</td>
<td>0.11 (0.01)</td>
<td>0.10 (0.01)</td>
<td>0.38 (0.05)</td>
</tr>
<tr>
<td>SSU_3</td>
<td>0.16 (0.03)</td>
<td>0.18 (0.02)</td>
<td>0.94 (0.14)</td>
</tr>
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<td>0.15 (0.01)</td>
<td>0.22 (0.02)</td>
<td>1.20 (0.18)</td>
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<td>0.39 (0.05)</td>
<td>0.38 (0.07)</td>
<td>1.34 (0.21)</td>
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<td>0.26 (0.03)</td>
<td>1.11 (0.07)</td>
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<td>0.16</td>
<td>0.22</td>
<td>1.11</td>
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<td>(0.10-0.38)</td>
<td>(0.38-1.34)</td>
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<td>0.08 (0.01)</td>
<td>0.44 (0.05)</td>
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<td>0.05 (0.005)</td>
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<td>0.15 (0.02)</td>
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<td>0.08</td>
<td>0.4</td>
</tr>
<tr>
<td>Range</td>
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<td>(0.05-0.15)</td>
<td>(0.22-0.68)</td>
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<td>0.47 (0.05)</td>
<td>0.98 (0.15)</td>
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<td>0.20 (0.02)</td>
<td>0.62 (0.12)</td>
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<td>1.00 (0.17)</td>
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<td>0.23</td>
<td>0.22</td>
<td>0.98</td>
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<tr>
<td>Range</td>
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<td>(0.12-0.47)</td>
<td>(0.62-1.03)</td>
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<td><strong>Soups:</strong></td>
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<td>0.39 (0.06)</td>
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<td>0.11 (0.01)</td>
<td>0.49 (0.10)</td>
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<tr>
<td>HO_8</td>
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<td>0.09 (0.01)</td>
<td>0.33 (0.06)</td>
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<td>0.41 (0.07)</td>
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<tr>
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<td>0.1</td>
<td>0.39</td>
</tr>
<tr>
<td>Range</td>
<td>(0.06-0.12)</td>
<td>(0.07-0.13)</td>
<td>(0.24-0.49)</td>
</tr>
<tr>
<td><strong>Lund University Hospital (LUH)</strong></td>
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<td></td>
</tr>
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<td>0.14 (0.02)</td>
<td>0.38 (0.03)</td>
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<tr>
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<td>0.36 (0.05)</td>
<td>0.43 (0.07)</td>
<td>0.94 (0.15)</td>
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<tr>
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<td>0.35 (0.04)</td>
<td>0.91 (0.04)</td>
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<td>0.43 (0.03)</td>
<td>0.52 (0.03)</td>
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<td>(0.12-0.43)</td>
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<td>(0.38-1.28)</td>
</tr>
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</table>
Bolivian and Swedish hospital were below the Swedish National Food Administration, European and US recommendation for dietary fiber.\textsuperscript{29-31}

In a recent study, reported from South Africa, the antioxidants and the nutritional levels were measured in meals for older people in a day care centre.\textsuperscript{11} The data from the present study shows similar protein content. However, the amounts of carbohydrates and fat were higher than in the present study.

The antioxidant levels were somewhat higher in the Swedish hospital meals. The reason could be due to the higher amount of dietary fiber, to which antioxidants are known to sometimes be associated.\textsuperscript{32} Furthermore, how the food is combined and processed could influence the antioxidant capacity.\textsuperscript{14-16,34}

In comparison with the data obtained from the South African study our values are in general lower. This may be explained as above considering food processing conditions etc.

The antioxidants and phenolic compounds measured in the whole meals were lower than those values obtained in individual uncooked foods.\textsuperscript{10-12} This fact is also reflected in the South African study. The reason could be due to the fact that the combination of items could give a whole matrix that inhibits the antioxidant capacity, and we also can expect some loss in antioxidant capacity due to cooking. We can conclude that antioxidant levels measured in individual food items can be overestimated in comparison to whole meals.\textsuperscript{13,15,36}

The present study is among the first that reflect the antioxidant levels and phenolic content in hospital meals. The results give an interesting indication of how antioxidant levels could be evaluated in hospital and other meals. The results give an interesting indication of how antioxidant levels and phenolic content in hospital meals. In summary, the antioxidant level in whole diets has a great importance, often overestimated by considering only individual uncooked ingredients. An interesting consideration is the dietary fiber content in the meals which can be an important source of antioxidants and non-extractable phenolic compounds.

Acknowledgements

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References


