

## Deallergisation Trials of Pure Celery Juice and Apple-Celery Juice Mixture by Oxidation

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### Abstract

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This work aimed to determine if it was possible to eliminate or reduce the content of the Api g1 allergen in celery juice by oxidation, utilising its natural polyphenol oxidase (PPO) content. We attempted to determine a possible relationship between the enzymatic browning of celery juice and the reactivity of the Api g1 allergen. Pressed celery juice was stirred, and samples for the colour measurement and allergenicity, determined using the Western Blot (WB) method, were collected at pre-defined times. Oxidation failed to eliminate the allergenicity of pure celery juice. Further trials were focused on celery allergen elimination in apple-celery juices mixtures in ratios of 3:1, 5:1, and 7:1. We selected the 5:1 ratio as the most acceptable from the sensory perspective, and monitored its allergenicity using the WB method, basophil activation test, and skin prick testing. The WB test showed that oxidation, caused by stirring for 120 min, reduced the allergenicity of the mixture. However, the basophil activation test showed no reduction in the allergic response to the oxidised juice mixture. Skin testing showed that the oxidised juice mixture stirred for 120 min exhibited a significantly lower reaction than the juice mixture stirred for 60 min or celery and apple juice stabilised with ascorbic acid. Due to the contradictory results in different tests, the method cannot be declared successful or safe, even for mixtures of apple-celery juices.

**Keywords:** celery; allergen; juice; oxidation; colour; Western blot; basophil activation; skin tests

Celery ranks among the most aggressive allergens in the world – although celery allergies are not particularly widespread, allergic reactions to celery tend to result in systemic symptoms, including anaphylactic shock. The seriousness of the celery protein allergenicity is amplified by the fact that, for particularly sensitive individuals, not only raw but also relatively well cooked celery can induce allergic reactions (FUCHS 2007).

Celery is a common cause of pollen-related food allergies, especially in European countries. In Switzerland and France, about 30–40% of patients with food allergies are sensitised to celery root, and in 30% of serious food product anaphylactic reactions (Food Safety Information Center), the patients list celery as the cause.

Celery (*Apium graveolens*) belongs to the *Apiaceae* family. The plant is formed by a fleshy top root

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and petioles (leaf stalks). Celery can be consumed both raw and boiled (as an ingredient in soups and sauces), or as a condiment (as an ingredient in various processed foods). Allergic reaction to the celery root is more common than the reactions to the stalk. Dried celery (used as a seasoning) can provoke allergic reactions as severe as raw celery.

Celery contains at least three groups of allergenic proteins:

- Api g1 – homologous with Bet v1, main allergen in birch pollen; it belongs to the so-called pathogenesis-related (PR) proteins;
- Api g4 (profilin) – homologous to the secondary allergen in birch and wormwood pollen;
- CCD (cross-reactive carbohydrate determinants), which include especially Api g5 (60 kDa), the proteoglycan with the largest molecular mass.

The research has also confirmed the presence of a celery lipid transfer protein (approx. 9 kDa).

Due to its cross-reactive nature, the sensitivity to celery is often connected with the allergy to birch or wormwood pollen, hence the commonly used term birch-wormwood-celery syndrome. Cross-allergy to celery and birch pollen is very common in Central Europe; allergic reactions to celery and wormwood are typical for Southern regions of Europe. German literature often speaks of the celery-carrot-wormwood-spice syndrome.

Allergen stability decreases with increasing heat, in the following order: celery LPT > Api g5 > CCD (N-glycan containing fucose and xylose) > profilin > Api g1 (most unstable). Some individuals can react negatively to cooked celery, even if it has been processed at high temperatures. Celery used as seasoning is allergenic for people who also have allergy to raw celery.

There is little data available regarding the safe threshold dose. Local symptoms will likely occur at a dosage of 0.7–2.7 g; systemic symptoms appear at doses between 7.5–31 g; the doses limits are similar for both raw and cooked celery. Celery, as a condiment, can provoke allergic reactions in even smaller doses (0.16–5.85 g).

A study performed by the Scientific Committee for Food (ANONYMOUS 2005) examined the effects of processing on the allergenicity of celery and showed that a certain degree of allergenicity is retained even if celery is boiled or exposed to microwave radiation. Nobody has ever reported a reaction to cooked celery without being reactive to raw celery as well.

Considering the significant nutritional qualities of celery, it would be useful to prepare a product, at as little processing as possible, which would be free of its main allergen, Api g1.

CHUNG *et al.* (2005) described successful attempt to reduce the contents of allergens Ara h1 and Ara h2 in a peanut extract by inducing a reaction with apple PPO (polyphenol oxidase). PPO produced the best reaction in combination with caffeic acid. CHUNG and CHAMPAGNE (2007) published another paper on this topic.

Independently of the above mentioned researcher, we established that it was possible to inactivate the main allergen in apples, Mal d1, and most likely also the main allergen in carrot, Dau c1 (KUČERA *et al.* 2009). The method involved polyphenol oxidase-supported oxidation of phenolic acids to quinones, which then polymerise with the resident proteins (VELÍŠEK & HAJŠLOVÁ 2009), which results in the formation of complexes that are no longer allergenic. However, the matrix contains various antioxidants, especially vitamin C, which may inhibit or completely prevent the oxidative reaction (carrot, carotenoids). This issue is closely related to the enzymatic browning of apples and apple juice caused by PPO, which has been frequently described in the literature (SHANNON & PRATT 1967).

TREJO-GONZALES and SOTO-VALDEZ (1991) studied the activity of PPO isolated from apples of the Anna variety, grown in Sonora, Mexico (a region known for its hot, arid climate). The apples are prone to enzymatic browning due to the high content of phenolic substances, with the enzyme being thermally stable between 35–60°C. The optimal pH (5.4) was determined for the maximum level of PPO activity isolated from this apple cultivar.

JOSHI *et al.* (2007) conducted a detailed evaluation of various apple varieties with regard to their suitability for industrial processing, with enzymatic browning used as the primary criterion. While trying to discover the browning mechanism, they discovered that the speed and intensity of browning depended on the substrate (phenolic substances), the enzyme (PPO), and presence of ascorbic acid. The ascorbic acid content in apples fluctuates over the course of apple ripening and storage and is the controlling factor of enzymatic browning (BARUAH & SWAIN 1953), and is even capable of reducing the already formed quinones back to diphenols (JOSHI *et al.* 2007; ÖZDEMİR 2007).

VELÍŠEK and HAJŠLOVÁ (2009) describes, in detail, the reactions which occur during enzymatic apple browning. The first reaction to occur is the oxidation of phenolic substances. In the apple flesh, these include primarily chlorogenic acid, epicatechin, and proanthocyanidin B2, which constitute more than 90% of the phenolic substances present. In unripened fruit, the concentration of these substances is the highest but it rapidly decreases as the fruit ripens and finally stabilises at a certain level. Oxidising reactions produce quinones which react with proteins to form dark-coloured polymeric products.

However, no research exists concerning apple browning with the relationship between PPO activity and allergen content.

In 2007, our research facility conducted experiments to establish if it was possible to reduce the allergenicity of apple juice using high-pressure treatment (400 MPa/3 min up to 550 MPa/10 min); ultimately, we established that the pressure treatment was ineffective in suppressing allergic reactions to apple juice. FERNANDEZ *et al.* (2009) used high-pressure treatment, up to 800 MPa, on an apple extract and arrived at the same conclusion as our team. However, they did establish that high temperatures rather than high pressures were more effective in producing irreversible changes.

HOUŠKA *et al.* (2009) tried to determine if it was possible to eliminate the allergenicity of the recombinant allergen rApi g1. We examined the effects of both high temperatures (30°C, 40°C, 50°C) for periods of 10 min and 20 min under the given high pressure of 500 MPa, as well as the effects of high pressure (400 MPa, 450 MPa, and 500 MPa) for 10 min at the given temperature of 50°C. We monitored structural changes by means of circular dichroism (CD) spectra. The application of high pressure (500 MPa) to the rApi g1 allergen caused minor conformational changes (in relation to the untreated control samples) in the protein structure; the higher the bath temperature during pressurisation, the more extensive the changes. The application of varying pressures (400–500 MPa) to the rApi g1 allergen demonstrably caused conformational changes in the protein structure; the higher the pressure applied, the more extensive the changes. Using Western Blot on the samples tested for the effect of high temperatures established that the pressure of 500 MPa and temperatures of 30°C, 40°C, and 50°C had no effect on the rApi g1 allergenicity.

This paper therefore attempts to verify whether celery (in the form of juice extracted from the plant) contains enough phenolic substances and low enough levels of antioxidants for the oxidising-polymerising reaction to occur and for the juice to lose its allergenic properties. With regard to pure celery juice, it does not demonstrate the ability to become deallergised by oxidation, therefore it was attempted to produce the reaction using a surplus of apple juice. Apple juice contains enough PPO and phenolic acids, and relatively little protective vitamin C, which results in fairly rapid enzymatic browning.

## MATERIAL AND METHODS

**Materials.** Pure and mixed juices were made using washed celery without haulm (top root), and the Golden Delicious cultivar of apples, since they have the highest content of the Mal d1 allergen that can be inactivated as well.

**Instruments used to prepare the oxidised juice.** Champion press (Plastaket MFG, Lodi, USA, delivered by Mipam bio Ltd., Czech Republic); Sentron pH meter (Sentron, the Netherlands); Refractometer 139611 (Carl Zeiss, Jena, Germany); Minolta CR 300 colorimeter (Minolta, Japan); MONO-THERM heated magnetic stirrer (VWR International, USA).

**Preparation of juice for measuring colour and allergenicity using the Western blot (WB) method.** To prepare pure celery juice for the colour measurements (repeated 3 times), we cleaned and chopped 2 large celery roots, pressed the pieces using a Champion press, and strained the juice through a sieve with 1.2 mm holes. We used a graduated cylinder to measure 350 ml of juice, which we then stirred in a glass beaker using a magnetic stirrer at 600 revolutions per minute for 90 minutes.

To prepare the apple-celery juices mixture, we first strained the juice from the celery, and then squeezed cut-up apples. We mixed both juices in the selected ratios (3:1, 5:1, 7:1) to achieve a final volume of 350 ml. We stirred the combined juices in a glass beaker, using a magnetic stirrer at 600 revolutions per min for 120 minutes. Each measurement was repeated 3 times.

**Juice colour measurement and evaluation.** Immediately after pressing, we measured 350 ml of juice in a graduated cylinder. 150 ml of juice was transferred into a white plastic PET container with

a diameter of 65 mm, and the colour was measured at six different places using a Minolta colorimeter (initial colour at 0 minutes). The measurements were done in triplicates. The celery juice was measured at 0, 15, 30, 45, 60, 75, and 90 minutes. The mixed apple-celery juice was measured at 0, 15, 30, 45, 60, and 120 minutes.

We evaluated the colour parameters using the CIE system, brightness  $L^*$ , red colour shade  $+a^*$ , and yellow colour  $+b^*$ . The colour change was comprehensively expressed by the  $\Delta$  parameter, defined as the distance from the reference point (comparison sample) in the colour space. The change in colour ( $\Delta$ ) is defined as follows:

$$\Delta = [(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2]^{1/2} \quad (1)$$

We performed a statistical evaluation to establish if the colours of the mixed juice collected at different points in time show any conclusive differences. For this purpose, we used Student's *t*-test with a significance level of 0.05.

**Preparation of samples for examination using the Western Blot technique.** From each celery juice sample, we prepared a sample for electrophoresis (ELF), then we heated all the samples in boiling water for 5 min, cooled them and refrigerated them until the following day.

We measured out 10 g of fresh juice to which we immediately added 5 ml of 10mM potassium phosphate buffer (pH = 7.0) with protective chemicals. We measured the pH, strained the juice using triple-layered folded gauze, and centrifuged it in a Hettich centrifuge at 18 000 revolutions per min (15 min at 8°C). We took 2 ml of the pure supernatant, added SDS, saccharose, mercaptoethanol (MeOH), and bromophenol blue. The protein content in the samples tested was predicted by the BRADFORD (1976) method based on the determination of the protein and Coomassie Brilliant blue 250 complex at 595 nm. Quantification was done by means of the standard bovine serum albumin (BSA) in the concentration range 2–10 µg/ml.

**Preparation of juices for the basophil activation test and skin testing.** The apples were washed and cored, then celery tubers were peeled and chopped into suitably-sized pieces. We prepared samples of pure celery juice from celery macerated in 3% solution of ascorbic acid; pure apple juice from the Golden Delicious apples, macerated in 3% solution of ascorbic acid. The maceration in ascorbic acid solution intentionally stabilised the allergens present in the control samples.

We also prepared samples of apple-celery juices mixture (5:1) and oxidised them by stirring for 60 min or 120 minutes. The juices were prepared using a Champion press, and strained through a sieve. Oxidation was achieved using a magnetic stirrer at 600 rev/min. Such revolutions created a central vortex, which gently drew air into the juice. The juice was stirred at room temperature (approximately 22°C). The celery juice had a pH of 6.6, and refraction of 7.4%. The apple juice had a pH of 3.7 and refraction of 13.1%. The mixed juice had pH of 4.1, and refraction of 11.6%.

The juice samples were poured into flat PE/PA bags, and immediately placed in a freezer (–18°C). We prepared sample batches for skin tests, as well as sample batches for the basophil activation test; the frozen samples were forwarded to laboratories for basophil activation testing, and to a clinical facility for skin prick testing. Using the above methods, we therefore prepared the following samples:

- apple juice stabilised with ascorbic acid;
- celery juice stabilised with ascorbic acid;
- mixed juice, apple-celery (5:1), oxidised for 60 minutes;
- mixed juice, apple-celery (5:1), oxidised for 120 minutes.

**Determination of PPO activity.** PPO activity was determined in the laboratories of the Faculty of Food and Biochemical Technology of the Institute of Chemical Technology in Prague. In order to determine PPO activity in celery, we supplied the lab with one whole tuber and four whole apples. The enzyme was extracted into pre-cooled phosphate buffer (0.1M Na<sub>2</sub>HPO<sub>4</sub>, pH = 6.5) with added polyvinylpyrrolidone, which binds phenols present in the apple flesh. After centrifugation, an aliquot of the supernatant was added to the solution of 0.05M catechol (substrate for the enzymatic reaction) and using a spectrophotometer, PPO activity was measured as a change in absorbance at 420 nm.

**Determination of vitamin C content.** In order to determine the content of vitamin C in celery, we provided one whole celery tuber and two apples. To ascertain the content of ascorbic acid, we used the titration method with 2,6-dichlorophenolindophenol (in accordance with CSN ISO 6557/2:1996), with potentiometric indication of the equivalence point. The extraction from the material was performed by using 3% solution of metaphosphoric acid. The usual repeatability of the

method, expressed by a relative standard deviation, is  $\pm 7\%$ . In the case of a lower content (around 1 mg per 100 g), the relative standard deviation may be up to 20%, but such figures come close to the determinable limits.

**Determination of overall polyphenol content.**

To determine the polyphenol content, we provided one whole celery tuber and four apples. The method is built on the reaction of the extract with Folin-Ciocalteu reagent and sodium carbonate solution. The final colouring of the solution (absorbance) was measured using a spectrophotometer at a wavelength of 765 nm. Total polyphenol content was expressed as the gallic acid equivalent. 10–15 g of the sample were extracted in 100 ml of acetone: water mixture (7:3) in a water bath, under a reflux condenser, at a temperature of 65°C (2 h). The extract was cooled and filtered, and a 1 ml aliquot was added to into a 50 ml measuring flask, followed by the addition of 2.5 ml of Folin-Ciocalteu reagent and 7.5 ml of 20% sodium carbonate solution. The content was mixed, distilled water was added to make up the volume to 50 ml; the mixture was left in rest for 2 h at room temperature when

the colour was fully developed. The calibration curve was prepared using gallic acid solutions at concentrations of 0.1–0.4 g/l.

## RESULTS AND DISCUSSION

### Colour Evaluation

**Celery juice.** Figure 1 shows the progress of the overall  $\Delta$  colour change during the course of oxidation, calculated using equation (1). Parameter  $\Delta$  significantly differed from zero already after 15 min of stirring. The parameter did not show any further statistically significant changes beyond 30 min of stirring.

**Mixed juice.** The overall change in the colour of the mixed juices, expressed as parameter  $\Delta$ , is shown in Figure 2. It is apparent that the formula had a negligible effect, and that all major changes in the parameter occurred within approximately 45 min from the beginning of the stirring process.

The progress of the colour change of the mixed juices was governed primarily by the changes in the

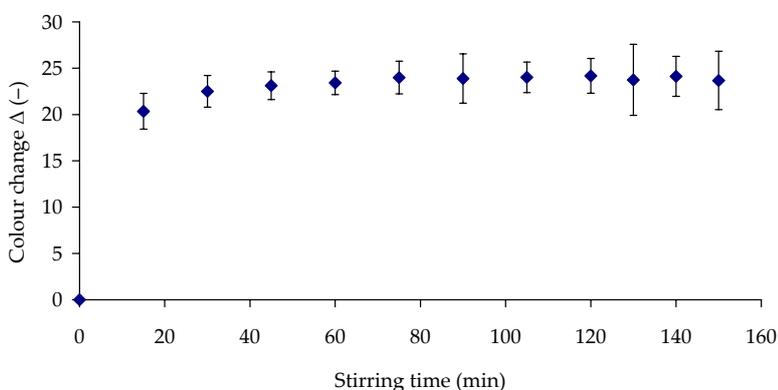


Figure 1. Relation of celery juice color change ( $\Delta$ ) to stirring time (an average of three experiments); volume of stirred sample: 300 ml; stirrer revolution: 600 revs per minute

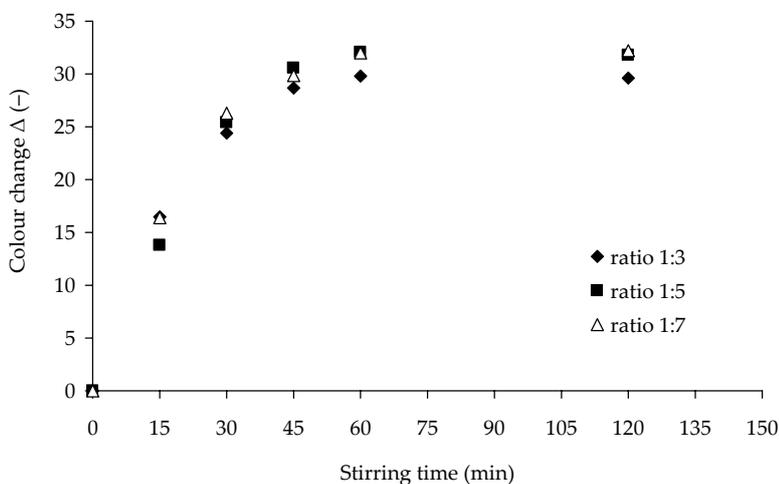
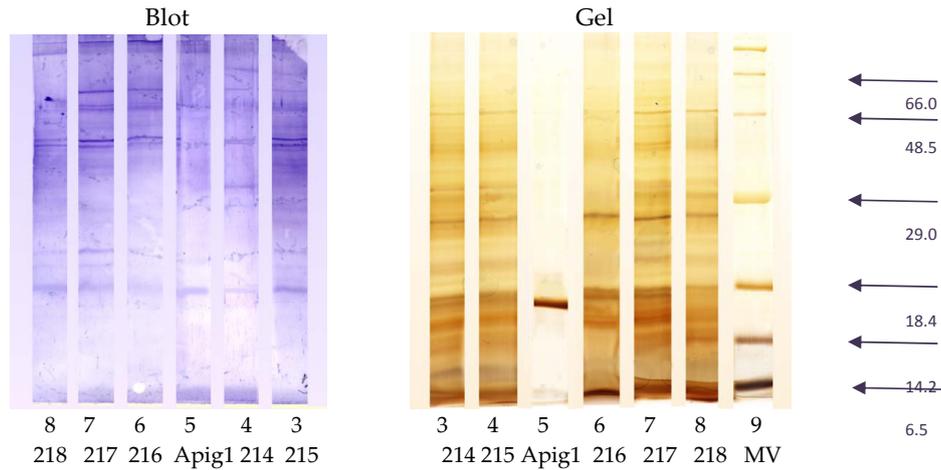


Figure 2. Relation of celery-apple juice (1:3, 1:5, 1:7) color change ( $\Delta$ ) to stirring time; volume of stirred sample: 350 ml; stirrer revolution: 600 revs per minute



214 – without stirring; 215 – 15 min stirring; 216 – 30 min stirring; 217 – 60 min stirring; 218 – maceration in 3% ascorbic acid water solution, no stirring; Line 5 in gel – pure allergen rApi g1; line 5 in gel indicates the place of this allergen in other lines; line 9 in gel – molecular weight standard

Figure 3. WB test performed on oxidised celery juice samples No. 214–218

prevailing apple juice. The colour change measurement may prove to be a suitable tool for monitoring the kinetics of the oxidising reactions involved, even in practical applications of this method.

Based on the above results, we concluded that the colour change is not a decisive criterion for finding an optimal formula for an oxidised juice mixture. The defining parameter in selecting the right ratio of apple to celery juices was the mixture palatability, established using a taste test. From this perspective, the ratio of juices chosen for further testing regarding the impact of oxidation on allergenicity using the WB method was 5:1.

### Results of the celery juice WB test

Figure 3 shows the results of the Western Blot test with oxidised celery juice. It was apparent from the blot (comparison with the strip in position 5 – pure allergen rApi g1), that the Api g1 allergen was present in all samples (214 to 218) of the oxidised celery juice. The gel and blot probably also showed other allergens, with a molecular mass of approximately 6.5 kDa. In general, we concluded that oxidation by stirring had no effect on the Api g1 allergen.

The results of the protein content determination are shown in Table 1. Sample 214 (i.e. fresh, unmixed pressed, out celery juice) showed the highest protein content. With prolonged stirring period the protein

contents in the samples decreased. One sample, which had been stirred for 60 min, had the lowest protein content. The protein content in the sample macerated for 10 min in 3% ascorbic acid solution was before pressing 22% lower than that in fresh juice (sample 214). We can explain the lowering of the protein content by the method used. We assume that the used BRADFORD (1976) method predicts only proteins that are available to form complexes with Coomassie Brilliant blue 250.

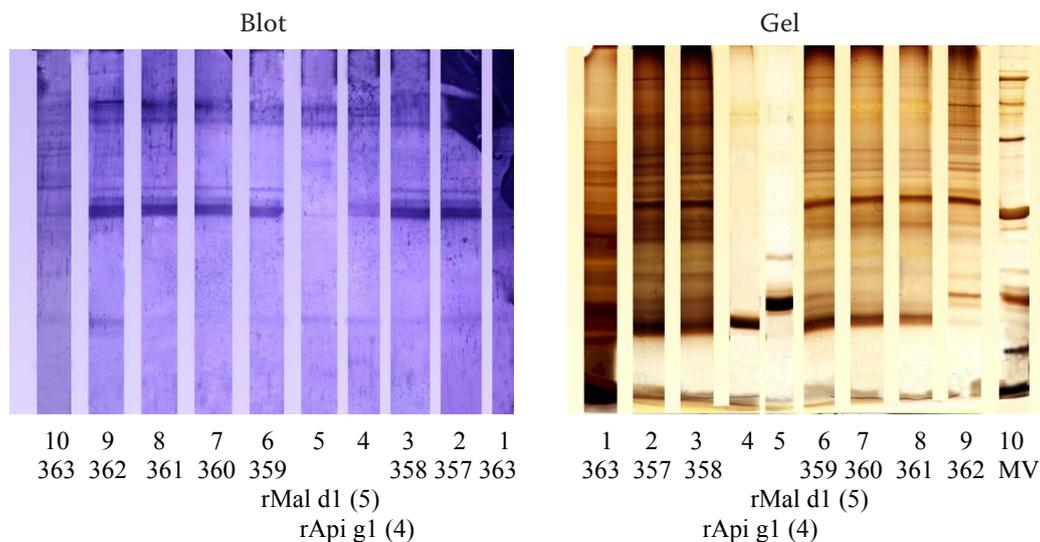
### Results of the mixed juice WB test

Figure 4 shows the results of the WB tests with the samples of mixed juice over the course of the

Table 1 Protein content in celery juice depending on oxidization time (stirring)

| Sample No. | Oxidation   | Proteins (mg/ml) |
|------------|---|------------------|
| 214        | without mixing  | 0.86             |
| 215        | mixing 15 min   | 0.74             |
| 216        | mixing 30 min   | 0.60             |
| 217        | mixing 60 min   | 0.51             |
| 218        | without mixing, raw material<br>macerated in 3% askorbic acid-water<br>solution | 0.67             |

Standard deviation of the method is 10%



357 – mixture apple: celery (5:1) without stirring; 358 – mixture apple: celery (5:1) 15 min of stirring; 359 – mixture apple: celery (5:1) 30 min of stirring; 360 – mixture apple: celery (5:1) 60 min of stirring; 361 – mixture apple: celery (5:1) 120 min of stirring; 362 – apple juice from apples macerated in 3% ascorbic acid solution; 363 – celery juices from celery macerated in 3% ascorbic acid solution

Figure 4. WB test performed on celery-apple juice (1:5) in the course of the oxidation process

oxidation process. It also includes the control samples of the juice stabilised with ascorbic acid, standard allergens in apples and celery, and molecular mass standards.

The right-hand side of the scheme shows protein distribution. It is apparent that the longer the stirring time, the lower the overall protein content (cf. the strip strength in samples 357–361).

The entire blot membrane was induced with a mixture of serums of four patients in ratio (4:2:2:2) showing reactions to the celery allergen. We can state with certainty that the allergen was present in the mixed juice samples 357, 358, and 359 (stirred for 0, 15, and 30 min). In samples 360 and 361 (stirred for 60 min and 120 min, respectively), the presence of the allergen is disputable. We saw evidence of the allergen trace amounts present in sample 361; however, it seems that no allergen was present in sample 360. No satisfactory explanation for this contradiction was found; however, we may reasonably assume that the oxidation of phenolic substances and the subsequent polymerisation of proteins, including the allergens, was effective after 60 minutes of oxidation. However, further tests will be necessary to verify this hypothesis (skin tests and basophil activation tests being the only available options). Because the celery allergen is potentially very dangerous, oral challenge tests were not applied.

### Results of the basophil activation test

The test was performed in the Imumed Ltd. laboratories using standard methods, with both Imumed and Faculty Hospital Kralovske Vinohrady (FNKV) patients' blood. The test results are outlined in Table 2. The differences were evaluated using the non-parametric Wilcoxon test (LIKEŠ & LAGA 1978).

The results showed primarily that there was no statistical difference between the reactions to oxidised mixed juices (whether oxidised for 60 min or 120 min) and those to celery juice stabilised by ascorbic acid; in fact, they produced a significantly stronger reaction than rApi g1 and the Stallergen standard.

### Skin prick test results

Skin prick tests were performed on the same group of patients as the basophil activation tests. From the test results, we chose to evaluate the average early reaction with wheal occurrence (we also determined average skin flare, but chose not to use this criterion since it was not considered sufficiently conclusive). We again used the non-parametric Wilcoxon test to evaluate the differences (LIKEŠ & LAGA 1978).

Table 2. Results of the basophil activation test using an oxidised mixed celery-apple juice (1:5) and its components

| Laboratory                           | Patient                         | Without<br>stimul | Ig E  | Celery<br>stallergen | Api g1 | Celery ju.<br>+asc. ac. | appl. j.+<br>asc. ac. | 60'oxid.<br>mixture | 120'oxid.<br>mixture |
|--------------------------------------|---------------------------------|-------------------|-------|----------------------|--------|-------------------------|-----------------------|---------------------|----------------------|
| FNKV                                 | 1                               | 4.1               | 89.0  | 82.0                 | 8.2    | 98.0                    | 97.0                  | 99.0                | 98.0                 |
|                                      | 2                               | 9.3               | 63.0  | 50.0                 | 55.0   | 92.0                    | 93.0                  | 97.0                | 99.0                 |
|                                      | 3                               | 3.9               | 19.0  | 12.0                 | 8.6    | 27.0                    | 18.0                  | 18.0                | 16.0                 |
|                                      | 4                               | 3.3               | 56.0  | 58.0                 | 71.0   | 79.0                    | 76.0                  | 74.0                | 72.0                 |
|                                      | 5                               | 7.7               | 73.0  | 79.0                 | 86.0   | 99.0                    | 99.0                  | 98.0                | 100.0                |
|                                      | 6                               | 14.0              | 48.0  | 58.0                 | 76.0   | 82.0                    | 93.0                  | 74.0                | 84.0                 |
| Imumed                               | 7                               | 13.0              | 86.0  | 86.0                 | 95.0   | 99.0                    | 99.0                  | 99.0                | 99.0                 |
|                                      | 8                               | 7.6               | 74.0  | 70.0                 | 5.8    | 96.0                    | 66.0                  | 52.0                | 55.0                 |
|                                      | 9                               | 11.0              | 66.0  | 6.7                  | 7.4    | 38.0                    | 4.8                   | 12.0                | 13.0                 |
|                                      | 10                              | 5.4               | 92.0  | 41.0                 | 84.0   | 96.0                    | 98.0                  | 98.0                | 99.0                 |
|                                      | 11                              | 3.5               | 82.0  | 76.0                 | 94.0   | 98.0                    | 94.0                  | 98.0                | 99.0                 |
|                                      | 12                              | 4.2               | 91.0  | 96.0                 | 81.0   | 96.0                    | 94.0                  | 99.0                | 99.0                 |
|                                      | median                          | 6.5               | 73.5  | 64.0                 | 73.5   | 96.0                    | 93.5                  | 97.5                | 98.5                 |
|                                      | 5% percentile                   | 3.4               | 35.0  | 9.6                  | 6.7    | 33.1                    | 12.1                  | 15.3                | 14.7                 |
|                                      | 95% percentile                  | 13.5              | 91.5  | 90.5                 | 94.5   | 99.0                    | 99.0                  | 99.0                | 99.5                 |
|                                      | mean                            | 7.3               | 69.9  | 59.6                 | 56.0   | 83.3                    | 77.7                  | 76.5                | 77.8                 |
|                                      | comparing lines<br>with columns |                   |       |                      |        |                         |                       |                     |                      |
| Wilcoxon test<br>> greater<br>< less | celery ju.+asc. ac.             | YES >             | YES > | YES >                | YES >  | –                       | NO                    | NO                  | NO                   |
|                                      | appl. j.+asc. ac.               | YES >             | YES > | YES >                | YES >  | NO                      | –                     | NO                  | NO                   |
|                                      | 60'oxid. mixture                | YES >             | NO    | YES >                | YES >  | NO                      | NO                    | –                   | NO                   |
|                                      | 120'oxid. mixture               | YES >             | NO    | YES >                | YES >  | NO                      | NO                    | NO                  | –                    |

celery ju. + asc. ac. – celery juice stabilised with ascorbic acid; appl. j.+asc. ac. – apple juice stabilised with ascorbic acid; 60'oxid. mixture – 60 min oxidation of mixed juice of celery and apple (1:5); 120'oxid. mixture – 120 min oxidation of mixed

The test results are shown in Table 3. Apart from the standard allergens and negative control samples, we also included other plant allergens, such as birch, wormwood, and timothy grass. The tested patient group exhibited strong reactions to these allergens.

The results show that the borderline conclusive reactions (wheals of average diameter of 3 mm) occurred after the application of mixed juice oxidised for 120 min, which exhibited a demonstrable statistical difference from the mixed juice oxidised for 60 min, and also from apple and celery juice stabilised by ascorbic acid. However, even the latter juice provoked a conclusive reaction in 4 out of 12 patients (33%). It seems therefore that

the response can vary dramatically from person to person.

#### PPO activity in celery and apples

The celery sample was divided into several layers and each layer was analysed separately; the results are shown in Table 4. It is apparent that PPO activity varies greatly in different layers of the root. The average activity level was 858 U/g/min, but there was a large variance due to the non-homogeneity of the roots. Standard variance calculated from all 6 figures was 394 U/g/minutes.

Table 3. Skin prick test results – average diameter of early reaction wheals (mm) celery-apple (1:5)

| Laboratory                              | Patient                         | Control             |                  | Celery<br>staller-<br>gen | Skin prick test – diameter of wheals (mm) |                      |                  |                  |                         | Celery ju.<br>+asc. ac. | Appl. j.+<br>asc. ac. |
|---|---------------------------------|---------------------|------------------|---------------------------|---|----------------------|------------------|------------------|-------------------------|-------------------------|-----------------------|
|   |                                 | negative            | pozitive         |                           | 60'oxid.<br>mixture                       | 120'oxid.<br>mixture | birch            | timothy<br>grass | worm-<br>wood           |                         |                       |
| FNKV                                    | 1                               | 0                   | 3                | 0                         | 6   | 3                    | 8                |                  | 12                      | 3                       | 8                     |
|   | 2                               | 0                   | 6                | 3                         | 6   | 2                    | 8                |                  | 5                       | 6                       | 8                     |
|   | 3                               | 0                   | 5                | 0                         | 0   | 0                    |                  |                  |                         | 0                       | 0                     |
|   | 4                               | 0                   | 5                | 4                         | 3   | 3                    | 10               | 8                | 6                       | 5                       | 0                     |
|   | 5                               | 0                   | 5                | 5                         | 10  | 6                    | 6                | 6                | 6                       | 8                       | 5                     |
|   | 6                               | 0                   | 6                | 6                         | 7   | 5                    | 6                |                  | 10                      | 8                       | 4                     |
| Imumed                                  | 7                               | 0                   | 5                | 5                         | 3   | 2                    | 5                | 15               | 0                       | 8                       | 6                     |
|   | 8                               | 0                   | 5                | 2                         | 5   | 5                    | 2                | 10               | 15                      | 5                       | 5                     |
|   | 9                               | 2                   | 5                | 2                         | 2   | 2                    | 5                | 6                | 10                      | 0                       | 0                     |
|   | 10                              | 0                   | 5                | 2                         | 5   | 3                    | 10               | 8                | 10                      | 5                       | 5                     |
|   | 11                              | 0                   | 8                | 3                         | 8   | 5                    | 15               | 10               | 25                      | 10                      | 15                    |
|   | 12                              | 0                   | 10               | 5                         | 5   | 3                    | 10               | 10               | 5                       | 5                       | 5                     |
| Median                                  |                                 | 0.0                 | 5.0              | 3.0                       | 5.0                                       | 3.0                  | 8.0              | 9.0              | 10.0                    | 5.0                     | 5.0                   |
| 5% percentile                           |                                 | 0                   | 4                | 0                         | 1   | 1                    | 4                | 6                | 3                       | 0                       | 0                     |
| 95% percentile                          |                                 | 1                   | 9                | 5                         | 9   | 5                    | 13               | 13               | 20                      | 9                       | 11                    |
| Mean                                    |                                 | 0.2                 | 5.7              | 3.1                       | 5.0                                       | 3.3                  | 7.7              | 9.1              | 9.5                     | 5.3                     | 5.1                   |
| Wilcoxon<br>test<br>> greater<br>< less | comparing lines<br>with columns | pozitive<br>control | celery<br>stall. | 60'oxid.<br>mixture       | 120'oxid.<br>mixture                      | birch                | timothy<br>grass | worm-<br>wood    | celery ju.<br>+asc. ac. | appl. j.+<br>asc. ac.   |                       |
|   | celery staller-<br>gen          | YES <               | –                | YES <                     | NO  | YES <                | YES <            | YES <            | YES <                   | NO                      |                       |
|   | 60'oxid. mixture                | NO                  | YES >            | –                         | YES >                                     | YES <                | YES <            | YES <            | NO                      | NO                      |                       |
|   | 120'oxid. mixture               | YES <               | NO               | YES <                     | –   | YES <                | YES <            | YES <            | YES <                   | NO                      |                       |
|   | birch                           | YES >               | YES >            | YES >                     | YES >                                     | –                    | NO               | NO               | NO                      | YES >                   |                       |
|   | timothy grass                   | YES >               | YES >            | YES >                     | YES >                                     | NO                   | –                | NO               | YES >                   | YES >                   |                       |
|   | wormwood                        | YES >               | YES >            | YES >                     | YES >                                     | NO                   | NO               | –                | YES >                   | YES >                   |                       |
|   | celery ju.+asc. ac.             | NO                  | YES >            | NO                        | YES >                                     | NO                   | YES <            | YES <            | –                       | NO                      |                       |
| appl. j.+asc. ac.                       | NO                              | NO                  | NO               | NO                        | YES <                                     | YES <                | YES <            | NO               | –                       |                         |                       |

celery ju.+asc. ac. – celery juice stabilised with ascorbic acid; appl. j.+asc. ac. – apple juice stabilised with ascorbic acid; 60'oxid. mixture – 60 min oxidation of mixed juice of celery and apple (1:5); 120'oxid. mixture – 120 min oxidation of mixed juice of celery and apple (1:5); YES – significant difference; NO – not significant difference

PPO activity in apples, of the Golden Delicious cultivar, was determined in a parallel experiment with apple storage. PPO activity in the apples reached the levels of 11771, 10630, and 9302 U/g/min. The results clearly show that PPO activity in celery is more than one order lower than in apples.

### Vitamin C contents in celery and apples

Vitamin C content in celery was determined to be  $8.8 \pm 1.1$  mg in 100 g sample (confidentiality interval at the significance level of 95%).

Vitamin C content in Golden Delicious apples was determined during a parallel storage experiment. The mean value of vitamin C content was 2.75 mg in 100 g of apple sample, which is considerably lower than in celery.

### Total polyphenol contents in celery and apples

The polyphenol content in celery, defined as an equivalent of gallic acid, was determined to be  $37.7 \pm 0.6$  mg in a 100 g sample (confidentiality interval at the significance level of 95 %).

Table 4. Results of determining PPO activity in celery root

| Sample             | Activity of PPO<br>(U/g/min) |      |
|--------------------|------------------------------|------|
| 369–3003-C         |                              |      |
| 1                  | 699                          | 490  |
| 2                  | 1250                         | 1422 |
| 3                  | 482                          | 803  |
| Mean of all values | 858                          |      |
| Standard deviation | 394                          |      |

The total polyphenol content in Golden Delicious apples was determined from a parallel storage experiment. The mean value of the total polyphenol content was 67.0 mg in 100 g of the apple sample, which is considerably higher than in celery.

## DISCUSSION

The kinetics of the colour change in the mixed celery/apple juices in the ratios of 1:3, 1:5 and 1:7 is not substantially different from the kinetics of the colour change in pure apple juice. This is caused by the prevalence of apple juice in the samples, and the different compositions of celery and apple juice matrices.

The results of the WB test performed on celery juice showed that oxidation did not reduce the allergenicity of the primary allergen Api g1; it appears that the patients' serums revealed and reacted to allergens of very low molecular mass (approx. 7 kDa).

What makes it impossible to de-allergise celery juice by oxidation like apple juice? Compared to apple juice, celery juice contains half the total polyphenols amount and its polyphenol oxidase activity is ten times lower. On the other hand, celery contains three times as much vitamin C as apple juice, which protects celery juice against oxidation. This may be the reason why celery juice fails to produce sufficient oxidation products for polymerisation and de-allergisation of the allergenic proteins present.

Following the unsuccessful attempt to oxidise celery juice, we attempted to induce oxidation by supplementing it with the substances contained in apple juice (celery/apple ratio 1:5). The WB test showed that oxidation of the prepared mixed juice by stirring caused a reduction of the protein content in the juice, but did not eliminate the allergic reaction to Api g1.

The basophil activation tests performed on the mixed juice showed no reduction in allergic reactions. The oxidised mixed juice stirred for 120 min showed a promising reduction in allergenicity in skin prick tests. Such juice produced a significantly weaker reaction than the mixture stirred for only 60 min, as well as a weaker reaction compared to celery and apple juices stabilised with ascorbic acid. Unfortunately, such juice still produced excessive reactions in 4 out of 12 patients tested (33%). Such results indicate that in most patients, oxidised mixed celery/apple juice (celery/apple ratio 1:5) need not cause an external reaction, but an individualised internal reaction cannot be avoided.

## CONCLUSIONS

The results of our experiments show that oxidation is not a suitable method for eliminating the primary allergen in celery juice. The method was applied to celery juice, in the presence of excess PPO and phenolic substances from apple juice. Deallergisation was not demonstrated using the WB test, and was essentially ruled out by the basophil activation test.

Deallergisation was partially demonstrated using skin prick testing. Because of the above given results, the method cannot be declared successful or safe, even for the mixtures of apple-celery juices.

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