A Sandwich ELISA for the Detection of Oat Protein in Foods Including Other Gluten-Containing Cereals

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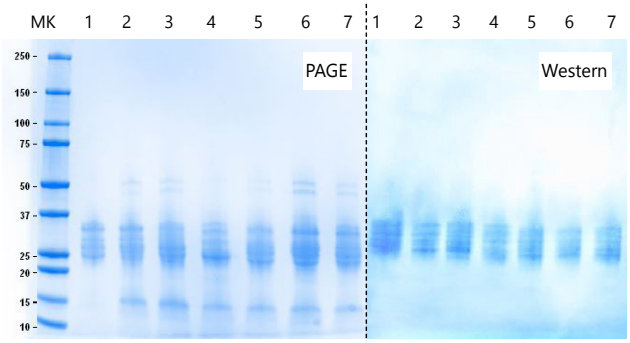
INTRODUCTION

The role of oat consumption in coeliac disease (CD) is unclear. Many CD sufferers can tolerate pure oats while in others it can trigger harmful effects. Many jurisdictions, including Australia and New Zealand, consider oats to be a gluten-containing cereal and do not allow gluten-free claims on any products including oats[1]. Most of the ELISA tests used to detect gluten have little or no activity against oats. Hence, a specific test for oats is necessary to substantiate gluten-free claims. In addition, a specific allergy to oats (separate to coeliac disease) has been described[2] thus creating a further need for oat testing.

Anti-Avenin Antibody

A polyclonal antibody was raised against oat avenin. A range of oat-containing products were extracted using 40 % ethanol and screened using PAGE and western blotting. The western blotting results show that a range of avenin proteins in the molecular weight range of 20-35 KDa are detected by the antibody. Avenins comprise the majority of the oat protein extracted using 40 % ethanol. Similar profiles were seen in oat flour and rolled oat samples and there was no obvious difference between American and Australian oat samples.

This antibody was used as the capture antibody and was conjugated with horseradish peroxidase to be used in the ELISA Systems ESOAT-48 sandwich ELISA.



Samples: (1) Purified oat avenin; (2) Gluten-free oat flour (USA); (3) Rolled oats (USA); (4) Oat flour (Aust); (5) Oat bran (Aust); (6) Fine powdered oats (Aust); (7) Quick oats (Aust)

The ESOAT-48 Assay

The ESOAT-48 Assay is calibrated in the range of 2.5 – 25 ppm oat protein. It has a Lower Limit of Application of 1.25 ppm Oat Protein.

The calibration of the ESOAT-48 assay was compared with the Kjeldahl nitrogen using 10 commercial oat flour samples. The average oat protein concentration as measured by the ESOAT-48 kit was 13.1 % (range 6.3 – 21.5 % w/w) while the average oat protein concentration as measured by Kjeldahl was 13.1 % (range 8.2 – 16.2 % w/w). This shows that while there is variability in both the oat protein and avenin levels in these samples, both methods measure in a similar range. The variability in the oat protein and the avenin levels could be based on a range of factors including cultivar and seasonality[3].

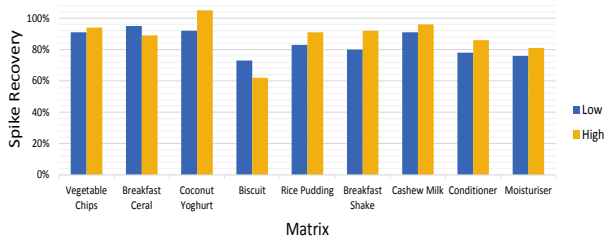
Cross-Reactivity

A panel of 68 foods were tested in the ESOAT-48 assay screening for false positive results. No false positive results were detected in any of the foods tested. Of particular note is that there were no false positive results recorded in seven gluten-containing food samples.

Gluten Cereals	Wheat	**Seeds**	Celery Seed	**Vegetables & Herbs**	Chilli Powder
	Rye		Sunflower Seed		Split Peas
	Barley		Poppy Seed		Date
	Couscous		Pumpkin Seed		Basil
	Spelt Flour		Mustard		Rosemary
	Burghul		Linseed		Peppercorn (black)
	Freekeh		Sesame Seed		Apricot (dried)
Non-Gluten Cereals	Cornflour		Cumin Seed	**Legumes & Pulses**	Lentil (flour)
	Polenta		Coriander Seed		Lima Bean
	Millet Flour		Cocoa		Pinto Bean
	Amaranth		Lupin Seed		Soy (bean, flour, milk)
	Teff Flour	**Nuts**	Macadamia		Black Turtle Bean
	Buckwheat		Cashew		Red Kidney Bean
	Rice (brown and white)		Hazelnut (roasted)		Adzuki Bean
	Corn		Brazil Nut		Mung Bean
	Sorghum Flour		Pine Nut		Peanut
	Tapioca		Pistachio	**Other**	Wine (red and white)
	Chia Flour		Pecan		Egg
	Chickpea Flour		Almond		Skim Milk Powder
	Quinoa Flour		Walnut		Balsamic Vinegar
	Hemp Flour		Coconut		Honey

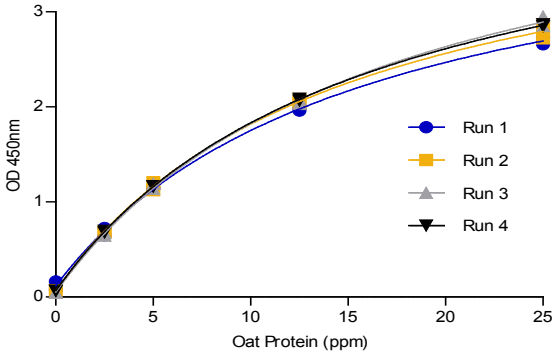
Spike Recovery

A range of food and cosmetic products were spiked with an ethanolic oat avenin extract. Samples were spiked with a low (5.6 ppm) or a high (15.2-15.9 ppm) level of oat protein and then assayed using the ESOAT-48 assay. The spike recovery was satisfactory for all of the matrices tested showing that the assay is suitable to measure a wide range of food and cosmetic samples.

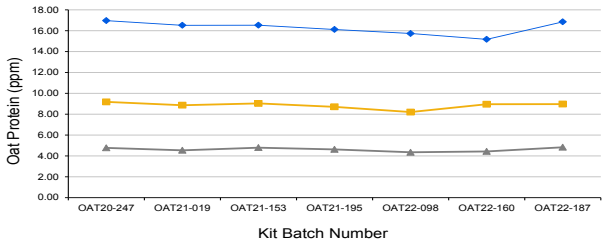


Variability and Reproducibility

The oat standard curve was run in duplicate in four independent assays. The four standard curves are shown in the graph below. As can be seen in the graph below the standard curve varied minimally between assays (RSD values for each of the positive standards < 4.0 %). When the standards were compared in a single assay similar results were obtained (RSD values for the positive standards < 6.0 %, data not shown).



To determine lot-to-lot variation, three oat-containing samples were run as check samples. The three are seen to be consistent across seven production batches with RSD calculated to be below 5.0 %, thus ensuring reproducible results.



SUMMARY

The ELISA Systems Oat Protein (ESOAT-48) assay can be used to detect oat protein (avenin) in foods and cosmetics. It does not detect other gluten containing cereals such as wheat, barley and rye. The ESOAT-48 assay is a sensitive (Lower Limit of Application 1.25 ppm) and specific assay for the detection of oat protein. It is a robust and reliable assay as evidenced by the low assay-to-assay and lot-to-lot variability. It is suitable for the measurement of oat protein in a wide variety of food and cosmetic samples.

REFERENCES

1. Food Standards Australia New Zealand. Food Standards Code: Schedule 4 (Nutrition, health and related claims)
2. Boussault, P., Léaute-Labrèze, C., Saubasse, E., Maurice-Tison, S., Perromat, M., Roul, S., Sarrat, A., Taieb, A., and Boralevi, F. (2007) Allergy 62: 1251-1256. DOI: 10.1111/j.1398-9995.2007
3. Mujico, J., Mitea, C., Gilissen, L., de Ru, A., van Veelen, P., Smulders, M., and Koning, F. (2011) J. Cereal Sci. 54: 8-12. DOI: 10.1016/j.jcs.2010.09.007