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Quanti-qualitative evaluation of pectins in the dietary fibre of 24 foods

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ABSTRACT
The dietary fibre of 24 foods was analysed for its proportions of insoluble and soluble (SDF) fractions and for its content of high methoxyl pectins (HM), low methoxyl pectins (LM) and protopectin. The fractional extraction and quantitative determination of pectins were performed on the total dietary fibre residue, following the procedure suggested by Robertson (1979). Total pectin content (TP), calculated as sum of the three fractions, ranged from 2.4 to 49.8 g/kg of dry matter. The variation coefficient of TP measurements repeated on the same foods were, on average, 2%. Total pectin content was 49.8 g/kg DM in dried beet pulp and averaged 33.8±0.3 g/kg DM in fruits and vegetables, 13.2±8.4 g/kg DM in legumes and tubers and only 2.8±0.5 g/kg DM in cereals. HM fraction prevailed in apple and pear samples (>40% of TP), while LM and protopectin largely prevailed in legumes and vegetables. A strong variability among foods was found for the TP/SDF ratio. In general, within each food category, increasing levels of SDF were associated with decreasing values of TP/SDF ratio. Since many foods contain low amounts of pectins, care in the development of calibration curves for spectrophotometric reading is required. Finally, the whole procedure for pectin extraction and quantification on dietary fibre of food is very complex and time-consuming.

Key Words: Foods, HM pectins, LM pectins, Protopectin, Dietary fibre

RIASSUNTO
VALUTAZIONE QUALI-QUANTITATIVA DEL CONTENUTO DI PECTINE NELLA FIBRA ALIMENTARE DI 24 ALIMENTI
Ventiquattro alimenti di interesse nell’alimentazione sia umana che animale sono stati caratterizzati per il contenuto di pectine ad alta metossilazione (HM), bassa metossilazione (LM) e protopectine. I processi di estrazione, frazionamento e quantificazione delle pectine sono stati condotti, seguendo la procedura indicata da Robertson (1987), a partire però dal residuo della fibra alimentare totale (TDF), in alternativa al residuo insolubile in alcool (AIR). Le letture di assorbanza sono state effettuate in spettrofotometro UV/visibile, dotato di un sistema “sipper” per l’introduzione del campione. Gli alimenti sono stati inoltre analizzati presso due laboratori per il contenuto di fibra alimentare insolubile (IDF) e solubile (SDF). Le misure di fibra alimentare totale, ottenute come somma dell’IDF e SDF nei due laboratori, hanno evidenziato un elevato coefficiente di correlazione (y_{IDF+SDF} = 0.999 x_{IDF+SDF}; RSD = 1.37 % DM; R² = 0.996). I risultati della prova suggeriscono che la determinazione delle pectine a partire dal residuo TDF, in alternativa al residuo insolubile in
Introduction

Pectins are found in the primary cell wall and middle lamella of the plant tissues as complex polymers of \(\alpha-D-(1\rightarrow4)\) galacturonic acid with occasional rhamnopyranosil residues bound in different points of the linear chain (McCready, 1970). They contribute to many functions in plants, affecting cell size and shape, tissue resistance, ion transport, water holding capacity, defense against pathogens and wounding (Voragen et al., 2001). At various stages of maturity of the plant the pectins are partially in the methyl ester form and may contain some acetyl groups (Jeraci and Lewis, 1989).

Three fractions of pectins can be distinguished: HM (pectin or pectinic acids or high methoxyl pectins and pectinates), LM (pectic acids or low methoxyl pectins and pectates) and protopectin (Thibault and Ralet, 2001). HM retains water and forms gel under acid conditions and in the presence of sugar; LM forms gel in the presence of divalent cations (i.e., calcium); and protopectin are converted to pectin or pectinic acids by alkaline hydrolysis (McCready, 1970).

A suggested procedure to evaluate the pectin fractions in foods is based on four steps (Thibault and Ralet, 2001). The first is a treatment of the feed sample with a solution (alcohol or alcohol-benzene) to achieve an insoluble residue (Selvendran and O’Neill, 1987; Esteban et al., 1992). The second is the extraction of the three fractions with three different solutions. The third is a treatment of the extracted fractions with a strong acid in order to obtain the decarboxylation of the galacturonosyl units (Theander and Westerlund, 1986) or a treatment with an aromatic chromogen to promote a colorimetric reaction (Blumenkrantz and Asboe-Hansen, 1973; Van Soest et al., 1991; Thibault and Ralet, 2001). The last step is the quantification of the fractions by the CO₂ determination and by spectrophotometric reading, respectively.

Since these substances are not digested by the mammalian enzymes they are considered to be an important constituent of the dietary fibre. The presence of different pectic fractions can markedly affect the chemico-physical and the biological properties of the dietary fibre (Voragen et al., 2001). However, limited and fragmentary information is available about the amount and the composition of pectins in the dietary fibre of foods and feeds, with some exceptions for fruits and vegetables (Robertson, 1979; Van Soest et al., 1991; Marlett, 1992; Lintas and Cappelloni, 1992). The purpose of this work was to evaluate dietary fibre and pectin content of 24 foods, applying the procedure of extraction not on the AIR but on the total dietary fibre (TDF) residue obtained following the AOAC procedure (AOAC, 2000).

Material and methods

Collection and preparation of foods

Twenty-four samples of human foods and animal feeds of different categories (cereal meals, cereal by-products, fibrous feeds, legumes, tubers and roots, vegetables, and fruits) were collected from the local market and from an animal feed firm. Foods were chosen in order to achieve a wide range of variability for insoluble and soluble dietary fibre. According to the procedure suggested by Marlett (1992), the inedible portion of human foods was removed (Table 1), with the exception of potato, apple and pear peels (Robertson, 1979; Marlett, 1992). The samples were oven dried at 60 °C (freeze-dried when mois-
ture was above 30%) and ground through a mill with a screen diameter of 0.5 mm (Cyclotec® 1093 Sample Mill, Tecator). Samples containing more than 10% of oil (whole soybean meal) were preliminarily defatted according to the procedure described by AOAC (2000).

**Pectic substance analysis**

Total pectic substances and their fractions were evaluated as proposed by Robertson (1979). However, the procedure of extraction was performed not on the AIR but on the total dietary fibre residue (TDF), obtained as described by AOAC (2000). TDF differs from AIR only as regards the presence of a chemical-enzymatic treatment of the sample that removes all the digestible compounds. The various steps of the fractional procedure of pectin extraction and quantification are summarized in figure 1.

**Achievement of the TDF residues**

The TDF residue was obtained using the Fibertec E System (1023 Fibertec® E, 1024 Shaking Water Bath, Tecator) according to the method suggested by AOAC (method n. 985.29) (AOAC, 2000). Three food samples, of 1.000 ± 0.005 g, for 3 replications and 3 blanks were subjected to the TDF procedure in each cycle (in total 12 flasks/cycle). In order to obtain TDF residues from all 24 food samples, 8 cycles were performed. In each cycle 3 TDF residues for each food and 3 TDF blanks were obtained. Two residues for each food (and 2 TDF blanks) were used to correct the TDF value for the contribution of protein and ash. The third residue (and 1 blank) was subjected to the procedure of extraction of HM, LM and protopectin (Robertson, 1979; Plessi et al., 1996).

**Pectin extraction**

Extraction was also performed in 8 cycles. The dried TDF residues were homogenized in approximately 5 mL of distilled water and placed into a centrifuge tube; then 35 mL of distilled water were added. The content of each tube was vigorously stirred for 10 minutes by bubbling air through a capillary pipe, and centrifuged at 1000 g for 15 minutes. The supernatant, containing the HM fraction, was collected in a 100 mL volumetric flask with 5 mL of 1N sodium hydroxide. The content of the flask was made up to volume with distilled water. After mixing, the solution was allowed to stand 15 minutes before being subjected to the colorimetric procedure.

In order to allow the homogenisation of the sample, 5 mL of a 0.75% ammonium oxalate solution was added to the pellet remaining in the centrifuge tube. In addition, 35 mL of the ammonium oxalate solution were added to the tube to completely dissolve the LM fraction and it was allowed to stand 15 minutes.

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**Table 1. Edible fractions (% on fresh matter) of food samples.**

| Discarded fraction | Edible fraction |
|--------------------|---------------|
| **Tubers and roots:** | | |
| Potato, with peel | Buds | 99.3 |
| Carrot | Peel | 93.5 |
| **Vegetables:** | | |
| Lettuce | External leaves | 78.8 |
| Chicory | Root | 91.2 |
| Spinach | Root | 94.4 |
| **Fruits:** | | |
| Apples, with peel | Peduncle and seeds | 98.4 |
| Pears, with peel | Peduncle and seeds | 96.5 |
The last pellet remaining in each centrifuge tube was washed and transferred with distilled water into a 100 ml volumetric flask. Five mL of a 1N sodium hydroxide solution were added and the flask content was made to volume with distilled water. After mixing the solution containing protopectin was allowed to stand 15 minutes and occasionally shaken. Then it was filtered through a Wathman paper n. 1 and the remaining solution was allowed to stand as describe above.

**Analysis of standard solutions for spectrophotometer reading**

A preliminary test of calibration was performed. Standard solutions containing from 0 to 80 µg/mL D (+) of galacturonic acid monohydrate (ACROS, cod. 22782-0050) were prepared and analysed. The absorbance was measured at 520 nm using an UV/visible single beam spectrophotometer (Lambda 11, Perkin Elmer). Readings for calibration curves were developed by using a “sipper” system as a tool to deliver the standard solutions to the spectrophotometer. This system was a spectrophotometric cell receiving a continuous flux of the sample solution by means of a peristaltic pump.

**Colorimetric procedure and reading**

From each of the 12 solutions obtained in each cycle, two sub-samples of about 0.5 mL were collected and placed into test tubes to be subjected to the colorimetric procedure. Another two sub-samples were collected to be used as blanks, without the chromogen reagent, for absorbance reading. Thus, in each cycle 24 samples (3 foods and 1 TDF blank x 3 pectic fractions x 2 replications) were subjected to the colorimetric reaction and another 24 samples were used as blanks for reading.

All 48 samples were placed into test tubes and put in a water-ice bath for 10 minutes. After cooling, 3 mL of 96% sulphuric acid was added into each
Results and discussion

Measurements of dietary fibre

The NDF, TDF, IDF and SDF measurements obtained for the 24 foods are given in Table 2. TDF ranged from 1.7 to 84.4% on dry matter and IDF and ranged from about 1.4 to 84.0% DM, while SDF ranged from 0.3 to 14.2% DM. On average, there was some agreement between the NDF (x) and IDF (y) measurements ($y = 2.736 + 0.099 x; R^2 = 0.945$), but the rather high RSD, 4.5% DM suggest caution in using NDF as an alternative to IDF. The regression achieved by running TDF values against IDF+SDF was $y_{TDF} = 0.98 x (IDF+SDF)$ ($RSD = 1.7% DM, R^2 = 0.994$) and that obtained by running the data collected from the other laboratory was: $y_{TDF} = 0.97 x (IDF+SDF)$ ($RSD = 1.55, R^2 = 0.994$). The two sets of IDF+SDF values obtained from the two laboratories showed a linear regression with a slope close to 1.00, an intercept close to 0 and a RSD of 1.37 % DM ($R^2 = 0.996$). These results, in agreement with those of international collaborative studies (Prosky et al., 1988; Prosky et al., 1992; Lee et al., 1992; Prosky et al., 1994), indicated a good reproducibility of the dietary fibre determination.

Absorbance measurements of foods and blanks samples

The procedure of colorimetric reaction and reading was performed in replication on the 24 food samples and the 8 TDF blanks. The mean values and standard deviation of the absorbance measurements obtained for HM, LM and protopectin solutions are given in Table 3. The mean value of absorbance obtained for the 48 solutions not treated with chromogen varied from 5.2 to 7.5% of that of the solutions containing the chromogen. The mean values and standard deviation of the absorbance measurements obtained for the three pectic fractions of the 16 TDF blank samples were low when compared to the corresponding values of the food samples. On average the incidence of the TDF blanks on the mean value of absorbance found for the food samples was only 3.7% for HM, and less than 1% for LM and protopectin. This suggests the possibility of reducing the number of TDF blank samples and the analytical cost.
Analytical results

The amount of TP found in the various food samples ranged from 2.44 to 49.82 g/kg of dry matter (Table 4). Except for carrots and chicory, the standard deviation of the measurements obtained for each food was maintained around 2% of the mean value. This indicated that the intermediate repeatability of the procedures of colorimetric method and reading was acceptable. TP/SDF ratio ranged from 10.9% (barley meal) to 96.2% (raw carrots). Table 4 shows the average incidence of pectins on the SDF was high (> 50%) for vegeta-

Table 2. Dry matter (%) and fibrous fractions (% DM) content of 24 foods.

| Dry matter | NDF  | TDF  | IDF  | SDF  | IDF+SDF |
|------------|------|------|------|------|---------|
| Cereal meals:                                       |      |      |      |      |         |
| Barley     | 88.6 | 24.8 | 21.0 | 20.0 | 2.3     | 22.3    |
| Wheat      | 87.6 | 10.3 | 8.9  | 9.8  | 0.7     | 10.5    |
| Corn       | 85.4 | 3.2  | 3.2  | 4.5  | 0.3     | 4.6     |
| Rice       | 85.7 | 1.4  | 1.7  | 1.4  | 0.3     | 1.7     |
| Cereal by-products:                                |      |      |      |      |         |
| Wheat bran | 87.5 | 45.0 | 43.2 | 43.1 | 3.0     | 46.0    |
| Wheat middlings | 87.6 | 3.2  | 4.4  | 4.4  | 0.5     | 4.9     |
| Corn gluten| 90.0 | 13.0 | 6.1  | 6.1  | 0.8     | 6.9     |
| Corn gluten feed | 87.9 | 32.9 | 33.0 | 32.6 | 1.3     | 33.6    |
| Corn seed, silage | 63.5 | 10.2 | 10.5 | 8.2  | 0.7     | 8.9     |
| Fibrous feeds:                                     |      |      |      |      |         |
| Barley straw | 91.3 | 84.4 | 84.4 | 84.0 | 0.6     | 84.6    |
| Alfalfa meal, dehydrated | 89.7 | 40.8 | 50.0 | 46.7 | 7.9     | 54.6    |
| Beet pulp, dried | 90.7 | 50.6 | 73.3 | 56.2 | 14.2    | 70.4    |
| Legumes:                                           |      |      |      |      |         |
| Peas       | 89.0 | 21.4 | 27.2 | 27.7 | 1.5     | 29.2    |
| Whole soybean, meal | 90.4 | 15.1 | 27.3 | 22.1 | 7.3     | 29.4    |
| Beans      | 92.1 | 11.1 | 19.7 | 18.2 | 3.5     | 21.7    |
| Broad beans | 89.2 | 18.7 | 22.7 | 20.3 | 4.4     | 24.7    |
| Tubers and roots:                                  |      |      |      |      |         |
| Potato, with peel | 18.8 | 4.7  | 8.5  | 6.9  | 2.6     | 9.5     |
| Cassava meal | 87.6 | 11.4 | 12.0 | 10.8 | 2.4     | 13.1    |
| Carrots raw, peeled | 9.6  | 10.8 | 24.3 | 20.7 | 3.3     | 24.0    |
| Vegetables:                                        |      |      |      |      |         |
| Lettuce    | 5.4  | 16.7 | 28.4 | 25.9 | 4.1     | 30.0    |
| Chicory    | 6.2  | 16.5 | 28.7 | 24.1 | 5.3     | 29.3    |
| Spinach    | 11.4 | 17.2 | 32.2 | 25.0 | 5.8     | 30.7    |
| Fruits:                                            |      |      |      |      |         |
| Apples, with peel | 15.1 | 8.2  | 13.9 | 10.7 | 4.8     | 15.5    |
| Pears, with peel | 14.7 | 15.1 | 20.4 | 16.1 | 5.3     | 21.5    |

NDF: Neutral Detergent Fibre; TDF: Total Dietary Fibre; IDF: Insoluble Dietary Fibre; SDF: Soluble Dietary Fibre.
bles and fruits and low (<50%) for the other categories of food, excluding two cereals (corn and rice), one fibrous feed (barley straw) and raw carrots, among tubers and roots. The lowest values of TP/SDF ratio were found for barley, meal, wheat bran and whole soybean meal indicating the presence of other compounds in these foods, i.e. gums and β-glucans (Prosky et al., 1994).

In addition, the TP/SDF ratio was strongly affected by the SDF level (Figure 2). In general, within each food category, increasing levels of SDF were associated to decreasing values of this ratio. A similar trend was found by plotting the TP/TDF ratio against TDF.

The lowest amounts of TP were found on cereal meals and cereal by-products, which contained on average 2.48 and 3.04 g/kg DM of TP, respectively. In these foods the three different pectic fractions were almost equally represented.

As expected, the TP content of barley straw was very low (5.00 g/kg DM of TP). Higher TP contents were observed for dried beet pulp (49.89 g/kg of DM) and dehydrated alfalfa meal (37.35 g/kg). Also in these two feeds the proportions of the various pectic fractions were similar.

Peas and whole soybean meal contained about the same amount of TP (7.95 and 8.44 g/kg of DM, respectively) with high proportions of LM and protopectin as respect to HM. Vidal-Valverde et al. (1982) found for whole soybean meal a TP content of 36.8 g/kg of DM. Bean and broad bean contained, respectively, 13.25 and 11.24 g of TP per kg of DM with a high proportion of protopectin (close to 50% of TP). Potato and cassava meal had an average TP content of 10.00 g/kg of DM, while for carrots this value was 31.76 g/kg DM. In these 3 feeds pectic fractions with a low degree of methoxylation prevailed.

Lettuce, chicory and spinach contained almost the same amount of TP (on average 33.69 g/kg of DM), but in lettuce and chicory the proportion of protopectin markedly prevailed (49 and 43 % of TP, respectively) with respect to LM and HM, while in spinach the 3 fractions were more equally represented.

Apples and pears showed TP contents (on average 33.92 g/kg of DM) close to those of vegetables but with a higher proportion of the HM fraction (50% of TP) and a lower proportion of protopectin (around 20% of TP). Vidal-Valverde et al. (1982)

| Sample             | Observations | A-B | Corrected absorbance |
|--------------------|--------------|-----|----------------------|
| **HM solutions:**  |              |     |                      |
| Foods              | 48           | 0.449 ± 0.549 | 0.415 ± 0.529 | 0.399 ± 0.531 |
| TDF Blanks         | 16           | 0.023 ± 0.007 | 0.015 ± 0.007 |                     |
| **LM solutions:**  |              |     |                      |
| Foods              | 48           | 0.496 ± 0.561 | 0.472 ± 0.538 | 0.468 ± 0.538 |
| TDF Blanks         | 16           | 0.007 ± 0.004 | 0.004 ± 0.003 |                     |
| **Protopectin solutions:** | |     |                      |
| Foods              | 48           | 0.531 ± 0.575 | 0.497 ± 0.551 | 0.493 ± 0.551 |
| TDF Blanks         | 16           | 0.007 ± 0.002 | 0.004 ± 0.002 |                     |
Table 4. Content of the total pectic substances (TP, g/kg of dry matter), high methoxyl pectins (HM), low methoxyl pectins (LM) and protopectin fractions (% of TP), and TP/SDF ratio (%).

| Cereal meals:        | TP Mean ± SD | Pectic fractions | TP/SDF ratio |
|----------------------|-------------|------------------|--------------|
|                      |             | HM   | LM   | Protopectin |              |
| Barley               | 2.51 ± 0.12 | 29.2 | 35.2 | 35.6        | 10.9         |
| Wheat                | 2.53 ± 0.00 | 36.4 | 31.3 | 32.3        | 34.1         |
| Corn                 | 2.44 ± 0.04 | 28.7 | 34.8 | 36.5        | 81.3         |
| Rice                 | 2.46 ± 0.02 | 35.2 | 30.9 | 33.9        | 82.0         |
| Cereal by-products:  |             |      |      |             |              |
| Wheat bran           | 3.47 ± 0.03 | 38.5 | 32.2 | 29.3        | 11.6         |
| Wheat middling       | 2.44 ± 0.06 | 35.9 | 29.8 | 34.2        | 48.8         |
| Corn gluten          | 2.52 ± 0.03 | 34.8 | 29.0 | 36.2        | 31.5         |
| Corn gluten feed     | 4.02 ± 0.03 | 38.5 | 30.7 | 30.8        | 30.9         |
| Corn seed, silage    | 2.74 ± 0.03 | 36.0 | 35.2 | 28.8        | 39.1         |
| Fibrous feeds:       |             |      |      |             |              |
| Barley straw         | 5.00 ± 0.11 | 40.7 | 35.9 | 23.4        | 83.3         |
| Alfalfa meal, dehy.  | 37.35 ± 0.40 | 30.0 | 37.7 | 32.3        | 47.3         |
| Beet pulp, dried     | 49.82 ± 0.04 | 30.8 | 34.9 | 34.3        | 35.1         |
| Legumes:             |             |      |      |             |              |
| Peas                 | 7.95 ± 0.01 | 28.6 | 33.8 | 37.6        | 53.0         |
| Whole soybean, meal  | 8.44 ± 0.07 | 23.7 | 38.5 | 37.8        | 11.6         |
| Beans                | 13.25 ± 0.05 | 21.5 | 26.6 | 51.9        | 37.9         |
| Broad beans          | 11.24 ± 0.04 | 20.5 | 31.4 | 48.1        | 25.5         |
| Tubers and roots:    |             |      |      |             |              |
| Potato, with peel    | 9.61 ± 0.21 | 21.0 | 27.5 | 51.5        | 37.0         |
| Cassava meal         | 10.38 ± 0.23 | 16.5 | 45.3 | 38.2        | 43.3         |
| raw, peeled          | 31.76 ± 1.57 | 16.7 | 5.7  | 47.6        | 96.2         |
| Vegetables:          |             |      |      |             |              |
| Lettuce              | 34.64 ± 0.42 | 13.2 | 37.9 | 48.9        | 84.5         |
| Chicory              | 33.96 ± 3.71 | 20.4 | 37.0 | 42.6        | 64.1         |
| Spinach              | 33.48 ± 0.26 | 37.3 | 31.1 | 31.6        | 57.7         |
| Fruits:              |             |      |      |             |              |
| Apples, with peel    | 34.29 ± 0.87 | 49.0 | 32.1 | 19.0        | 71.4         |
| Pears, with peel     | 33.54 ± 0.77 | 44.7 | 32.9 | 22.5        | 63.3         |
reported TP contents from about 43.1 to 36.0 g/kg of dry matter for apples and 24 g/kg DM for pears. However, in literature there is a huge variability (Table 5) with values for apples ranging from 3.8 to 18.1 g/kg of fresh matter, as a result of differences due to apple varieties, stage of ripening, fruit dry matter content, as well as to methods of sampling, extraction and analysis.

Conclusions

In this paper 24 human foods and animal feeds have been characterized by their content of insoluble and soluble dietary fibre and different pectic fractions. Results indicate that the fractional extraction and the quantitative determination of pectins in foods can easily be performed by using their TDF residue instead of their AIR residue. This offers the opportunity for a better characterization of the indigestible constituents of foods. In this paper some indications suggest that there may be some correlations between soluble dietary fibre and total pectin contents within food categories. Further experiments with a larger number of foods within each category should be carried out in order to verify these correlations.

Table 5. Pectin content of apple (g/kg of edible fraction as fed) by different Authors (Vidal-Valverde et al., 1982, mod.) and present experiment.

| References                  | Pectins   |
|-----------------------------|-----------|
| Belo and DeLumen (1981)     | 18.1      |
| Campbell and Palmer (1978)  | 7.1 - 8.4 |
| Hardinge et al. (1965)       | 6.0       |
| Krause and Bock (1973)       | 4.4 - 4.5 |
| Money and Christian (1950)   | 5.5 - 5.3 |
| Southgate (1976)             | 3.8       |
| Vidal-Valverde et al. (1982) | 5.0 - 5.6 |
| Present experiment           | 5.1       |

Figure 2. Relationships between the TP/SDF ratio and SDF content of cereal meals (●), cereal by-products (■), fibrous feeds (+), legumes (x), tubers and roots (*), vegetables (●), and fruits (▲).
Since many foods contain low amounts of pectins, a special care in the methodological approach, and, in particular, in the development of good calibration curves for spectrophotometric reading is required.

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