Article:
Orfila, C, Chu, J and Burley, V (2014) Analysis of dietary fibre of boiled and canned legumes commonly consumed in the United Kingdom. Journal of Food Composition and Analysis, 36 (1-2). 111 - 116 (6). ISSN: 1096-0481

https://doi.org/10.1016/j.jfca.2014.06.010

Analysis of dietary fibre of boiled and canned legumes commonly consumed in the United Kingdom

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Abstract

The use of different analytical methods to measure the dietary fibre content of foods complicates the interpretation of epidemiological studies. The aim of this study was to determine the total (TDF) and insoluble (IDF) fibre content of 14 boiled and canned legumes commonly consumed in the UK using the Association of Official Analytical Chemists (AOAC) enzymatic gravimetric method. The fibre values obtained were compared to non-starch polysaccharide (NSP) values. The results showed that mean values for TDF (2.7 - 11.2 g/100g) were higher than NSP (2.6 - 6.7g/100g), with a mean NSP: TDF ratio of 1:1.43. TDF was correlated with NSP (r= 0.6; p= 0.02). Canning significantly reduced TDF and IDF by an average of 30% and 26% compared to boiling respectively. However, IDF represented at least 60% of the TDF in both boiled and canned samples. In conclusion, fibre values are affected by the processing and analytical method used.

Keywords: Dietary Fibre; Insoluble Fibre; Legume;; AOAC method; Cooking Methods; Food Analysis; Food composition; Pulses; Non-Starch Polysaccharides; Canning

1. **Introduction**

Legumes are a rich source of dietary fibre as well as providing a good source of energy from starch and protein (Trinidad et al., 2010) . The beneficial effects of legumes have been reported in the results of a pooled analysis which showed an improvement in fasting blood glucose concentration in both diabetic and non-diabetes subjects (Sievenpiper et al., 2009). The hypoglycaemic effects of legumes have been attributed to their high content of dietary fibre (Trinidad et al., 2010).

The health benefits of a diet rich in dietary fibre have been reported (Lunn and Buttriss, 2007). Prospective studies were inconclusive regarding the protective effect of high dietary fibre intake on the risk of type 2 diabetes mellitus (Hopping et al., 2010; Barclay et al., 2007).. Inconsistency in the results may be explained partly by differences in the analytical method used to estimate the dietary fibre intake and to errors arising from the dietary assessment tool that is commonly used in the prospective studies.

There are two analytical methods that are commonly used for dietary fibre analysis: the enzymatic chemical method developed by Englyst (Englyst et al., 1982) and the enzymatic gravimetric methods (985.29 and 991.43) (Lee et al., 1992) endorsed by the Association of Official Analytical Chemists (AOAC). Both methods have been used to generate fibre data for food composition tables (Food Standard Agency, 2002; -DeVries and Rader, 2005). The Englyst method (Englyst et al., 1982) is based on the chemical analysis of alcohol-insoluble cell wall polysaccharides remaining after the enzymatic degradation of starch. Some residual starch glucose may also be included in the Englyst NSP values, and the acid hydrolysis step may result in the loss of some acid-labile cell wall sugars (Wolters et al., 1992). Alternatively, the AOAC method is based merely on the gravimetric measurement of the alcohol-insoluble solid residue remaining after enzymatic degradation of starch and protein. The AOAC method does not only provide a measure of plant cell wall polysaccharides, but also includes other indigestible substances such as digestion-resistant starch and protein, lignin and high molecular weight polyphenols (Englyst et al., 2007). Neither method takes into account low molecular weight, ethanol-soluble indigestible oligosaccharides such as the raffinose-like oligosaccharides. For practical reasons, both methods use microbial enzymes for the degradation of starch, which may not give a true representation of starch digestibility *in vivo*. Figure 1 shows the relationship between the main components of dietary fibre that are measured by the Englyst and AOAC methods. Updated dietary fibre definitions include components other than non-starch polysaccharides and therefore the AOAC analytical methods may more closely estimate the dietary fibre content of foods and have been adopted in many countries to provide fibre values for food composition tables and food labelling purposes

(DeVries and Rader, 2005). In the UK, the Englyst method has been used to determine non-starch polysaccharides (NSP) for food composition tables and remained the recommended method for nutrition and food labelling until 1999 (Food Standard Agency, 2002). After that, the Food Standard Agency (FSA) accepted the role of resistant starch and lignin as being part of dietary fibre and adopted the use of the AOAC method to generate fibre values for labelling purposes. The sixth edition of *McCance and Widdowson's The Composition of Foods* (Food Standard Agency, 2002) lists total dietary fibre (TDF) derived by AOAC values for 47 food items, including 27 values for the cereal group, 13 for the milk group, 4 for meat group, 2 for the fish group and a single item from vegetable dishes. There are no TDF values listed for any legume consumed in the UK. Most epidemiological studies undertaken in the UK still use NSP values, and it is therefore difficult to compare UK studies to those conducted in the rest of the world. In order to address this issue, a mean ratio of TDF:NSP of 1:1.3 was generated for all food groups (Lunn and Buttriss, 2007). However, the legumes were not highly represented in this ratio. A study by Reistad and Frolich (1984) suggested a ratio between 1.1–1.4 for vegetables, but this study did not include legumes in the analysis. A ratio that includes legumes may be useful to convert NSP to TDF values for populations with high consumption of legumes, such as Asian ethnic minorities and vegetarians. The aim of the current work was to determine TDF by the AOAC enzymatic gravimetric method for selected legumes commonly consumed in the UK. The study aimed to investigate the effects of common cooking methods (boiling and canning) on the TDF and IDF content of legumes. The second aim was to establish a NSP:TDF ratio for the legume group which would be of interest to nutritional epidemiologists.

2. Materials and methods

2.1. Materials

The tested samples were selected based on commonly consumed legume products listed in the National Diet and Nutrition Survey (NDNS) (Henderson L 2002) and frequency data derived from the UK Women Cohort Study (Cade et al., 2004). A descriptive analysis of a Food Frequency Questionnaire (FFQ) was used as part of the UKWCS showed that 88% of women in the cohort reported some legume consumption. The most frequently consumed pulses (at least once a week) were green beans (62%), peas (60%), baked beans (39%), lentils (15%), and mung and red kidney beans (12%), butter beans (9%) and chickpeas (8%). The women in the UKWCS reported eating legumes both in the boiled and canned forms, and therefore raw samples were not analysed.

Fourteen pooled samples of legumes were derived from different brands purchased from UK supermarkets and retailers (appendix A & B). Composite samples were obtained according to the sampling protocol used in the UK food composition table (Food Standard Agency, 2002). Six types of legumes were included, namely yellow chickpeas (*Cicer arietinum L*), red kidney beans (*Phaseolus vulgaris*), red lentils and green and brown lentil (*Lens culinaris*), butter beans (*Phaseolus lunatus L*), green peas (*Pisum sativum*), and green beans (*Phaseolus vulgaris*), baked bean in tomato sauce (haricot or navy beans; *Phaseolus vulgaris*) and mung beans (*Vigna mungo*). All chemicals were of analytical grade and were purchased from Sigma-Aldrigh (Dorset, UK) unless otherwise stated.

2.2. Sample preparation

Dried legumes were processed prior to analysis. Processing included soaking overnight in tap water (1:5 w/v) at room temperature, followed by draining and then cooking in tap water at boiling temperature according to the UK food composition description in *McCance and Widdowson's The Composition of Foods* (Food Standard Agency, 2002). When cooking instructions were not available in the aforementioned book, packet instructions were followed as per normal domestic practice. Then, samples were drained and homogenised prior to analysis. Canned samples were drained and homogenised prior to analysis.

2.3. TDF analysis by the AOAC method (991.43)

Food samples were analyzed for TDF and IDF following an AOAC (1995) official method (991.43) with two minor modifications that speeded up recovery of the fibre residue (centrifugation prior to filtration, and replacement of the sintered glass filter by three layers of Miracloth filter). A fibre assay kit (K-TDFR 03/2009) was used (Megazyme International, Bray, Ireland). TDF was determined in triplicate with a starting sample weight of 1.000±0.005 g.

The sample was suspended in MES/TRIS buffer, pH 8.2 at 24°C, 40 mL. Enzyme hydrolysis was performed by incubating the sample in a water bath at 95° – 100ºC with 150 IU of heat stable α-amylase (E-BLAAM; 3,000 Ceralpha U/ml) with shaking for 35 minutes, followed by incubation at 60ºC with 35 IU of protease (E-BSPRT; 50mg/ml) for 30 minutes with shaking, followed by pH adjustment to 4.5 and incubation at 60°C with 640 IU amyloglucosidase (E-AMGDF; 3200 U/ml) for 30 minutes in a shaking water bath for further starch and maltodextrin hydrolysis. After that, the digested mixture was precipitated with four volumes of 95% ethanol that had been preheated to 60°C. The precipitated sample was centrifuged using a Beckman Coulter J2 Centrifuge using 250ml Beckman tubes at 3840 g for 30 minutes at 20°C. This modification from the original protocol was included to facilitate separation and reduce the filtration time. The supernatant was removed, and the residue filtered through 3 layers of Miracloth (Calbiochem, La Jolla, California, USA). This mode of filtration was found to ease the recovery of the fibre residue without compromising yields. The residue was washed with ethanol, then acetone and dried in an oven at 103°C until constant weight was achieved. One residue was analyzed for nitrogen content by the Kjeldahl method (Bradstreet, 1965). Nitrogen content was multiplied by a conversion factor of 6.25 to calculate protein content. Another residue was used for ash analysis by combustion in a furnace at 550ºC until a constant weight was achieved. TDF values were recorded after subtracting protein and ash.

IDF from the same legume samples was also determined. Triplicate samples of boiled and canned legumes were gelatinized and treated with enzymes as above. The IDF residue was filtered through three layers of Miracloth and washed with 95% ethanol and acetone, dried and weighed. IDF value was obtained after subtracting protein and ash from the weighed residue as described above. The SDF content was determined by the difference between TDF and IDF values.

3. Statistical analysis

Statistical software (*Stata Statistical Software: Release 12*. College Station, TX: StataCorp LP) was used to test the significance of results at 95% confidence. Student t-test and analysis of variance (ANOVA) tests were performed as appropriate to analyse the effect of cooking method on fibre values. Coefficient of variation was calculated for comparing the degree of variation from one batch to another for the each legume type.

4. Results

4.1 TDF values for boiled and canned legumes

Fourteen legume samples (8 boiled, 6 canned) that are the most commonly consumed in the UK were selected for TDF analysis. The results are presented in Table 1 as grams of TDF per 100 grams legume (wet weight as eaten). The boiled legumes showed a range of TDF values from 3.6% in green beans to 11.2% in red kidney beans, with an overall mean TDF of 7.2%. The coefficient of variation for the boiled legumes ranges from 2.09% to 6.40%. The canned legumes showed a range of TDF values from 2.7% in canned green beans to 7.4% for canned chickpeas, with a mean TDF of 5.2g/100g. The coefficient of variation (CV) for canned legumes ranges between 1.37% to 5.73%. A collaborative study (Kanaya et al., 2007) showed a CV% range between 0.89 – 6.26% for fibre rich food from different food groups. This indicates that the repeatability of the TDF analysis in this study was within the acceptable range. The TDF values for boiled legumes were on average 31% higher than for the equivalent canned legume, and ANOVA analysis showed that boiled legume values were significantly higher than canned legumes by 2.57g/100g ($p<0.01$). The greatest difference was found in red kidney beans, with TDF values in canned samples (5.5 g/100 g) being half of the boiled equivalent (11.2 g/100 g). The present findings seem to be consistent with other research which found processing such as cooking and frying of chickpeas yielded varied amount of dietary fibre (Perez-Hidalgo et al., 1997). This indicates a significant effect of cooking method on the TDF content of the analysed legumes.

4.2. Insoluble and soluble dietary fibre content of boiled and canned legumes

The measured IDF and calculated SDF values for boiled and canned legumes are presented in Table 1. The results show that IDF values in boiled legumes ranged from 2.65% for green beans to 8.89% for red kidney beans, and in canned legumes from 1.96% green beans to 6.42% for yellow chickpeas.

The IDF represents at least 60% of TDF for all tested legumes with the remaining representing the SDF fraction. ANOVA was used to compare between IDF content in boiled versus canned legumes. The values were significantly higher in boiled legumes by 1.7g/100g compared to their canned equivalents (p= 0.02). Similar findings were observed in a previous study that found that IDF in boiled soaked beans was higher than in canned beans with a difference of 1g/100g (Kutos et al., 2003).

However, the proportion of IDF: SDF did not vary significantly with cooking method (p=0.3), indicating that both fractions (soluble and insoluble) decrease by the same proportion during canning and boiling. This is in contrast to a previous study that found changes in fibre fractions of green beans after cooking and autoclaving (Anderson and Clydesdale, 1980).

4.3. A comparison of TDF and NSP values

The results presented in Table 2 indicate that TDF values for all cooked legumes were found to be on average 43.3% higher than published NSP (Food Standard Agency, 2002) values. TDF values were 67.6% and 18.9% higher than NSP in boiled and canned legumes respectively. Only two legume samples, boiled green beans and canned kidney beans, showed slightly lower TDF values compared to NSP. Similar observations were found in some food items in the UK food composition table (Food Standard Agency, 2002) where unexpectedly, 5 of out of 47 food items had slightly lower TDF values compared with NSP values. A previous analysis of ten food groups showed that TDF fibre was higher than NSP by 20% (green vegetables) to 77% (other vegetables) which supports the current findings (Englyst H.N, 1996).

On average an NSP: TDF ratio of 1.43 was calculated for the cooked legume group (n=14). For the whole group, the TDF content of legumes was significantly correlated with NSP (r= 0.6, 95% CI: 0.101 to 0.872; p= 0.02). The ratio for the boiled legumes (1:1.68) was higher than for the canned legumes (1:1.19). This indicates that NSP: TDF ratio is dependent on the cooking method.

5. Discussion

The results presented in this paper show that the fibre content of legumes is affected by both the processing method and the method of analysis. Legumes preserved by canning were found to have significantly lower TDF values. Previous studies showed that boiling and microwaving did not affect the NSP content of legumes (Reistad and Frolich, 1984), but boiling and autoclaving affected TDF significantly (Li and Cardozo, 1993). This indicates that canning affects mostly non-cell wall polysaccharides, most likely resistant starch. Enzyme-resistant starch is one of the components of dietary fibre that is included in the TDF gravimetric measurement and to a lesser extent in Englyst's chemical method. The physical and chemical properties of legume starch provide an explanation for its poor digestibility in comparison with cereal starch and the high amount of resistant starch in cooked legumes (Sandhu and Lim, 2008) . Legume starch is relatively high in amylose (28-33%) which requires higher temperatures and longer heating times to gelatinise and shows higher propensity to retrogradation (Sandhu and Lim, 2008). It was suggested that there is a positive correlation between amylose and resistant starch content (Sandhu and Lim, 2008). An *in vitro* study showed that legume starch digestibility increased to 91% by heating at 121ºC (Rehman and Shah, 2005), suggesting that heating to high temperatures (e.g. canning) increases the availability of legume starch to amylase degradation, and therefore will reduce the amount of resistant starch residual in the fibre fractions. Preliminary results suggest that starch is around 10 to 20% more accessible to hydrolysis in canned butter beans and chickpeas compared to boiled samples (data not shown).

It was demonstrated in a previous study that exposure to high temperatures led to a breakdown of pectic substances (Anderson and Clydesdale, 1980), which may partly explain the minor non-significant differences in NSP values between boiled and canned legumes.

On the other hand, canning did not significantly change the proportion of IDF to SDF compared to boiled legumes. IDF was consistently around 60-80% of TDF values, suggesting that canning affects both fibre subgroups. IDF is insoluble in buffer, and is thought to consist mainly of cellulosic and hemicellulosic cell wall polysaccharides, lignin, resistant starch (Saura-Calixto et al., 2000). It is likely that canning affects resistant starch, making it available for amylase digestion. Hemicellulosic polysaccharides may become soluble and recovered in the SDF fraction. Other components of IDF are likely to be unaffected. Meanwhile, SDF which is soluble in buffer and thought to consist mainly of pectic polysaccharides and soluble hemicelluloses. As mentioned earlier, canning may lead to the breakdown or solubilisation of pectic polysaccharides (Kutos et al., 2003).

A ratio of 1:1.43 was obtained for the legume group, which is slightly higher than the published ratio of 1:1.33 for ten major food groups (Lunn and Buttriss, 2007). This ratio could be used to calculate TDF values from NSP values, providing an opportunity to estimate TDF intake and use the values to compare cohort studies in populations with high legume consumption. Moreover, the ratio for boiled legumes was dramatically higher than the ratio for canned legumes. Therefore, caution must be taken when applying the ratio without knowledge of the types of legume (boiled/canned) consumed. Characteristics of the studied population should be evaluated before considering the NSP: TDF ratio. For example, boiled legume ratio may be more suitable for studies which focus on minority ethnic group in UK, where boiled legumes are mostly consumed, compared to the rest of the UK general population which is more likely to consume canned legumes (Schneider, 2002). More research on the NSP: TDF ratio derived from a wide range of food items needs to be undertaken to understand the association between TDF and NSP more clearly. Furthermore, structural and functional characterisation of undigested TDF components is needed to explain the physiological effects of legume fibre.

6. Conclusion

This is the first report of AOAC-fibre data for legumes commonly consumed in the UK. Fibre values are affected by the processing and analytical method used.

Acknowledgements

The authors would like to thank the principle investigators of the UK Women's Cohort Study who provided us with the UKWCS data. This work was funded by a PhD scholarship from the Ministry of Health, State of Kuwait to MA. Authors responsibilities were as follows; MA preformed laboratory analyses, analyzed the data; MA, CO and VB wrote the manuscript; CO and VB were project leaders. There are no conflicts of interest to report.

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Figure Caption

Fig.1. Constituents of Total dietary fibre measured by the Association of Organic Analytical Chemists (AOAC) method and non-starch polysaccharides (NSP) measured by Englyst method (adapted from (British.Nutrition.Foundation, 1990).

Table 1. Mean and standard deviation (SD) of total dietary fibre (TDF), non-starch polysaccharides (NSP), insoluble dietary fibre (IDF) and soluble dietary fibre (SDF) for cooked and canned legumes (g/100g w/w) and their ratio. Values are the mean of triplicate analyses from pooled samples (n >3).

Legumes	TDF g/100g	NSP* g/100 g	IDF g/100g	SDF g/100g	IDF%: SDF%
			Boiled legumes		
Red kidney beans	11.22(0.14)	6.70	8.89(0.67)	2.34(0.70)	79 : 21
Butter beans	8.42(0.35)	5.20	6.96(0.48)	1.46(0.68)	83 : 17
Yellow chickpeas	9.19(0.46)	4.30	5.45(0.55)	3.74(0.67)	59 : 41
Green beans	3.66(0.05)	4.10	2.65(0.30)	1.00(0.31)	73 : 27
Green peas	5.92(0.16)	5.10	4.57(0.51)	1.35(0.61)	77 : 23
Red lentil	9.23(0.21)	1.90	8.17(0.03)	1.06(0.23)	89 : 11
Green brown lentil	5.24(0.11)	3.80	4.88(0.26)	0.35(0.14)	93 : 7
Mung beans	4.43(0.07)	3.00	3.64(0.57)	0.79(0.56)	82 : 18
			Canned legumes		
Red kidney beans	5.49(0.44)	6.20	3.84(0.73)	1.65(0.36)	70 : 30
Butter beans	4.48(0.14)	4.60	3.49(0.28)	0.98(0.14)	78 : 22
Yellow chickpeas	7.41(0.34)	4.10	6.42(0.15)	0.99(0.23)	87 : 13
Green beans	2.72(0.07)	2.60	1.96(0.36)	0.76(0.30)	72 : 28
Green peas	5.19(0.13)	5.10	4.27(0.22)	0.92(0.27)	82 : 18
Baked beans in tomato sauce	5.96(0.17)	3.70	3.34(0.60)	2.61(0.43)	56 : 44
Mean for all legumes	6.33	4.31	4.9	1.43	77:23

*NSP values are from McCance and Widdowson's The Composition of Foods (2002)(Food.Standard.Agency, 2002)

Table 2. Means of measured total dietary fibre (TDF) and non-starch polysaccharides (NSP) for canned and cooked legumes (g/100g) and their ratio with a percentage of the mean difference.

Variables (mean g/100g)	Boiled legumes	Canned legumes	Average legumes
AOAC-fibre	7.14	5.21	6.18
NSP-fibre	4.26	4.38	4.32
Mean difference	2.88	0.83	1.86
AOAC:NSP ratio	1.68	1.19	1.43
% difference	67.6	18.9	43.3

Appendix A. List of dried legumes purchased from local supermarkets

Legumes name	N°	Brands	NSP* g/100g	Code*
Dried legumes				
Yellow chickpeas	1	Sainsbury chickpeas dried		
	2	Tesco chickpeas dried	4.3	13-077
	3	Waitrose chickpeas dried		
	4	Chanadal chickpeas dried		
Red kidney beans	1	Morrison's whole food red kidney beans		
	2	Great scot red kidney beans	6.7	13-110
	3	Natco red kidney beans		
Mung beans	1	Moong whole heeva		
	2	Natco mung beans	3.0	13-097
	3	Tesco mung beans		
Red lentil	1	East End red lentil		
	2	Indus red lentil	1.9	13-092
	3	Tesco red lentil		
	4	Great Scot red lentil		
Butter beans	1	Whitworths butter beans		
	2	Whole food butter beans	5.2	13-071
	3	Great Scot butter beans		
Green brown lentil	1	East End Green lentil		
	2	Brown lentil Heera	3.8	13-090
	3	Waitrose green lentil		
Green peas frozen	1	Morrison green peas		
	2	Sainsbury's basic British garden peas		
	3	Bird's Eye field fresh garden peas	5.1	13-134
	4	British garden peas by Sainsbury's		
	5	Cooperative farm British garden peas		
Green beans frozen	1	Tesco sliced green beans	4.1	13-084
	2	Sainsbury's very fine whole green beans		
	3	ASDA sliced green beans		

* non-starch polysaccharides (NSP) from McCance and Widdowson's (FSA 2002)

Appendix B. List of canned legumes purchased from local supermarkets

Legumes name	N°	Brands	NSP*g/100g	Code*
		Canned legumes		
Baked beans in tomato sauce	1	Sainsbury's baked beans	3.7	13-044
	2	Heinz baked beans		
	3	Tesco light baked beans		
	4	ASDA Baked Beans in tomato sauce		
	5	Organic baked beans		
Yellow chickpeas	1	Sainsbury's chickpeas	4.1	13-078
	2	Tesco chickpeas		
	3	Waitrose chickpeas		
	4	Morrison chickpeas		
	5	Morrison organic chickpeas		
Red kidney beans	1	Tesco red kidney beans	6.2	13-111
	2	Waitrose red kidney beans		
	3	Tesco whole food red kidney beans		
	4	Morrison red kidney beans		
	5	Sainsbury's red kidney beans		
	6	Organic Tesco red kidney beans		
Butter beans	1	Morrison butter beans	4.6	13-72
	2	Essential Waitrose butter beans		
	3	Sainsbury's butter beans		
Green peas	1	Sainsbury's green peas in water	5.1	13-135
	2	Co-operative green peas		
	3	ASDA green peas		
	4	Daucy garden peas		
	5	Morrison green peas		
	6	Tesco garden peas		
Green beans	1	Bandwelle green beans in water	2.6	13-85
	2	Sainsbury's whole French green beans		
	3	Morrison cut green beans		
	4	Morrison whole green beans		
	5	Tesco whole green beans		
	6	Batchelor's cut green beans		

* non-starch polysaccharides (NSP) from McCance and Widdowson's (FSA 2002)