



<b>Title</b>	Dietary Fibre - Some Properties of Alcohol-insoluble Solids Residues from Apples
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<b>Publication date</b>	1981
<b>Publication information</b>	Gormley, T. R. (Thomas Ronan). "Dietary Fibre - Some Properties of Alcohol-Insoluble Solids Residues from Apples." Wiley, 1981. <a href="https://doi.org/10.1002/jsfa.2740320413">https://doi.org/10.1002/jsfa.2740320413</a> .
<b>Publisher</b>	Wiley
<b>Item record/more information</b>	<a href="http://hdl.handle.net/10197/6914">http://hdl.handle.net/10197/6914</a>
<b>Publisher's version (DOI)</b>	<a href="https://doi.org/10.1002/jsfa.2740320413">10.1002/jsfa.2740320413</a>

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## Dietary Fibre—Some Properties of Alcohol-insoluble Solids Residues from Apples

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(Manuscript received 2 November 1979)

Alcohol-insoluble solids (AIS) material prepared from apples contains about 80% dietary fibre and has therapeutic properties. The AIS content of seven apple cultivars was tested, three from a soil management experiment and four from a cultivar trial. Values for AIS content were different ( $P < 0.001$ ) for cultivars in both trials, ranging from 2.89 to 4.41%. The grass soil management treatment gave higher ( $P < 0.05$ ) AIS levels in the fruit than the overall herbicide treatment. In-vitro water holding capacities (WHC) of the AIS materials were not significantly different even though values ranged from 20.8 to 25.5 g water bound  $g^{-1}$  AIS. However, calcium binding capacities (CBC) were different for cultivars, with ranges of 3.69–4.38 mg Ca  $g^{-1}$  AIS material ( $P < 0.001$ ) and 3.25–4.08 mg ( $P < 0.05$ ) in the two trials. Pectin content of the AIS material was different ( $P < 0.05$ ) for cultivars in the soil management trial, ranging from 20.7 to 22.5%. Method of soil management had no effect on WHC, CBC or pectin content of the AIS material. Values for WHC, CBC and pectin based on 100 g fresh apple samples, as eaten, were significantly different in both trials, with grand means of 80.9 g, 14.2 mg and 0.76 g, respectively. The grass treatment gave higher CBC and pectin content in fruit than the overall herbicide treatment. These data indicate that AIS material from different apple cultivars varies in both amount and properties. The method of soil management had a lesser effect.

### 1. Introduction

The effect of dietary fibre (DF) on human health has been discussed previously<sup>1-3</sup> and the DF content of many foods has been quantified by Southgate.<sup>4</sup> Properties of DF from different foods have been investigated by a number of workers.<sup>5-8</sup> However, little information is available on the DF content (and properties) of different types of the same food e.g. apple cultivars, or on the effects of method of husbandry.

Ingestion of DF usually refers to DF in a food as consumed rather than the use of DF isolated from foods in a 'pharmaceutical' sense. However, there are two approaches; one as seen by the nutritionist and the other taken by the pharmaceutical industry which sees a therapeutic need.<sup>9</sup> Various fibre sources have been used in pharmaceutical preparations including Ispaghula (dried seeds of *Plantago ovato*), Psyllium (seeds of *P. psyllium* and *P. arinaria*) and Sterculia, which is a gum extracted from *Sterculia* spp.

Recently, Cummings *et al.*<sup>10</sup> studied the colonic response to DF prepared from carrot, cabbage, apple, bran and guar gum while Mayne *et al.*<sup>11</sup> found reduced haemoglobin A<sub>1</sub> values in subjects with late-maturity-onset diabetes who consumed alcohol-insoluble solids (AIS) material prepared from apples. A number of other workers<sup>12-15</sup> have found that pectin is a hypocholesterolaemic agent when consumed in fairly large quantities.

In the present study the AIS content of a number of apple cultivars, some of them grown under different methods of soil management, was investigated. Tests were conducted on the ability of the AIS to bind water and calcium *in vitro* and the pectin content of the AIS material was also measured. In non-starchy fruits such as mature apples, AIS material contains about 80% DF; an AIS preparation thus represents one of the cheapest and easiest to prepare forms of DF for use in human studies.

The in-vitro data for the AIS residues were extrapolated to give the pectin content and also the potential water and calcium-binding power (CBP) of 100 g of fresh apples as eaten. Apples were chosen for this experiment because of their hypocholesterolaemic effect<sup>16</sup> and also in view of the encouraging results noted in the case of diabetics.<sup>11</sup>

## 2. Experimental

### 2.1. Source of apples

Fruit of the cultivars Golden Delicious, Red Jonathan and Cox's Orange Pippin were obtained from a soil management experiment which commenced in 1965 at the Pomology Research Station of the Agricultural Institute at Ballygagin, Co. Waterford.<sup>17</sup> Samples from overall herbicide and grass treatments were used and there were five replicates.

Samples of the apple cultivars, Idared, Malling Kent, Malling Suntan, and Karmijn de Sonnaville were obtained from cultivar trials at Ballygagin. There were four replicates.

### 2.2. Preparation of AIS

Samples (1 kg) of mature, unpeeled, cored apples were comminuted at time of harvest in a Boku high speed chopper and were slow frozen and stored at  $-30^{\circ}\text{C}$  for 1 week. Prior to freezing, sub-samples were removed and dried in a vacuum oven at  $70^{\circ}\text{C}$  and 200 mmHg to constant weight to determine dry matter (DM) content. The samples were thawed and sufficient absolute ethanol added to 500-g portions of the thawed product to ensure a final aqueous ethanol concentration of 80% (v/v). The mixture was boiled for 2 min and left standing overnight. The AIS was collected, washed with 1 litre of boiling 80% aqueous ethanol and dried at a temperature below  $40^{\circ}\text{C}$ .

The dried samples were extracted again, this time under reflux for 0.5 h using AIS and aqueous ethanol in the ratio 1:20. The purified AIS material was collected, washed and dried as described and then passed through sieves to give a particle size of 1–2 mm. This material was used in all further tests.

### 2.3. Tests on AIS

Water holding capacity (WHC) of the AIS material was measured using the method described by McConnell *et al.*<sup>5</sup> which involved soaking AIS material (1 g) in water overnight followed by centrifugation at 14 000g.

The ability of the AIS material to bind calcium was tested using the procedure described by James *et al.*<sup>18</sup> The estimation was performed at pH 7.4 using 0.5 g samples of AIS and a 50 parts  $10^{-6}$  calcium solution.

The pectin content of the AIS material was measured using the method described by Warren and Woodman.<sup>19</sup> Protein content was measured by the Kjeldahl procedure and ash using a standard furnace procedure.

## 3. Results

### 3.1. AIS and DM content

Both AIS and DM values were significantly different for apple cultivars within the soil management trial and within the cultivar trial (Table 1). The highest AIS values were found in fruit of the cultivars Malling Kent and Golden Delicious and the lowest in that of Cox and Karmijn (Table 1).

Method of soil management also had an effect, with apples from grass plots having significantly higher AIS and DM values than those from overall herbicide plots.

### 3.2. Properties of AIS material

WHC values of AIS material from apples from the two trials were not significantly different nor did method of soil management have an effect (Table 2). Values for WHC varied from  $25.7\text{ g g}^{-1}$  AIS for Malling Suntan to  $20.8\text{ g g}^{-1}$  for Karmijn (Table 2).

Table 1. Alcohol-insoluble solids and dry matter content of apples from soil management and cultivar trials

	AIS (%)	DM (%)
<i>Soil management trial</i>		
Golden Delicious	3.64	17.0
Red Jonathan	3.28	14.8
Cox's Orange Pippin	2.89	14.2
<i>F</i> -test	$P < 0.001$	$P < 0.001$
S.e.	0.06	0.28
Overall herbicide	3.18	14.9
Grass	3.36	15.7
<i>F</i> -test	$P < 0.05$	$P < 0.05$
S.e.	0.05	0.23
<i>Cultivar trial</i>		
Idared	3.45	14.0
Malling Kent	4.41	16.7
Malling Suntan	3.60	16.4
Karmijn de Sonnaville	3.08	15.1
<i>F</i> -test	$P < 0.001$	$P < 0.01$
S.e.	0.16	0.59

Values for CBC of the AIS material were significantly different in the two trials with the highest value of 4.38 mg Ca g<sup>-1</sup> AIS being found for AIS material from Cox and the lowest at 3.25 mg g<sup>-1</sup> for Malling Kent (Table 2). Method of soil management had no effect.

### 3.3. Composition of AIS material

Pectin levels in the AIS material were significantly different for cultivars in the soil management trial (Table 3) but not in the cultivar trial. Method of soil management had no effect (Table 3). Values for the pectin content of the AIS material varied from 23.1% for Idared to 20.7% for Golden.

A similar situation was found for the protein and ash content of the AIS material, i.e. there was a significant effect for the cultivars in the soil management trial but not in the cultivar trial. Again,

Table 2. Water holding capacity and calcium binding capacity of alcohol-insoluble solids material from apples grown in soil management and cultivar trials

	WHC (g g <sup>-1</sup> AIS)	CBC (mg Ca g <sup>-1</sup> AIS)
<i>Soil management trial</i>		
Golden Delicious	24.4	3.69
Red Jonathan	22.2	4.30
Cox's Orange Pippin	22.7	4.38
<i>F</i> -test	NS	$P < 0.001$
S.e.	1.13	0.087
Overall herbicide	22.9	4.15
Grass	23.3	4.10
<i>F</i> -test	NS	NS
S.e.	0.92	0.071
<i>Cultivar trial</i>		
Idared	25.5	4.08
Malling Kent	21.5	3.25
Malling Suntan	25.7	3.50
Karmijn de Sonnaville	20.8	3.90
<i>F</i> -test	NS	$P < 0.01$
S.e.	2.10	0.173

Table 3. Pectin (as anhydrouronic acid), protein, and ash content of AIS material from apples grown in soil management and cultivar trials

	Pectin (%)	Protein (%)	Ash (%)
<i>Soil management trial</i>			
Golden Delicious	20.7	8.7	1.89
Red Jonathan	22.5	13.0	1.70
Cox's Orange Pippin	22.1	10.2	2.25
<i>F</i> -test	$P < 0.05$	$P < 0.001$	$P < 0.05$
S.e.	0.45	0.71	0.15
Overall herbicide	21.6	11.2	1.85
Grass	22.0	10.1	2.04
<i>F</i> -test	NS	NS	NS
S.e.	0.37	0.58	0.12
<i>Cultivar trial</i>			
Idared	23.1	9.6	1.55
Malling Kent	21.1	8.6	1.95
Malling Suntan	22.0	9.9	1.48
Karmijn de Sonnaville	21.9	11.1	1.75
<i>F</i> -test	NS	NS	NS
S.e.	1.04	2.48	0.39

the method of soil management had no effect (Table 3). Values for protein in the AIS ranged from 13.0 to 8.6% for Red Jonathan and Malling Kent, respectively, and ash from 2.25% for Cox to 1.48% for Malling Suntan.

#### 3.4. WHC, CBC and pectin content of apples as eaten

Calculated values for the WHC, CBC and pectin content of apples as eaten were obtained by extrapolating data for the AIS material to the fresh apples. Results (Table 4) show that WHC values were significantly different for cultivars in both of the trials, with values ranging from 94.9 g water 100 g<sup>-1</sup> fresh apple for Malling Kent to 63.5 g for Karmijn. Significant effects were also found for CBC and pectin content, with maximum values of 14.3 mg Ca 100 g<sup>-1</sup> apple and 0.92%, respectively,

Table 4. Water holding capacity, calcium binding capacity and pectin content of apples (as eaten) grown in soil management and cultivar trials (values for fresh apples obtained by calculation from data in Tables 1-3)

	WHC (g 100 g <sup>-1</sup> apple)	CBC (mg Ca 100 g <sup>-1</sup> apple)	Pectin (%)
<i>Soil management trial</i>			
Golden Delicious	89.4	13.38	0.75
Red Jonathan	72.9	14.11	0.74
Cox's Orange Pippin	65.8	12.78	0.65
<i>F</i> -test	$P < 0.01$	$P < 0.001$	$P < 0.01$
S.e.	4.60	0.205	0.02
Overall herbicide	73.2	13.13	0.69
Grass	78.9	13.72	0.74
<i>F</i> -test	NS	$P < 0.05$	$P < 0.05$
S.e.	3.78	0.168	0.02
<i>Cultivar trial</i>			
Idared	87.7	14.07	0.79
Malling Kent	94.9	14.28	0.92
Malling Suntan	92.0	12.58	0.79
Karmijn de Sonnaville	63.5	12.02	0.67
<i>F</i> -test	$P < 0.001$	$P < 0.05$	$P < 0.001$
S.e.	5.97	0.841	0.02

(cultivar Malling Kent) and minimum values of 12.0 mg Ca 100 g<sup>-1</sup> and 0.65% (cultivars Karmijn and Cox) (Table 4). The method of soil management did not significantly effect WHC, but the grass treatment resulted in significantly higher CBC and pectin contents (Table 4).

#### 4. Discussion

AIS content reflects maturity and texture of fruit and vegetables, and values for many crops have been given in the literature.<sup>20-27</sup> However, with increasing emphasis on the significance of complex carbohydrate in the diet,<sup>28</sup> the importance of AIS material assumes an additional dimension as it represents most of the complex carbohydrate fraction in fruit and vegetables. Extraction, purification and analysis of cell-wall material (AIS) from plant tissues has been performed by Selvendran.<sup>29,30</sup>

In non-starchy fruit and vegetables, e.g. mature apples, AIS material contains about 80% DF and so represents a useful fraction for testing as a 'fibre concentrate' in a therapeutic sense. Cummings *et al.*<sup>10</sup> have used DF isolated from carrots and other sources in human studies, but preparation of this material required an expensive process including fluidised-bed drying and extensive extraction with methanol and acetone; AIS material is cheaper to prepare.

AIS levels in this experiment varied from 2.89 to 4.41%. The values are slightly higher than those described by Toldby and Wiley<sup>31</sup> and by Kertesz *et al.*<sup>32</sup> The method of soil management affected AIS content of the fruit and this suggests that other aspects of husbandry could also influence AIS content. In-vitro values for WHC of the AIS material were not significantly different in either trial, even though the values ranged from 20.8 to 25.7 g of water bound g<sup>-1</sup> AIS in the cultivar trial. This may be due to the method<sup>5</sup> used to measure WHC, which is technically difficult resulting in high standard errors. Values found for WHC were higher than those reported<sup>5,33</sup> from an apple insoluble solids residue prepared using acetone rather than ethanol.

The grand mean in-vitro CBC value for AIS material from the two trials was 3.87 mg Ca (0.097 mmol Ca) bound g<sup>-1</sup> AIS material. This is within the range found by James *et al.*<sup>18</sup> of 0.05-0.42 mmol Ca bound g<sup>-1</sup> DF from a number of plant sources, but below the mean value of 0.22 mmol. The differences may be due to the fact that James *et al.*<sup>18</sup> used finely-divided DF material for the binding test whereas AIS material of particle size 1-2 mm was used in these tests. In this experiment the CBC was only measured at pH 7.4 and it has been shown that calcium binding,<sup>18</sup> and that of Cu, Zn and Fe,<sup>34</sup> are influenced by pH. However, the fear that people on a high DF intake could be deficient in Ca and other elements may be less than originally thought, in view of findings in Third World countries where intakes of phytates and uronic acids are such that all dietary Ca should be bound. However, satisfactory calcification has been found in children and also in women who have had many pregnancies and long lactations.<sup>35</sup>

It is important to stress that, while the in-vitro tests for WHC and CBC show differences in properties of AIS material from apple cultivars, the results cannot be extrapolated directly to an in-vivo situation where there are so many more variables. However, it is possible that the AIS materials could exert different pharmacological effects *in vivo*.

Pectin levels in the AIS materials were similar, with a mean value of 21.9%. In view of the reported hypocholesterolaemic<sup>12-15</sup> and other effects<sup>36</sup> of pectin it is likely that AIS material could cause similar effects in view of its pectin content.

The AIS materials used in these tests were prepared from apples at the time of harvest. However, changes occur during apple storage which could alter the properties of the AIS material. For example, various changes take place in the pectin fraction<sup>37</sup> and Strandzhev *et al.*<sup>38,39</sup> have shown that the amounts of total pectin, protopectin and soluble pectin decreased during refrigerated storage of apples.

The amount of cellulose in the AIS material was not investigated in the present study, but it is likely that it would vary for different cultivars—as described by Paton,<sup>40,41</sup> who showed values for percentage cellulose in AIS material ranging from 34.7 to 24.4 for different apple cultivars. He found that the sodium carboxymethylether of the isolated cellulose component from different apple cultivars showed a high viscosity in aqueous solution and extreme sensitivity towards divalent metal ions. These findings suggest that the cellulose fraction of apple AIS could have pharmacological properties.

## 5. Conclusions

This experiment indicates that the AIS material prepared from apples can vary in amount, composition and in properties depending on cultivar and other factors. It has been noted that aspects such as apple maturity and storage can affect the composition and properties of the AIS material. The AIS material prepared is fluffy and light in texture with a low bulk density suggesting that it has a good physical structure. It is also palatable when taken as a slurry with water. It is possible that AIS material may have an important role in relation to blood lipids, treatment of diabetes and as a DF agent which could be of special benefit to subjects that cannot tolerate DF of cereal origin such as bran. It is suggested, therefore, that more extensive research needs to be performed on AIS material from a wide range of fruit and vegetables to investigate possible beneficial pharmacological effects.

## Acknowledgements

The author would like to thank Mr S. Egan and Mr P. Walshe for technical assistance.

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