

# DETECTION OF CELERY (*Apium graveolens*) IN FOOD

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## Introduction

Celery is a frequent cause of pollen-related allergy, particularly in Germany, Switzerland and France. About 30 to 40% of patients with food allergy in these countries are sensitized to celery root (celeriac) and one third of severe food reactions are thought to be due to celery. Allergy to celery root is highly associated with birch and mugwort pollen allergy.

Celery (*Apium graveolens*) belongs to the *Apiaceae* (earlier *Umbelliferae*) family. Several allergens from celery have been identified and characterized, Api g 1 a 15 kDa protein homologous to Bet v 1 (birch pollen allergen), Api g 4, a 17 kDa profilin, cross-reactive carbohydrate determinants (CCD) of 32-70 kDa and recently a 58 kDa protein. Profilin and CCDs are heat resistant while Api g1 is heat labile.

Celery is commonly consumed as a root (raw or cooked), sticks, and leaves or as a spice. The dose causing allergic reactions varies from parts of gram up to gram levels. In celery spice, the amount of protein is higher, causing reactions at lower doses.

Celery and products thereof must always be declared according to the European labeling directive (2003/89/EC). The access to reliable methods for the detection of celery is thus needed.



## Limit of detection (LOD)

The absolute detection limit of the PCR system was determined by analysing serial dilutions of celery DNA. Results obtained showed that the absolute LOD was between 2-20 pg, which approximately corresponds to 1-7 haploid celery genome copies.

The relative detection limit was assessed on heated (72°C) reference samples of celery in meat, produced by the Federal Centre for Meat Research, Kulmbach, Germany. The sample containing 0.01% celery was readily detected. By additional mixing of the zero sample with different amounts of the sample with 0.01% celery, even celery levels of 0.001% could be detected.

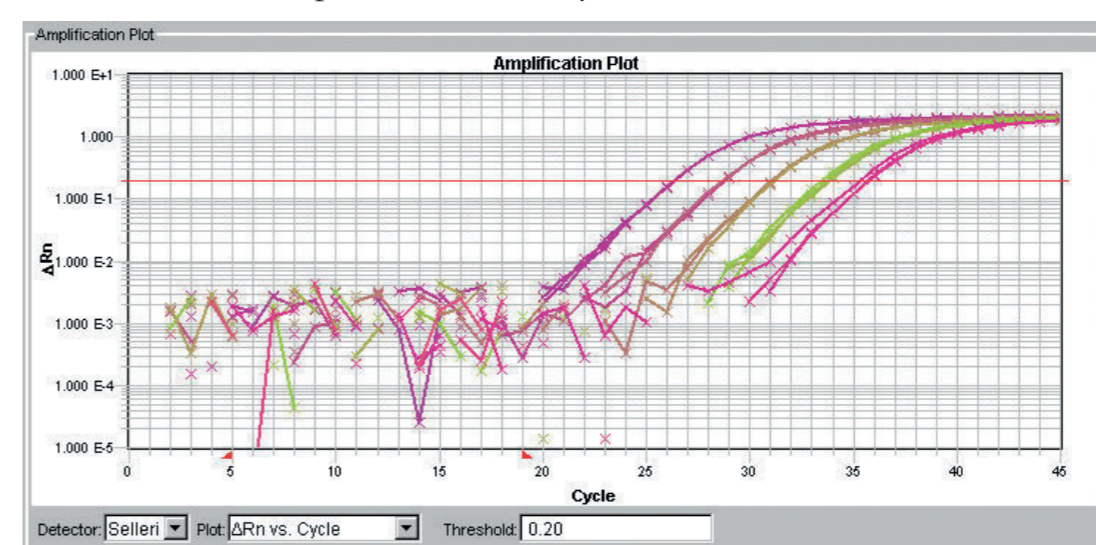
No signal was achieved in any of the samples with the celery ELISA.

Celery in meat (%)	Number of replicates	Number of positive amplifications
1	6	6
0.2	6	6
0.1	6	6
0.05	6	6
0.01	10	10
0.005	4	4
0.0025	4	3
0.0010	4	2
Zero sample	10	0

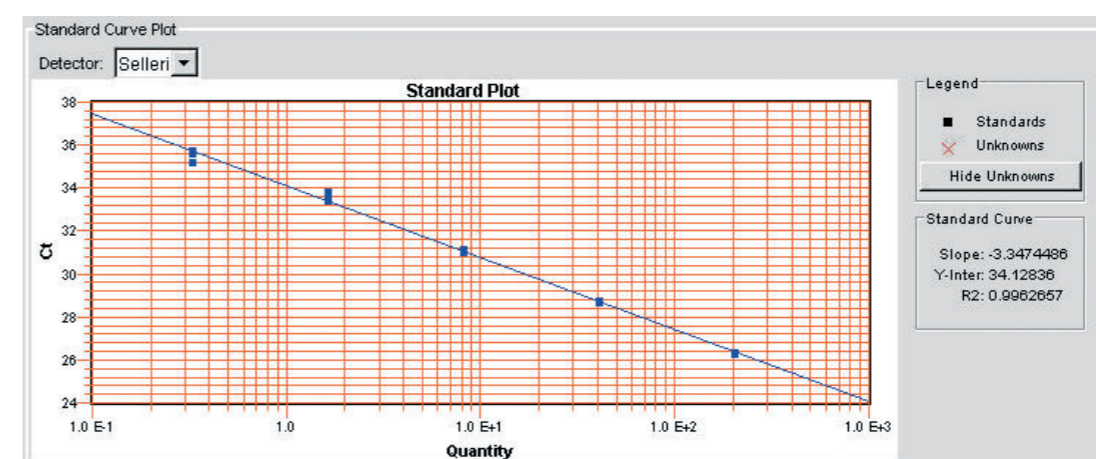
Result of PCR analysis of heated reference meat samples, containing different levels of celery.

## Amplification efficiency

By analysing serial dilutions of celery DNA a standard curve was created. The slope of the standard curve revealed an amplification efficiency of 1.99 which is close to the maximum value of 2.0.



Amplification plot generated by serial dilutions of celery DNA.



Standard curve generated from the amplification data.

## Analysis of food products

Seven food products, all declaring the presence of celery, were analyzed, both with the PCR method and the ELISA method.

In six out of seven products celery was detected with PCR. In one sample 'Sweet and sour sauce' no PCR product was obtained, despite the declaration of celery. Perhaps the amount of celery was below the detection limit or the DNA in the product may have been degraded.

Food product	PCR	ELISA	Other ingredients listed, which cross-reacts in the ELISA
Sauce mix with garlic and herbs	+	+	Parsley
Instant soup with broccoli and leek	+	+	Carrot, parsnip
Herbamare herbal salt	+	+	Parsley
Vegetarian beef	+	+	Carrot, parsnip
Sweet & sour sauce	-	+	Carrot
Meat casserole, baby food	+	+	Potato, carrot
Meat soup with vegetables	+	+	Carrot, cabbage, parsnip

Food products, all declaring celery on the ingredient list, analyzed by PCR and ELISA.

All samples were positive with the celery ELISA. However, in most samples carrot was an important ingredient, which cross-reacts in the antibody assay. Also potato, cabbage, parsley and parsnip cross-reacts with the celery ELISA. The positive result in the immunoassay can thus not be considered specific to celery since other cross-reacting ingredients could be responsible for the signal.

## Conclusion

A specific DNA method for celery was developed, using mannitol dehydrogenase as target sequence. The PCR method was shown to be specific for celery, producing a 113 base pair fragment with three different celery varieties. Negative results were achieved with closely related species.

The limit of detection was 2-20 pg DNA, corresponding to 1-7 haploid genome copies. A detection limit of 0.001% celery i.e. 10 ppm (mg/kg) was determined in heated reference samples of celery in meat. Six out of seven food products declaring celery on the ingredient list were correctly identified.

All samples analyzed with the DNA method were also analyzed with a celery ELISA. None of the heated reference products gave signals in the ELISA while all food products appeared positive. However, the antibodies were not specific to celery. Cross-reactivity to carrot, parsnip, parsley and potato or cabbage might be responsible for the result since all these were also ingredients in the food products.

## Material and methods

Total DNA from plant species of the *Apiaceae* family and from food samples was extracted using a DNeasy™ Tissue Kit from Qiagen, with some minor modifications. PCR was performed on an ABI PRISM 7900HT Real Time PCR instrument and the fluorescence from the TaqMan probe was monitored cycle-by-cycle.

Rabbit celery antibodies were raised against celery root powder (Nat. Vet. Inst., Oslo, Norway). From the immune serum the IgG-fraction was purified on a HiTrap protein A column.

## Results

### Design of the PCR system

A TaqMan Real-time PCR system (113 bp) was designed to the target gene mannitol dehydrogenase (Mtd) from celery using the Primer Express software (Applied Biosystems). No significant similarities to DNA sequences from other species than celery could be noted when the primers and probe were checked with a BLAST search. The concentrations of primers and probe were optimized to get the best reaction conditions.

Target sequence	Primers/probe	Amplicon length
Mtd gene of <i>Apium graveolens</i> (GenBank:AF067082)	AgMD2637F:	AGCCTGTTCCCGTACGAGAT
	AgMD2749R:	CTCATCACACCGTAATCCAACAT
	AgMD2690-Taq:	FAM-TACACGCTCATCGTACTCAGCA-TAMRA
		113 bp

### Specificity and selectivity

The specificities of the detection systems were tested on the most important agricultural plants of the family *Apiaceae*. Only DNA from three different varieties of celery (*Apium graveolens* var. *dulce*, var. *rapaceum*, var. *secalinum*) was amplified. Negative results were achieved with all other closely related species.

PCR on spiked samples demonstrated that lack of amplification in other samples than celery was not due to PCR inhibition.

When DNA from celery was mixed with DNA from other members of the *Apiaceae* family the PCR system could selectively identify celery. No amplification occurred when celery DNA was omitted from the mixture.

Plant species	Scientific name	PCR result	ELISA result
Celery	<i>Apium graveolens</i> var. <i>dulce</i>	+	+
Celeriac	<i>Apium graveolens</i> var. <i>rapaceum</i>	+	+
Leaf celery	<i>Apium graveolens</i> var. <i>secalinum</i>	+	+
Carrot	<i>Daucus carota</i>	-	-
Parsnip	<i>Pastinaca sativa</i>	-	+
Parsley	<i>Petroselinum crispum</i>	-	+
Lovage	<i>Levisticum officinale</i>	-	-
Dill	<i>Anethum graveolens</i>	-	+
Aniseed	<i>Pimpinella anisum</i>	-	+
Fennel	<i>Foeniculum vulgare</i>	-	(+)
Caraway	<i>Carum carvi</i>	-	-
Coriander	<i>Coriandrum sativum</i>	-	-
Chervil	<i>Anthriscus cerefolium</i>	-	-
Sweet cicely	<i>Myrrhis odorata</i>	-	not tested

The specificities of the detection systems tested on the most important foods from the *Apiaceae* family.

Screening with a variety of botanically related as well as non-related plants and seeds using an optimized sandwich ELISA indicated cross-reactivity with carrot, parsnip, parsley, dill and aniseed, all members of the *Apiaceae* family. Cross-reactivity was also seen with cabbage, cooked potato and pine nuts.

Prior to analysis with the sandwich ELISA, all food extracts were diluted 1:20 in assay buffer. This corresponds to 100 percent of a food matrix and a high protein concentration. For some foods showing cross-reactivity, these high amounts may have less significance in analysis of the actual food samples. Adsorption of the serum using tosyl-activated Dynabeads M-280 coupled to carrot and potato did not abolish the cross-reactivity.