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Safety and wholesomeness of oats for coeliac people - analytical aspects

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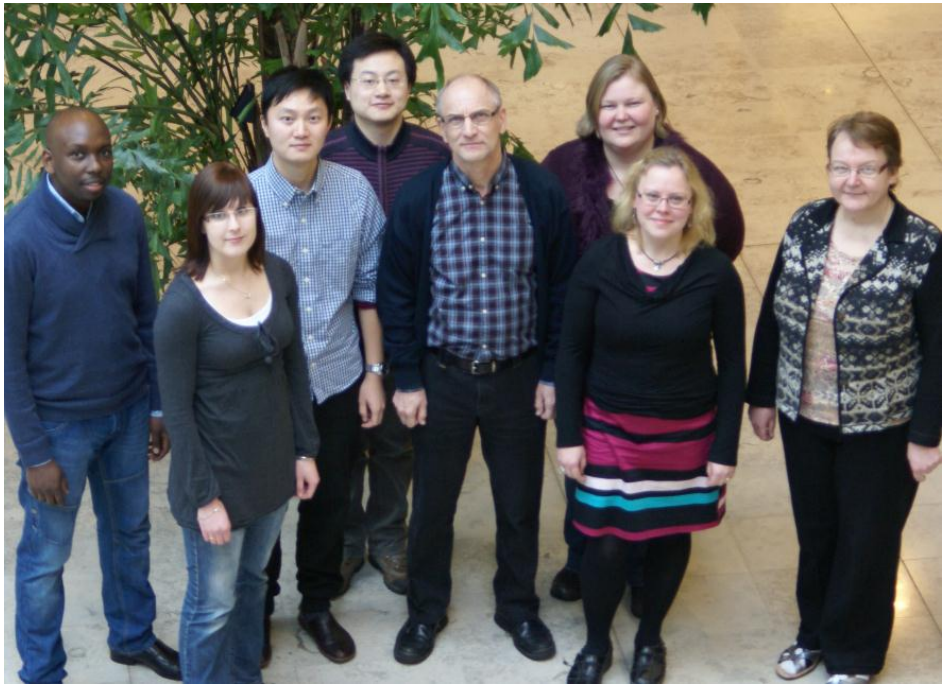


Safety and wholesomeness of oats for coeliac people - analytical aspects

The message :

Wholesomeness of oats is not fully exploited by the coeliac people

Contents:

- ***Wholesomeness, health claims***
- ***GF-regulation of oats***
- ***Analysis***
- ***Safety***

These will be discussed on the basis of the experience on oats that we have in Finland

Oat flakes and oat bran – basic composition

	Oat flakes/ Whole grain oat flour	Oat bran
Protein	12-17	70-80
Starch and sugars	56-62	10-50
Fat	4-9	5-14
Total dietary fibre	11-17	15-32
Beta-glucan	4-7	6-20

Benefits of oats in (gluten-free) diet

Oats provide increased

- variety
- palatability, and
- specific beneficial effects – 2 EFSA health claims ! (cf. rice, maize, etc.)

Oats are a good source of

- Dietary fibre (β-glucan) - cf EU health claim*
- B-complex vitamins (thiamin, niacin and riboflavin)
- Vitamin E and antioxidants (tocols)
- Good quality protein

'It is not ethical to prohibit oats in GFD if not shown harmful'

EFSA Journal 2009; 7(9):1254 (October **2009**)

Beta-glucans related health claims

SCIENTIFIC OPINION:

'On the basis of the data available, the Panel concludes that a cause and effect relationship has been established between the consumption of beta-glucans and the reduction of blood cholesterol concentrations. The following wording reflects the scientific evidence: "Regular consumption of beta-glucans contributes to maintenance of normal blood cholesterol concentrations". In order to bear the claim, foods should provide at least 3 g/d of beta-glucans from oats, oat bran, barley, barley bran, or from mixtures of non-processed or minimally processed beta-glucans in one or more servings. The target population is adults with normal or mildly elevated blood cholesterol concentrations. '

**EFSA 2011:*
'Oat beta-glucan reduces the cholesterol level in the blood. The lowering of the blood cholesterol level can reduce the risk of coronary heart disease'

http://www.efsa.europa.eu/en/efsajournal/doc/2207.pdf

EFSA Journal **2011**;9(6):2207

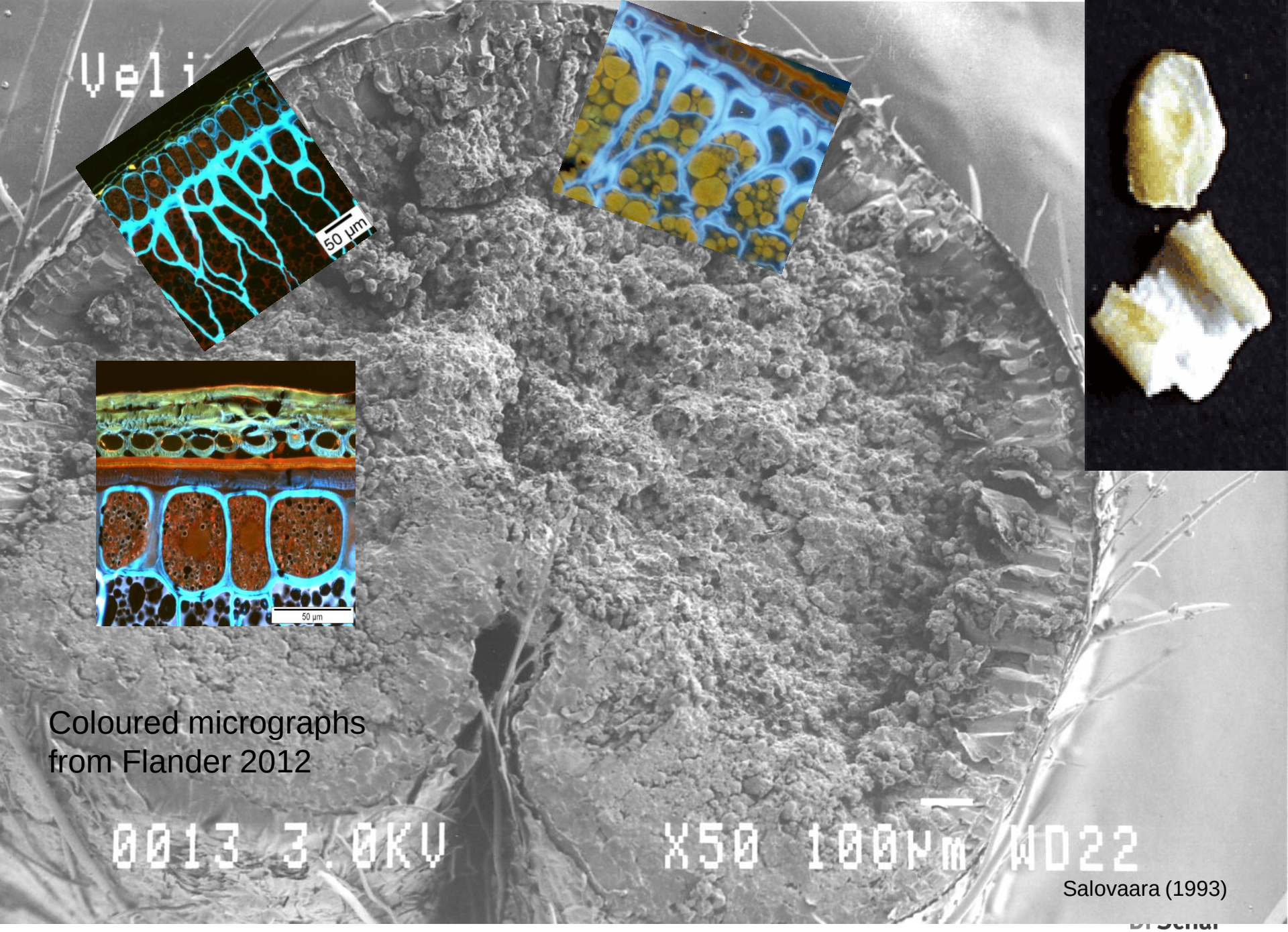
SCIENTIFIC OPINION

The following wording reflects the scientific evidence: “Consumption of beta-glucans from oats or barley contributes to the **reduction of the glucose rise after a meal**”.

In order to obtain the claimed effect, **4 g of beta-glucans** from oats or barley for each 30 g of available carbohydrates should be consumed per meal.

The target population is individuals who wish to reduce their post-prandial glycaemic responses.

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)
European Food Safety Authority (EFSA), Parma, Italy



Coloured micrographs
from Flander 2012

Salovaara (1993)

Background and history of oats in gluten-free diet

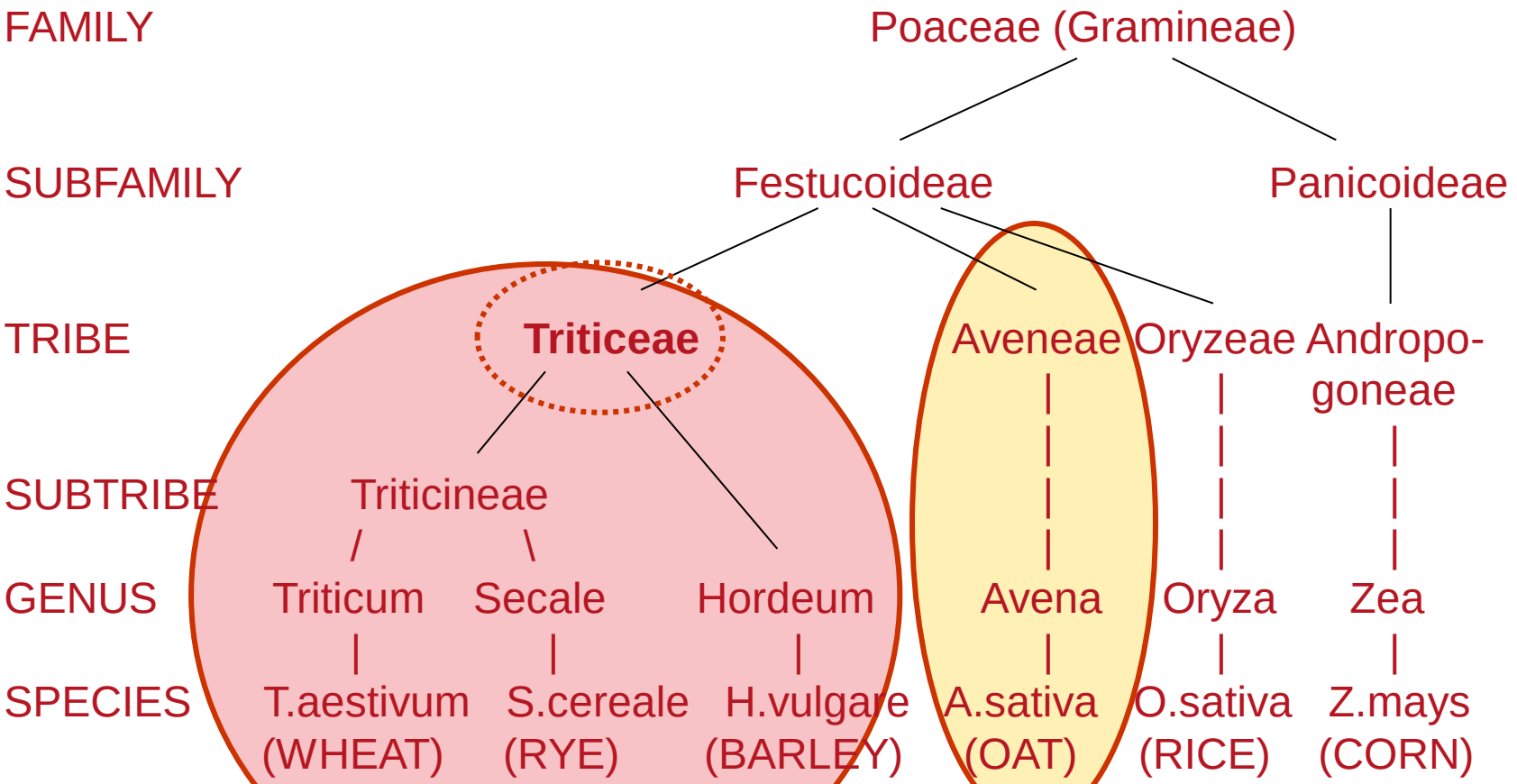
Oats are different in

- Phylogenetics (taxonomy)
- Protein composition

- The uncertainty on the suitability of oats since 1950's was reflected in regulation
- However, since 1995 clinical studies internationally show: oats not harmful

  (least 13 clinical studies 1995-2007)

Oats are phylogenetically different from Triticeae cereal grains



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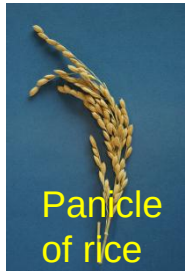


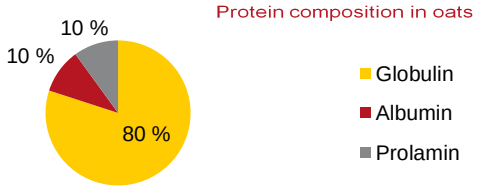
Oats are different in their protein and prolamin composition

TRIBUS	**Triticeae**			Aveneae	Oryzeae	Andropo-goneae
GENUS	Triticum	Secale	Hordeum	Avena	Oryza	Zea
SPECIES	T.aestivum WHEAT	S.cereale RYE	H.vulgare BARLEY	A.sativa OAT	O.sativa RICE	Z.mays CORN
% PROLAMIN	**70-80[1]**	**30-50[2]**	**30-50[3]**	**5-15[4]** (~10)	3-5	60
% Q	33	6(30)	3(30)	34	20	19
% P	17	17	20	10	5	10

1) Gliadin (and glutenin), 2) Secalin, 3) Hordein, 4) Avenin

Q = glutamine, P = proline

The Triticeae prolamins!



Data from Kasarda (1997) and Shewry & Tatham (1999)

Oat protein fractions according to Osborne protein solubility[1]

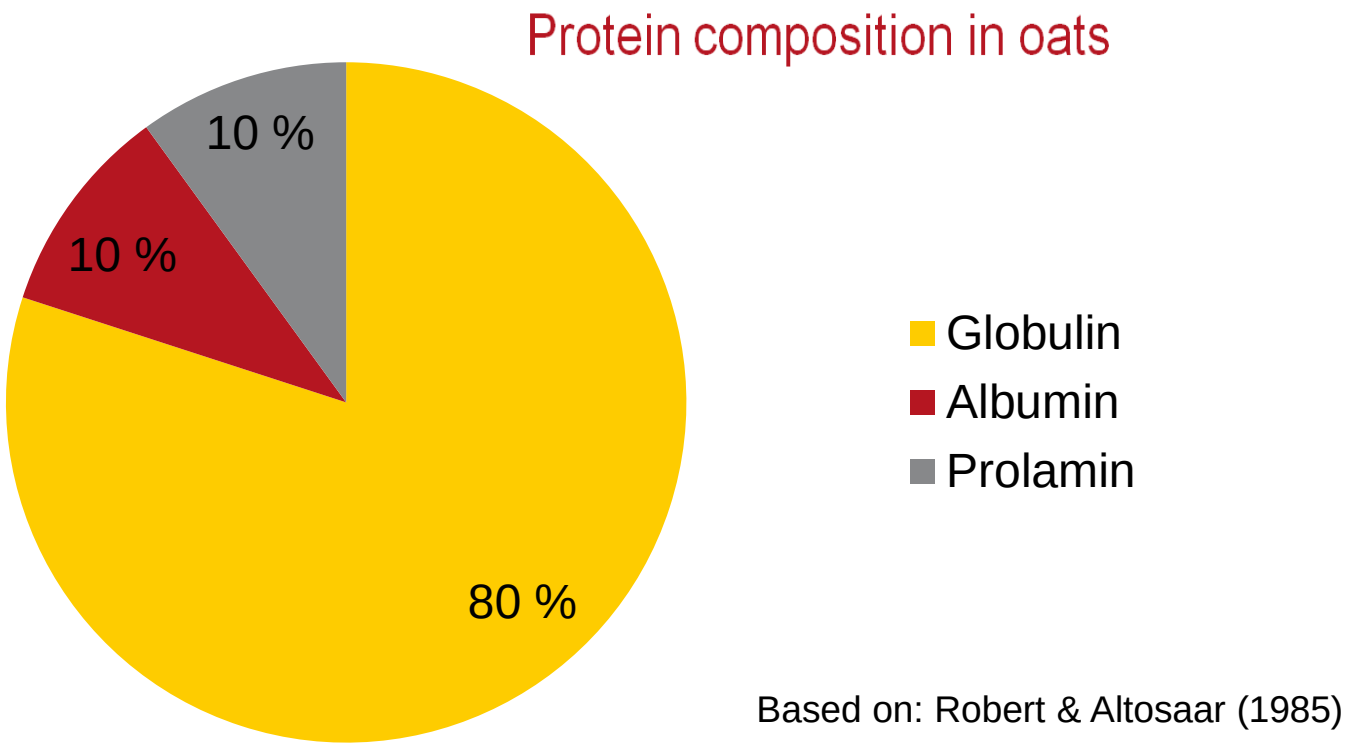
Osborne fraction	MW (kDa)	% of Total Protein*	Solubility
Globulins	53-58	70-80	Soluble in dilute aqueous salt solutions
Albumins (mostly enzymes)	Most of 15 kDa, also of ~36 and 22 kDa	9-20	Water soluble
Prolamins (avenin)	23.5 and 15.5 kDa (also some of 36 kDa)	4-14	Soluble in aqueous ethanol
Glutelins (residue)	9	<10	Soluble in dilute acids or alkalis

[1]Table remodelled from references Klose & Arendt 2011, Ma & Harwalkar 1984, Robert 1985
*shows wide differences depending on the cultivar and growth conditions (climate, soil quality etc.)

Globulins are the major proteins in oats

- only 10% prolamins (glutelins) (avenins)

- the amino acid sequence is different from Triticeae prolamins



Based on: Robert & Altosaar (1985)

Don Kasarda in 1996 at 7th ISCD in Tampere:

The prolamins in wheat, rye barley and oats

a. Active α-gliadin peptide (in vivo) **L-G-Q-Q-Q-P-F-P-P-Q-Q-P-Y-P-Q-P-Q-P-F**

b. Active α-gliadin peptide (in vivo) **L-G-Q-Q-Q-P-F-P-P-Q-Q-P-Y**

c. Active α-gliadin peptide (in vivo) **P-Q-P-Q-P-F-P-S-Q-Q-P-Y**

d. **Q-I-Q-V-F-P-S-G-Q-V-Q-W-P-Q-Q-Q-Q-P-F-P-** Act. γ-gli. pep. (in vitro)

Sequence found in wheat, rye, barley **-Q-Q-Q-P-F-P-**

Seq. found in wheat, rye, barley, oats **-Q-Q-Q-P-F-**

Seq. found in rice, maize, and many other proteins **-Q-Q-Q-P-**

Q = glutamine **F** = phenylalanine **P** = proline

Cf R5 antibody recognises QQPFP

Figure 6. Amino acid sequences of peptides tested for activity in coeliac disease and selected sequences from wheat, rye, barley, and oat prolamins. Sequence a is from Sturgess et al. (19). Sequences b and c are from Marsh et al. (21). Sequence d is from Fluge et al. (18). Potential core sequences are underlined in sequences a, b, c, and d.

(Kasarda 1997, in *Coeliac Disease*)

R5 antibody recognizes the pentapeptide QQPFP*

This is a repetitive sequence in gliadins, hordeins, and secalins, but not in avenins.

MKTFLIFVLLAMAMKIATAARELNPSNKELQSPQQSFSYQ**QQPFP**QQPYPQQPYP
SQQPYPS**QQPFP**TPQQQFPEQSQQPFTQPQQPTPIQP**QQPFP**QQPQQP**QQPFP**
QP**QQPFP**WQP**QQPFP**QTQQSFPLQP**QQPFP**QQP**QQPFP**QPQLPFPQQSEQIIP
QQL**QQPFP**LQP**QQPFP**QQP**QQPFP**QPQQPIPVQPQQSFPQQSQQSQQPFAQP
QQLFPELQQPIPQQP**QQPFP**LQP**QQPFP**QQP**QQPFP**QQPQQSFPQQPQQPYPQ
QQPYGSSLTSIGGQ

*QQPFP =glutamine-glutamine-proline-phenylalanine-proline

Avenin is very different by its sequence compared to wheat gliadin.

- However, oat avenins also contain a sequence relatively high in proline
  - a potential immuno-stimulatory sequence?
  - happens to be found by the antibody?
  - needs not to be an indication of toxicity?

avenin

```
  1 MKTFLIIALL AMAVATATAT TTVQYNPSEQ YQPYPEQQEP FVQQQQPFVQ QQQPFVQQQQ
 61 MFLQPLLQQQ LNPCKQFLVQ QCSPVAAVPF LRSQILRQAI CQVTRQQCCR QLAQIPEQLR
121 CPAIHSVVQS IILQQQQQQQ QFIQPQLQQQ VFQPQLQLQQ QVFQPQLQQQ VFQPQLQQVF
181 NQPQMQGQIE GMRAFALQAL PAMCDVYVPP QCPVATAPLG GF
```

α-gliadin

```
  1 MVRVPVPQLQ PQNPSQQQPQ EQVPLVQQQQ FPGQQQPFPP QQPYPQPQPF PSQQPYLQLQ
 61 PFPQPQLPYP QPQLPYPQPQ LPYPQPQPFR PQQPYPQSQP QYSQPQQPIS QQQQQQQQQQ
121 QQKQQQQQQQ QILQQILQQQ LIPCRDVVLQ QHSIAYGSSQ VLQQSTYQLV QQLCCQQLWQ
181 IPEQSRCQAI HNVVHAIILH QQQQQQQQQQ QQPLSQVSFQ QPQQQYPSGQ GSFQPSQQNP
241 QAQGSVQPQQ LPQFEEIRNL ALETLPAMCN VYIPPYCTIA PVGIFGTNYR
```

Figure 5.
Amino Acid Sequence of an Avenin (gi 82331, JQ1047) and an α-Gliadin (α2-Gliadin, AJ133612)

Arentz-Hansen H, Fleckenstein B, Molberg Ø, Scott H, et al. (2004) The Molecular Basis for Oat Intolerance in Patients with Celiac Disease. PLoS Med 1(1): e1. doi:10.1371/journal.pmed.0010001

PLOS | MEDICINE

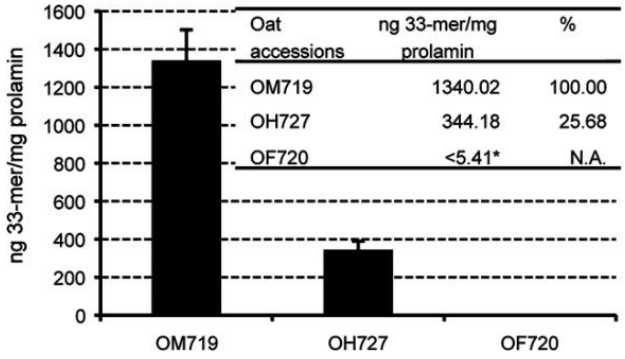
The oats issue: a question was raised: Could there be a risk in some oat varieties?

- Silano et al. (2006) *in vitro* studies suggested: some oat varieties may be toxic

- Comino et al. (2011) using G12 antibody ('33mer'):
  - Variation in cultivars: high – medium – 'zero'
  - G12 finds differences in varieties – tool for selection
  - (but at least 40-400 x less reactive than gliadin!)

- Mujico et al. (2011) – γ-gliadin reactive T-cell lines:
  - Reactive avenin epitopes in oat lines but not in all
  - Oat varieties can be selected

Detection of the concentration of the 33-mer peptide in different oat varieties.



Oat accessions	ng 33-mer/mg prolamin	%
OM719	1340.02	100.00
OH727	344.18	25.68
OF720	<5.41*	N.A.

Comino I et al. Gut 2011;60:915-922

Safety to be proven in clinical tests

Status of oat products in GF diet. Historical milestones

Year	Description
1950	Dicke finds wheat to cause CD, and later (1953) suggests oats harmful also
1953-1974	Conflicting results from very few studies with oats 1953 – 1974
1974	First study with oats applying biopsy. Result: oats not harmful
1976	Codex Alim . standard 118-1981 for 'gluten-free foods', amended in 1983, includes oats among cereals containing gluten (wheat. rye, barley, [oats]) (Oats in brackets to show uncertainty)
1976-1995	No clinical studies found on oats and CD in literature
1995	First Kuopio oat challenge study: : oats not harmful (Janatuinen et al. 1995), followed by others, also elsewhere, also long-term: oats not harmful
1997, 1998 2000	Oats endorsed by the Scientific Advisory Board of the Finnish Coeliac Society for adult CDPs in 1997, for adult DHPs in 1998, and for children with CD in 2000
2000	First pure oat products enter the market

Status of oat products in GF diet. Historical milestones. Analysis.

Oat beta-glucan health claims

Year	Description
2000-2003	Mendez et al develop the new R5 ELISA method gluten analysis that detects prolamins of wheat, rye and barley, but not oat avenins
2006	The R5 method is reported to greatly exaggerate barley contaminants in oats (Kanerva et al. 2006)
2006, 2008	Codex standard 118-1979 endorsed the R5 ELISA method for GF analysis
2009	New EU Commission regulation (41/2009); oat accepted only if 'pure oats' and if below 20 mg/kg; earlier national regulations to cease by 2012
2009, 2011	EFSA approves oat beta-glucan health claims on 1) cholesterol maintenance (2009) and 2) on low glycaemic response (2011)
2013 NOW!	Only relatively few oat-based GF foods fulfilling the <20 mg/kg requirement are on the market. Price of 'pure oatmeal' is 4-6 times that of regular oatmeal.

The European Commission Regulation EC 41/2009

- declared oats suitable for people intolerant to gluten

However, particular strict special requirements were applied

- Oats must meet the 20 mg/kg requirement
- Oats must be specially produced, prepared and/or processed in a way to avoid contamination (**= ’pure oats**’)
(Art. 3:3)

Note: similar requirements are not applied to any other non-Triticeae cereal or crop

Safety and wholesomeness of oats for coeliac people - analytical aspects

The pure oats issue

The problem: Barley contamination in oats and the oversensitivity of the ELISA R5 analysis to barley

Barley contamination in oats?

- *Oats and barley often grown on the same farm*

- *Pure oats can be grown only on specialized farms growing no other cereal*

- *0-tolerance to barley because of specific analytical issues*

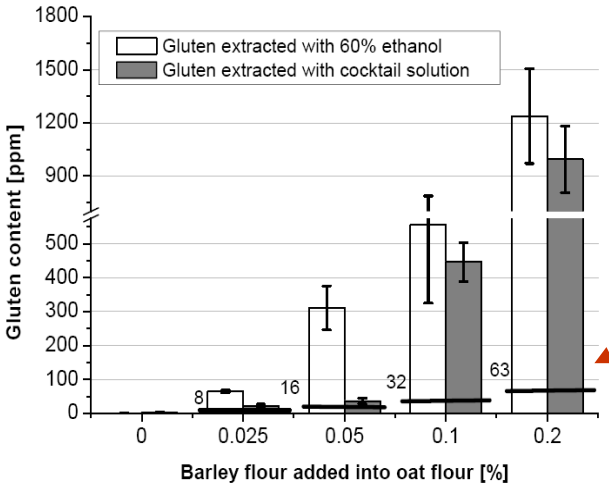
The **R5 ELISA** is fine in most situations.

However, the R5 antibody overreacts with barley.
→ Barley contamination in oats heavily overestimated.

- Oat samples were deliberately contaminated with barley flour
- Analyzed with the R5 ELISA method

- Failed to show contamination right
- Overestimated barley contamination
  15x (7-30 fold)
- With a hordein standard it worked fine

Note:
The problem with the older ω-gliadin ELISA method acc. to Skerritt was underestimation of barley



Gluten contents of barley-contaminated oat samples as detected by the method based on the R5 antibody. The dark lines show the level of hordein added to the samples.

(Kanerva et al. 2006)

The reason for the overreactivity of the R5 method with barley is in barley C hordein amino acid sequence

C hordein consists mainly of repeating units of eight amino acids PQQPFPQQ (Tatham and Shewry 2012).

The PQQPFPQQ contains the QQPFP

For barley there is 0-tolerance in gluten-free oats!

- A barley standard is needed (where barley is the expected source of contamination)

There are gluten-free pure oat products on the market – but at a high price

Price of gluten-free pure oatmeal (flakes) vs regular is - 4 to 6 times higher

Examples:

UK: Sainsbury's freefrom Pure Oats (7.80 €/kg) vs

(1.40 €/100g) → 6x

Sweden: Pris:22,00 kr 500 g Semper 5.06 €/kg

Havregryn 1,5kg AXA 1.68 €/kg → 4.5x

Finland: Elovena 1.40 € /kg, Provena 5.88 €/kg (Raisio) → 4.2x

Puuppolan Aitokaura 5.41 €/kg → 3.9x

Perhaps interesting to note that

*much of the **evidence*** of gluten-free diet comes from the l5-yr Kuopio oat challenge , which was made*

with **regular oatmeal** (Elovena Oat flakes) meeting the max 200 mg/kg gluten content requirement

(as determined with the Skerritt ω-gliadin ELISA of the time)

*(Janatuinen et al. 1995, 2002)

Now we have **pure oat** products of high price on the market. However, the volumes are low and the assortment is limited.

→ The coeliac people do not benefit from the wholesomeness of oats.

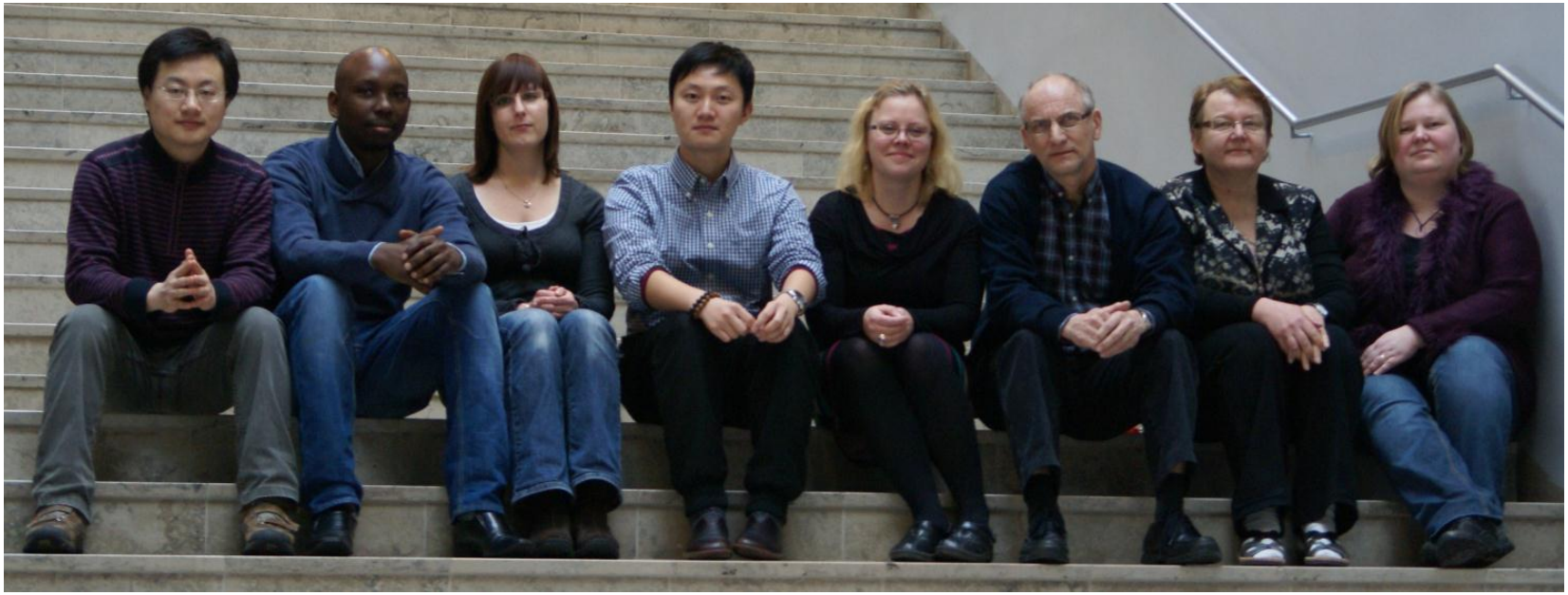
What kind of ’gluten-free diet policy’ should be followed?

- Compliance to the GF diet is the key factor → Support compliance!
    - Compliance is supported by attractive products available
        - → Wheat starch !
        - → Oat products !
- Poor compliance is more harmful than minor contamination
- It appears that coeliac people in Finland are doing better than CDPs in many other countries that apply more strict rules

Conclusions based on the Finnish experience:

- Oat products have been used by CDPs for 15 years
- Today 86% of CDPs are using oat products;
  amounts maybe small
- A massive oat challenge, almost 20 000 CDP's ongoing
- No adverse clinical effects reported
- No harmful oat varieties known

Thank you



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