Universidad Zaragoza
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This is to confirm to

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that within a study (reports March 26th,2015; June 18th, 2015; July 16th, 2015) conducted by Universidad Zaragoza (Plant Foods Research Group) on behalf of BSH Hausgeräte GmbH at

three different household steam ovens, representing a number of identically constructed ovens (see attached list at the end of this document):

- a full size steam oven (Bosch HSG856XS1)
- a compact full steam oven (Neff C17FS42N0)
- a compact pure steam oven (Siemens CD634GBW1)

in which potatoes and peppers of different shapes and sizes were steamed and the retention of Vitamin C (potatoes and peppers) as well as nicotinamide and riboflavin (only potatoes) was measured.

Within the used test conditions (see next pages) following findings concerning nutrient retention can be stated:

- Vitamin C retention in potatoes varies between 70 and 79 % for the full steam ovens and between 87 and 70 % for the pure steam oven.
- The nicotinamide retention in potatoes varies from 90 to 95 % and the riboflavin retention from 92 to 97 % for all ovens.
- The vitamin C retention in peppers varies from 78 to 73 % for full size full steam oven, from 85 to 91 % for the compact full steam oven and from 84 to 90 % for the pure steam oven.

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The aim of the study was to analyze the nutrients retention in the steaming of some vegetables. Therefore *Agata* potatoes and *California* peppers were steamed using three different ovens:

- a full size steam oven (Bosch HSG856XS1)
- a compact full steam oven (Neff C17FS42N0)
- a compact pure steam oven (Siemens CD634GBW1).

For all tested ovens identically constructed appliances from BSH series EOX6 of the brands Bosch, Siemens and Neff are available. An according list of identically constructed appliances is attached to this report.

The tests were carried out according to the analysis method described in an attached document as follows:

The potatoes, adjusted to 20 ºC, were peeled off, washed, drained and cut into cubes of approximately 2 cm or 3 cm of lateral length or into slices of 3 cm diameter and 1 cm thickness. The peppers at the refrigerator temperature (around 6 ºC) were washed, dried and cut into stripes (0.3-0.7 cm × 3-5 cm) or squares (3-4 cm × 3-4 cm). The products were put in a perforated gastronorm container without salt or seasoning. The amount of potatoes was 250g or 500 g, and 350 g for peppers. In the pure steam oven and in the full size full steam oven the product was located at level 3 and at level 2 in the compact full steam oven. In all cases the heating mode was “Steam 100 ºC”. The cooking time was the shortest time needed to have completely cooked vegetables. That is 16 min for potatoes cut in 2 cm cubes or slices and 21 min for 3 cm cubes, independently of the oven. The cooking time for peppers was 7 min for stripes and 5 min for squares when the pure steam and full size full steam ovens were used, while shorter cooking times were used for compact full steam oven (6 min for stripes and 4 min for squares).

Vitamin C was analyzed in potatoes and peppers, and nicotinamide and riboflavin were also quantified in potatoes. To obtain the percentage of retention, the vitamins were analyzed in raw and in cooked vegetables. The vitamins were determined by a high performance liquid chromatography method (HPLC) using a Hewlett Packard (Series 1100) chromatograph.

The results obtained in the analysis of potatoes, reveal that the percentage of retention of vitamin C varies between 70 and 79 % when the full steam ovens were used and neither the shape nor the weight of potatoes influences the retention of vitamin C. For the pure steam oven the vitamin C retention varies between 87 and 70 %. In this case, the shape and the amount of the potatoes appear to influence the retention of this nutrient. The cubes of 2 cm and the slices were steamed during the same time, but the cooking slices retain less amount of vitamin C. The losses of vitamin C are more pronounced for 500 g than for 250 g although these differences are only significant

($p<0.05$) for 2 cm cubes. The cause for this fact might be the different area of the perforated tray covered by the potato pieces and as a consequence the condensation of the steam over the product and the leaching could be take place in a different extent.

The nicotinamide retention in potatoes varies from 90 to 95 % and from 92 to 97 % for riboflavin retention, regardless, in both cases, of steaming conditions and oven.

From the results of the pepper analysis can be concluded that the retention of vitamin C does not depend significantly on the shape of the product, and varies from 78 to 73 % for full size full steam oven, from 85 to 91 % for compact full steam oven and from 84 to 90 % for pure steam oven.

List of identically constructed appliances

Identically constructed pure steam ovens:

Appliance	Description	Series	Brand	
CD634GBS1	Height 45 cm	EOX6	Siemens	
CD634GBS1B	Height 45 cm	EOX6	Siemens	
CD634GBS1W	Height 45 cm	EOX6	Siemens	
CD634GBW1	Height 45 cm	EOX6	Siemens	Tested model
CD834GBS1	Height 45 cm	EOX6	Siemens	
CD834GBB1	Height 45 cm	EOX6	Siemens	
CDG634BB1	Height 45 cm	EOX6	Bosch	
CDG634BB1W	Height 45 cm	EOX6	Bosch	
CDG634BS1	Height 45 cm	EOX6	Bosch	
CDG634BS1B	Height 45 cm	EOX6	Bosch	
CDG634BS1W	Height 45 cm	EOX6	Bosch	
CDG634BW1	Height 45 cm	EOX6	Bosch	
CDG634BW1W	Height 45 cm	EOX6	Bosch	

Identically constructed full steam ovens (60 cm):

Appliance	Description	Series	Brand	
HS636GDS1	Height 60 cm	EOX6	Siemens	
HS636GDS1C	Height 60 cm	EOX6	Siemens	
HS636GDS1W	Height 60 cm	EOX6	Siemens	
HS636GDW1W	Height 60 cm	EOX6	Siemens	
HSG636BB1	Height 60 cm	EOX6	Bosch	
HSG636BS1	Height 60 cm	EOX6	Bosch	
HSG636BW1	Height 60 cm	EOX6	Bosch	
HSG636ES1	Height 60 cm	EOX6	Bosch	
HSG636ES1C	Height 60 cm	EOX6	Bosch	
HSG636ES1W	Height 60 cm	EOX6	Bosch	
HSG636XS6	Height 60 cm	EOX6	Bosch	
HSG656RS1	Height 60 cm	EOX6	Bosch	
HSG656XS1	Height 60 cm	EOX6	Bosch	
HSG656XS6W	Height 60 cm	EOX6	Bosch	
HSG856XB6	Height 60 cm	EOX6	Bosch	
HSG856XS1	Height 60 cm	EOX6	Bosch	Tested model
HSG856XS6	Height 60 cm	EOX6	Bosch	

Identically constructed full steam ovens (45 cm):

Appliance	Description	Series	Brand	
C15FS22N0	Height 45 cm	EOX6	Neff	
C15FS24N0	Height 45 cm	EOX6	Neff	
C17FS32N0B	Height 45 cm	EOX6	Neff	
C17FS42N0	Height 45 cm	EOX6	Neff	
C17FS52N0	Height 45 cm	EOX6	Neff	Tested model
C87FS22N0	Height 45 cm	EOX6	Neff	
C87FS32N0	Height 45 cm	EOX6	Neff	
C87FS32N0B	Height 45 cm	EOX6	Neff	
CS636GBS1	Height 45 cm	EOX6	Siemens	
CS656GBS1	Height 45 cm	EOX6	Siemens	
CS656GBS1B	Height 45 cm	EOX6	Siemens	
CS656GBS1W	Height 45 cm	EOX6	Siemens	
CS656GBW1	Height 45 cm	EOX6	Siemens	
CS856GBS1S	Height 45 cm	EOX6	Siemens	
CS856GDB6S	Height 45 cm	EOX6	Siemens	
CS856GDW6S	Height 45 cm	EOX6	Siemens	
CS856GPS1	Height 45 cm	EOX6	Siemens	
CS856GPB6	Height 45 cm	EOX6	Siemens	
CSG636BS1	Height 45 cm	EOX6	Bosch	
CSG636BS2	Height 45 cm	EOX6	Bosch	
CSG656BS1	Height 45 cm	EOX6	Bosch	
CSG656BS1B	Height 45 cm	EOX6	Bosch	
CSG656BS1I	Height 45 cm	EOX6	Bosch	
CSG656BS1W	Height 45 cm	EOX6	Bosch	
CSG656RB1	Height 45 cm	EOX6	Bosch	
CSG656RB6	Height 45 cm	EOX6	Bosch	
CSG656RS1	Height 45 cm	EOX6	Bosch	
CSG656RS1A	Height 45 cm	EOX6	Bosch	
CSG656RS6	Height 45 cm	EOX6	Bosch	
CSG656RW1	Height 45 cm	EOX6	Bosch	
CSG656RW6	Height 45 cm	EOX6	Bosch	
CSG856NS1	Height 45 cm	EOX6	Bosch	
CSG856RB6	Height 45 cm	EOX6	Bosch	
CSG856RS1	Height 45 cm	EOX6	Bosch	
CSG856RS6	Height 45 cm	EOX6	Bosch	

ANALYSIS METHOD

Vitamin C analysis:

Apparatus:

The vitamins were determined by a high performance liquid chromatography method (HPLC), using a Hewlett Packard (Agilent-Series 1100) chromatograph equipped with the following modules:

- Degasser (G1322 A, 1100 Series)
- Quaternary pump (G1311 A, 1100 Series)
- Autosampler (G1313 A, 1100 Series)
- Photodiode array detector (DAD) (G1315 B, 1100 Series)
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Chromatography column: Discovery®RP Amide C16, 5µm, 15 cm x 4.6 mm supplied by Supelco Analytical.

Analysis conditions:

- Injection volume: 20 µL
- Flow-rate of the mobile phase:1 mL/min
- Time of analysis: 10 min
- Temperature: 40 ºC
- Mobile phase: HPLC grade water with metaphosphoric acid to pH2.2

Commercial standards: Ascorbic acid 99.7 % (Merck, Ref:1.00127.0100)

Calibration:

Commercial Standard	λ (nm)	Intercept	Slope (L/mg)	Interval	Correlation coefficient
Ascorbic acid (time=2.6 min)	245	7.463±0.635	94.458±1.363	1-50 mg/L	0.995

Extraction procedures:

Potatoes:
Five grams of homogenate were mechanically stirred in 10 mL of a 4.5 % (w/v) solution of metaphosphoric acid for 15 min. The mixture was vacuum-filtered. The permeated was mixed with other 10 mL of the same metaphosphoric acid solution and vacuum filtered again. The filtrate was diluted until obtaining a total final volume of 25 mL with metaphosphoric acid solution. An aliquot was filtered through a 0.45 µm Millipore filter prior to injection into the chromatographic column. To ensure that the samples were representative, all pieces of potatoes were mixed doing a paste and

afterwards 6 samples of 5 g were analysed separately. Two or three replicates were performed for each condition.

Peppers:
Five grams of peppers were cut into small pieces with a ceramic knife. 10 mL of a 4.5 % (w/v) solution of metaphosphoric acid was added. The mix was homogenized in an "ultraturrax" for 2 min. The weight of the samples was balanced with other 10 mL of the same metaphosphoric acid solution. The samples were centrifuged at 4000 rpm for 20 min. The upper liquid was vacuum filtered. The filtrate was diluted until obtaining a total final volume of 25 mL with metaphosphoric acid solution. An aliquot was filtered through a 0.20 μm Millipore filter prior to injection into the chromatographic column. To ensure that the samples were representative, the squares or the stripes of raw or cooked peppers, were cut into small pieces and afterwards 6 samples of 5 g were analysed separately. Two replicates were performed for each condition. Therefore, 12 samples for each cooking condition were analysed.

Vitamin B analysis:

Apparatus:
The vitamins were determined by a high performance liquid chromatography method (HPLC), using a Hewlett Packard (Agilent-Series 1100) chromatograph equipped with the following modules:

- Degasser (G1322 A, 1100 Series)
- Quaternary pump (G1311 A, 1100 Series)
- Autosampler (G1313 A, 1100 Series)
- Photodiode array detector (DAD) (G1315 B, 1100 Series)

Chromatography column: Discovery®RP Amide C16, 5μm, 15 cm x 4.6 mm supplied by Supelco Analytical.

Analysis conditions:

- Injection volume: 100 μL
- Flow-rate of the mobile phase:1 mL/min
- Time of analysis:47 min
- Temperature: 25 ºC
- Solvents: Initial isocratic step with 10 mM phosphate buffer at pH 6.5 for 13 min followed by a linear gradient to acetonitrile-buffer (6:94 v/v) mixture during 1 min, this mixture being held for 6 min. Then a second linear gradient to acetonitrile-buffer (12:88, v/v) mixture 1 min and held for 15 min.

Commercial standards:

- Nicotinamide ≥ 99.5 % HPLC. Sigma-Aldrich Chemie GmbH
- Riboflavin 98 %. Alfa Aesar Gmb&CoKG.

Calibration:

Commercial Standard	λ (nm)	Intercept	Slope (L/mg)	Interval	Correlation coefficient
Nicotinamide	266	2.279±0.107	122.920±0.842	0.5-20 mg/L	0.999
Rivoflavin	266	2.901±0.297	370.369±12.886	0.02-0.4 mg/L	0.996

Extraction procedures:

Samples (10 g) were subjected to successive hydrolysis with hydrochloric acid (60 mL, 0.1 M). The suspensions were homogenized for 30 s and then heated in a water bath at 100 ºC for 30 min. The suspensions were cooled in an ice-water bath during 5 min. When they were cold, the pH was adjusted to 4 using 2 M sodium acetate. Taka-diastasa (0.5 g) was added for the enzyme hydrolysis. The samples were maintained at 50 ºC during 16 h. Then, 1 mL of 50% (w/v) trichloroacetic acid was added and the samples were again introduced into a water bath at 90 ºC during 10 min. When the samples were cold, the pH was adjusted to 6.5 with 10 M potassium hydroxide, and they were quantitatively transferred to a 100 mL calibrated flask using the mobile phase buffer (10mM potassium hydroxide pH 6.5). Aliquots were centrifuged at 4000 rpm for 15 min, filtered through a 0.2 μm polyamide Millipore chromatographic filter and injected into the chromatograph. To ensure that the samples were representative, all pieces of potatoes were mixed doing a paste and afterwards six samples of 10 g were analysed separately. Two or three replicates were performed for each condition.