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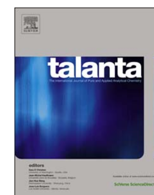

# *In situ* quantitative $^{1}$H nuclear magnetic resonance spectroscopy discriminates between raw and steam cooked potato strips based on their metabolites

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A direct quantitative proton nuclear magnetic resonance spectroscopy method was developed for the measurement of saccharides, organic acids and amino acids in potato (*Solanum tuberosum* L.) tuber filaments, a complex gel-like food matrix. The method requires minimal sample preparation. It is thus a faster alternative compared to liquid sample matrices, as well as an extension to methods analyzing only selected metabolites in the sample. The samples in this study were either raw or steamed potato strips that were either measured as $D_2O$ extracts or directly without extraction or derivatization steps (*in situ* technique). A total of 22 compounds were identified in extracts and 18 in potato strips. Of these, 20 compounds were quantifiable in potato extracts and 13 compounds in potato strips. The effect of thermal processing was reflected in the profile of analyzed compounds. One example was fumaric acid, which was completely lost in steamed samples in both measurement techniques. Additionally, the content of γ-aminobutyric acid in steamed potato strips was lower. In potato extracts, the contents of additional 7 compounds were statistically different. The raw and steamed samples separated into two groups with multivariate models both in extracts and potato strips, and these groups were linked to changes in aforementioned compounds. These results demonstrated that the *in situ* quantitative $^{1}$H NMR technique is a useful tool to analyze potato metabolites. This technique could be further applied to any gel-like complex matrix, meaning that lengthy sample pretreatment could be skipped.

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## 1. Introduction

Current typical food analysis methods rely on lengthy extractions. For example, both chromatography [1–3] and NMR [4] based analysis protocols for potato metabolites call for time and solvent consuming or multi-step sample pretreatment. The steps can cause modifications to and extraction artifacts in the sample material.

Recently, it has become common to measure liquid samples directly, with matrices ranging from fruit juices [5] to urine [6]. However, as one of our authors has presented before, there is no theoretical reason why direct quantitative NMR would be inapplicable to gel-like vegetable matrices [7]. One example of this was an *in situ* quantitative $^{1}$H NMR (*is*qNMR) study for carrot (*Daucus carota* L.) roots, in which the saccharides of dried, whole tissues of samples were measured [8]. More recently, it was shown that the method has a coefficient of variation $< 0.05$ and that samples ranging from 250 $mm^3$ can be measured [9]. This kind of protocol could demonstrate what kind of changes extraction causes to complex gel-like samples. However, the published method quantified only one type of metabolites. Additionally, carrots are still a relatively simple matrix, as there is little starch and protein present.

On the other hand, potato (*Solanum tuberosum* L.) tubers are highly complex as they have high levels of polysaccharides. They also have high levels of free amino acids [10] which makes them different from many other typical vegetables. Low field NMR instruments have been employed first in 1950 to measure the water

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content of potato strips [11] and later in 2000 to predict the sensory texture of cooked potatoes [12]. However, while extensive analysis of water-soluble potato metabolites with NMR has been published [4], there is no literature on the direct measurements of metabolites potato strips with NMR. Therefore, it has been difficult to compare whether this extracted sample is representative.

In this study, we describe an extended *is*qNMR method for the analysis of potato strips. In this method, saccharides, organic acids and free amino acids are measured simultaneously. The analysis requires no derivatization or extraction steps; air drying of the strips and rehydration with $D_2O$ was the only required pretreatment. This *in situ* analysis was compared to an isolated fraction. The performance of the method is displayed with compound level changes in potato samples as a result of cooking. We present a multivariate model in which the processing type of samples is predicted based on taste compounds alone.

## 2. Materials and methods

### 2.1. Chemicals

$D_2O$ (99.96%) was from VWR (Radnor, PA) and 3-(trimethylsilyl)-2,2′,3,3′-tetradeuteropropionic acid sodium salt (TSP, 98%) was from Sigma-Aldrich (St. Louis, MO). Starch was commercial food grade potato starch manufactured by Finnamyl Oy (Kokemäki, Finland), and additionally pure amylopectin from potato starch was bought from Fluka Biochemika, (Buchs, Switzerland; $<10\%$ $H_2O$).

Additionally 18 reference compounds were used: L-alanine, L-glutamine, L-isoleucine, L-phenylalanine, L-threonine and L-valine were from Serva Feinbiochemica GmbH & Co (Heidelberg, Germany). D-glucose, L-asparagine, L-leucine and malic acid and were from Merck (Darmstadt, Germany). Sucrose and citric acid were from J.T. Baker (Deventer, the Netherlands). L-arginine was from MP Biomedicals (Illkirch Cedex, France). Fructose, L-aspartic acid and L-glutamic acid were from Sigma-Aldrich (St. Louis, MO). L-proline was from Applichem (Darmstadt, Germany). Fumaric acid was from FlukaChemie AG (Buchs, Switzerland). All reference compounds were of analytical or greater grade.

### 2.2. Potato samples

Potato samples of cultivar Rikea were used for this study. The potato crop was cultivated in Karijoki in Finland (62°18′ N, 21°42′ E) in a fine sand soil at pH 5.9 in 2012. The crop was fertilized with N, P and K and sprayed with fungicides against potato blight three times during the growing season. The crop was planted at May 15th and harvested on September 25th. The growing season at the growing site was up to 0.5 °C colder and had a 110–125% higher precipitation compared to the 1981–2010 average according to the Finnish Meteorological Institute (Helsinki, Finland). After harvesting, the potatoes were stored at 7–8 °C until November 30, after which the storing temperature was 3 °C. The storing humidity was 80–90%. After receiving the samples for analysis in late June 2013, they were stored at 7–8 °C at 60–70% humidity for two weeks, during which all samples were analyzed.

### 2.3. Pilot study for the measurement protocol

Before the main test series, there was a pilot study that examined the sources of measurement uncertainty in the *in situ* measurement. The pilot data was used to determine the final protocol parameters. The data set consisted of 4-factor full-factorial triplicate data on the variation between tubers, within tubers, between NMR measurement of the sample and within peak integration, totaling 81 observations of data. The sample preparation was comparable to the one described below, but sample mass was only 1/3 of that in the main measurements. Data analysis and a summary of the pilot study results is included in Supplementary data (Fig. S1).

### 2.4. Sample preparation

Contents of starch, saccharides, and amino acids have been reported to differ up to twofold between different tissues in the potato tuber [13–15]. To minimize this tissue-based variation, all samples were taken from the inner pith in the center of the tuber. The extracts had samples from three potatoes for both treatments and potato strips were from four potatoes for both treatments, respectively. All analyses were done in triplicate.

Sectors from the longitudinal ends of the tubers were cut out with a scalpel to set the length of the potato equal to the height of 600 μL of liquid sample in a 5 mm NMR tube (about 45 mm). This also confirmed the inner morphology of each potato sample to allow for repeatable sample-taking. Next, 4 mm diameter cylinders with a crescent shaped cross section were cut from the center of the potato with a house made hollow steel drill (Supplementary data, Fig. S2). The resulting strips were washed with deionized water to remove starch from the surface, dabbed clean with tissue paper and then either steam cooked or dried.

For steam cooking, the cut and washed potato strips were placed over boiling water in a closed container for 10 min on wave-shaped, steel mesh racks (Fig. S2). After processing, the strips were allowed to cool on the racks at ambient temperature for 5 min and then dabbed dry from condensed moisture. After cooling, potato strips to be analyzed without extraction were dried in a commercial food dehydrator (Orakas 5510, 1100 W, Marlemi OY, Lemi, Finland). The temperature in the dehydrator was $29\pm2$ °C and relative humidity $42\pm9\%$. Strips were weighed periodically and dehydration was stopped when the mass of the strips was $<20\%$ of the initial mass (to keep them pliable for transfer into the NMR tubes).

The samples for extraction were cut in smaller pieces, placed in Eppendorf tubes with perforated stoppers, frozen with liquid nitrogen and freeze-dried overnight with a Virtis benchtop K freeze-dryer (SP Industries, Warminster, PA) at $-79$ °C, $p<0.3$ mbar.

The dried potato strips were transferred into a 5 mm NMR tube (product 634-0450, VWR, Radnor, PA). $D_2O$ was added to cover the strips completely, typically about 500 μL. The NMR tube was then sonicated for 10 min and further incubated for 15 min to promote rehydration of the strips. Finally, an inner capillary tube containing TSP in $D_2O$ (for locking and reference) was added in the NMR tube to the concave side of the potato strip. This external standard method was used to prevent reactions of TSP with the compounds in the sample solution [16]. Altogether, the sample preparation until NMR measurements took approximately 3 h.

For extraction, the freeze-dried samples were ground to a fine powder with a mortar and pestle. 70 mg of powder was mixed with 1000 μL of $D_2O$ and shaken for one hour. The suspensions were centrifuged at 8000*g* for 10 min. The supernatant was separated and the extraction procedure was repeated for the pellets. The uncooked sample supernatants were filtered through a 0.45 μm regenerated cellulose syringe filter. The membrane was nonpermeable to steamed samples, so they were instead filtered through a cotton-filled Pasteur pipette. The filtrates were freeze-fried and resuspended to 350 μL of $D_2O$. The suspensions of the two extractions were combined, centrifuged like before and 550 μL of the supernatant was transferred to a NMR tube with an inner capillary tube containing TSP dissolved in $D_2O$. The extraction protocol and preparation for NMR measurements required a minimum of 20 h.

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