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The role of drying methods on enzymatic activity and phenolic content of impregnated dried apple

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ABSTRACT

Infusion of antioxidants into vegetables is a new food strategy managed by matrix processing. Raw and blanched apple were air or freeze dried. In the case of freeze-dried samples, different freezing methods were previously applied: conventional (-28 °C), blast freezing (-30 °C) and liquid N_2 (-196 °C). Afterwards, air and freeze-dried samples at the different conditions were impregnated with a concentrated (40 °Brix) tea extract and finally, air dried for their stabilization. Total phenolic content (TPC), antioxidant capacity (AC), enzymatic activity and microstructure were analyzed. Regardless pre-treatments, the impregnation and the further drying improved the antioxidant potential. Samples with the most porous microstructure free of degradative enzymes provided high AC (78.5±0.9 mg Trolox/g dried matter) and TPC (16.7±0.2 mg GAE/g dried matter).

Keywords: blanching, freezing, infusion, enzymatic activity, antioxidant capacity.

1. Introduction

Apple polyphenols are important because of their contribution to sensory traits, being also recognized for their health promoting bioactive properties.[1, 2, 3] In addition, its structure with great number of air spaces makes apple a suitable fruit material to be infused with bioactive solutions. These facts and the growing tendency to its consumption in the world, in the form of fresh fruit, juice or dried product, including snack preparations, integral breakfast foods and other varieties,[4, 5] make apple a suitable raw material to develop new foods with higher bioactive content. Recent studies have illustrated the production of this kind of foods by the infusion of olive leaf extracts,[6, 7] grape phenolics compounds[8] or even probiotics[9] into solid vegetable matrixes. For this purpose, not only the bioactive potential of the solution being infused is relevant but also how the raw solid material is processed before and after the infusion.[10] In this way, blanching [11], freezing [12] and drying [13, 14] are essential by their impact on the native structure and compounds, such as enzymes, polyphenols and cell wall components [15].

Blanching is a common pre-treatment for vegetable products. It not only induces the thermal inactivation of undesirable enzymes in vegetable tissue, including polyphenol oxidase,[16] but also causes structural changes at a cellular level that result in a cell separation[17] influencing the mass transfer phenomena during drying.[18]

In general terms, it is known that freezing rate determines the ice crystal size and the nucleation, which is extracellular or intracellular for slow and fast rates, respectively[19] . Thus, it is commonly accepted that fast freezing better

preserves native structure due to the production of a large number of small ice crystals that cause less migration of water and less breakage of cell walls, and consequently less texture deterioration. However, if the process is too fast it can provoke breakage at the product level.[20, 21, 22] Therefore, depending on the freezing method the material will show different structural properties, which should be relevant for further infusion.

The removal of water by prior drying of the raw material could facilitate the infusion of the extracts into fruit matrixes. Nevertheless, drying could also negatively affect not only the nutritional quality but also the microstructure, being this dependent on drying conditions and technique employed.[23] Among the most relevant structural modifications, cell shrinkage should be considered because it causes the major modification in the global structure of the product [24,25] creating a more compact and close matrix, which could strongly affect the impregnation process hindering the entrance of the liquid. Moreover, from a technological point of view and aiming to long shelf-life foods, dehydration is the final step for the product stabilization.[26]

Vacuum impregnation has received increasing attention as potential process for the design of new enriched fruit and vegetable products. It makes possible to introduce dissolved or suspended substances directly into the product porous structure, allowing fast compositional and structural changes.[27] Although, as already mentioned, infusion capacity is mostly dependent on how the raw material was processed before.

Taking into account the aforementioned factors, the aim of this work was to evaluate how some processing steps (blanching, freezing and drying) affect

the phenolics retention of infused extracts with high antioxidant potential into apple, paying special attention to the role of the microstructure and enzymatic activity.

2. Materials and methods

2.1. Raw material

A concentrated (40 °Brix) tea extract, previously obtained in Unilever laboratories, was used for the impregnation. Before impregnation, the extract was pasteurized for 5 min at 75 °C and diluted in water (1:50, v/v) to obtain the tea impregnation solution.

An homogeneous apple (*Malus domestica* cv. Jonagold) batch (20 kg) was purchased in a local market, presenting an average total solid content of 11±3 g/100 g and 10.3±0.7 ºBrix. Cubes of 10 mm were obtained from the apple flesh by using a cutting machine (CL50 Ultra, Robot Coupe USA, Inc., Jackson, MS, USA) and immediately processed. The half of the fresh samples were blanched by immersion in boiling water for 90 s and afterwards, rinsed in cold water (4 ºC) for 10 seconds.

2.2. Apple drying

In order to obtain dehydrated samples to be impregnated, both fresh (non-blanched, NB) and blanched (B) apple cubes were dried by means of two different methods: freeze drying (FD) and hot air drying (HAD). Once the samples were impregnated, further dehydration was carried out by HAD. A

scheme of the experimental design and the nomenclature employed is shown in Figure 1.

In FD experiments, apple cubes were frozen using three different procedures: a conventional freezer (-28 ºC), a blast freezer (-30 ºC) and liquid N_2 (-196 °C). FD was stepped from -30 °C up to 50 °C at a constant pressure of 0.4 mbar (Zirbus Technology, Bad Grund, Germany). For the HAD, apple samples were dried in a pilot-scale convective drier (Mitchell Dryers LTD, Carlisle, UK) with parallel flow at 60 °C, 0.5 m/s and relative humidity lower than 10%.

In both FD and HAD, the initial mass load used was 3.5 kg, being the mass load in both driers of 5.6 kg/m^2. Drying was extended until the samples lost 89 ± 3 % of the weight for fresh and blanched apple, while impregnated apples lost 95.8 ± 0.3 %.

2.3. Impregnation

For impregnation, 6 g of dried apple cubes were immersed in 300 mL of the tea solution at 25 °C using a flask protected from light. The impregnation was carried out in two steps, a vacuum period of 14 h (- 600 mm Hg) followed by 55 min at atmospheric pressure. Apple cubes were blotted with tissue paper to remove the excess of surface tea solution before being weighed and processed. Experiments were conducted in triplicate.

2.4. Apple extracts for analysis

136 Apple samples (0.25-1 g) were mixed with distilled water (40 mL) and
137 blended (Variable Speed Laboratory Blender, Waring Laboratory, USA) for
138 5 min. Afterwards, the extracts were filtered (nylon filters of 0.45 µm) and placed
139 in opaque vials at 4 °C until analysis of the antioxidant potential. In the case of
140 enzymatic activity determination, the extracts were filtered twice by using paper
141 filters (MELB 1077, 185 mm) and a PD-10 desalting column (Amersham
142 Pharmacia Biotech, NJ, USA). The desalted sample extracts were stored at
143 4 °C until being analyzed.
144
145 *2.5. Total phenolic content measurement (TPC)*
146 The phenolic content was determined by the Folin-Ciocalteu method.[28]
147 Briefly, 100 µL of sample were mixed with 200 µL of Folin-Ciocalteu's phenol
148 reagent (Sigma-Aldrich, Madrid, Spain) and 2 mL of distilled water. After 3 min
149 at 25 °C, 1 mL of Na_2CO_3 (Panreac, Barcelona, Spain) solution (Na_2CO_3-water
150 20:80, w/v) was added to the mixture. The reaction was kept in the dark at room
151 temperature for 1 h. Finally, the absorbance was read at 765 nm using a
152 spectrophotometer (Helios Gamma, Thermo Spectronic, Cambridge, UK). The
153 measurements were carried out in triplicate. A calibration curve was previously
154 prepared using solutions of a known concentration of gallic acid hydrate
155 (Sigma-Aldrich, Madrid, Spain) in distilled water. Results were expressed as mg
156 of gallic acid (GAE) per g of dried matter (d.m.). Following this procedure, the
157 TPC of the concentrated tea extract was also measured (0.100 g GAE/mL
158 concentrated extract).
159

160 *2.6. Antioxidant capacity measurement (AC)*

161 The antioxidant capacity was determined by using the Ferric-reducing
162 ability power (FRAP) method,[29,30] which is a simple method used to estimate
163 the reduction of a ferric-tripyridyltriazine complex method. Briefly, 900 µL of
164 freshly prepared FRAP reagent were mixed with 30 µL of distilled water and
165 30 µL of test sample or water as appropriate reagent blank and kept at 37 °C for
166 30 min. The FRAP reagent contained 2.5 mL of a 10 mM TPTZ (Fluka,
167 Steinheim, Germany) solution in 40 mM HCl (Panreac, Barcelona, Spain) plus
168 2.5 mL of 20 mM $FeCl_3 \bullet 6H_2O$ (Panreac, Barcelona, Spain) and 2.5 mL of 0.3 M
169 acetate buffer (Panreac, Barcelona, Spain), pH 3.6. Readings at the maximum
170 absorption wave length (595 nm) were taken using a spectrophotometer (UV-
171 1800, Shimadzu, 's-Hertogenbosch, The Netherlands). Four replicates were
172 made for each measurement. The antioxidant capacity was evaluated through a
173 calibration curve, which was previously determined using water solutions of
174 known Trolox (Sigma-Aldrich, Madrid, Spain) concentrations and expressed as
175 mg Trolox per g of dry matter (d.m.). Following this procedure, the AC of the
176 concentrated tea extract was also measured (0.528±0.088 g Trolox/mL
177 concentrated extract).

178

179

180 *2.7. Peroxidase (PO) activity*

181 The PO activity was determined monitoring the increase in the
182 absorbance (UV-1601, Shimadzu, 's-Hertogenbosch, The Netherlands) at
183 414 nm and 25 °C with ABTS (Sigma-Aldrich, Madrid, Spain) as substrate. The

184 reaction mixture consisted of 100 µL of ABTS 10 mM, 100 µL of Na-acetate
185 buffer (Sigma-Aldrich, Madrid, Spain) 100 mM pH 5 and 790 µL of desalted
186 sample extract. The reaction was started with the addition of 10 µL of
187 0.1 M H_2O_2, the optical density was recorded on-line for 10 min. The PO activity
188 was expressed as units of enzymatic activity (UEA) per g of dried matter (d.m.).
189 One UAE was defined as the amount of enzyme needed to produce an increase
190 of 0.001 optical density unit/min in a 1 cm cuvette under our standard assay
191 conditions. Measurements were replicated three times.
192
193 *2.8. Polyphenol oxidase (PPO) activity*
194 The activity of PPO was measured by monitoring for 10 min the increase
195 in the absorbance (UV-1601, Shimadzu, 's-Hertogenbosch, The Netherlands) at
196 400 nm and 25 °C with epicatechin (Sigma-Aldrich, Madrid, Spain) as substrate.
197 The reaction mixture consisted of 500 µL of epicatechin 2 mM in MES buffer
198 (Sigma-Aldrich, Madrid, Spain) pH 6 and 500 µL of desalted sample extract.
199 The PPO activity was expressed as units of enzymatic activity (UEA) per g of
200 dried matter (d.m.). One UAE was defined as the amount of enzyme needed to
201 produce an increase of 0.001 optical density unit/min in a 1 cm cuvette under
202 our standard assay conditions. Measurements were replicated three times.
203
204 *2.9. Scanning electron microscopy (SEM)*
205 A piece of dried apple was cut into two halves in such a way that a cross-
206 section was obtained. In the case of dried apple, a very thin slice was cut off
207 from the surface with a razor blade to obtain a high quality cross-sectional

surface of the remaining piece of dry tissue. Obtaining the thin slice was not possible in the case of impregnated and dried apple so, the analysis focused only on the surface of the cross-section. In both cases, sample surface was sputter coated with platinum for better SEM imaging quality. The Pt coated sample was inserted into a scanning electron microscope (Jeol 6490LA, Tokyo, Japan) and both the peripheral and central areas were imaged at several magnifications: 25×, 50×, 100×, 250× and 500×.

2.10. Statistical analysis

Analysis of variance (ANOVA) were conducted (significance level of 95 %) in order to statistically identify the effect of the variables under study by using the Statgraphics-Plus software 5.1 (Statistical Graphics, Rockville, MD, USA). Homogeneity of variance was analyzed by comparing standard deviations and least significance difference (LSD) were computed to compare groups.

3. Results and discussion

3.1. Influence of processing on the microstructure of dried apple

Changes at microstructural level were induced in apple by combining blanching, freezing and drying methods, as illustrated in Figure 1, being structural modifications shown in Figure 2.

Microstructural analysis showed that every pre-treatment greatly affected the microstructure of the dried apple. Therefore, it was possible to obtain samples with different structural properties (Figure 2). In SEM micrographs,

bright regions correspond to cell walls and membranes whereas intra and intercellular spaces appear as dark zones.

Prior blanching to drying (Figure 2a, c, e and g) promoted remarkable changes on the microstructure of dried apple, being characterized by a more porous structure. The pectin substances are the main components of the middle lamella, a region which maintains cell to cell packing in fruit tissue.[31, 32] During blanching, modifications of pectins and hemicelluloses may contribute to the collapse of the cell walls, resulting in cell separation and the increase of intercellular spaces.[33] Moreover, blanching causes homogenization of sugars and other solutes over the tissue due to the disruption of membranes.[34]

The loss of membrane integrity facilitates free water permeation, giving no preference for extracellular nucleation during freezing.[35] Thus, B-FD samples presented smaller ice crystals and more homogeneous distribution (Figure 2c, e and g) than NB-FD samples (Figure 2d, f and h). As noticed, this effect linked to blanching was more remarkable as the freezing rate increased. These results were consistent with the ones obtained in carrot, where blanching before freezing at -150 °C resulted in smaller pores which were more homogeneously distributed whereas in freezing at -28 °C, the effect was less noticeable.[36]

In the case of HAD apples, the enhancement of microstructure by blanching prior to drying was also observed (Figure 2a and b). During drying, the structure and interactions with solid matrix affect diffusion of gases and liquids. Moreover, concentration gradients impose stresses on the material and diffusion can be accompanied by shrinkage and deformation.[37] Nevertheless,

these effects could be minimized by a previous blanching due to its abovementioned effects on cell structure. The more free water movement in blanched apples would facilitate the water leaving and would contribute to reduce the stress,[38] giving rise to a less collapsed structure in B-HAD apple.

As regards freezing pre-treatment in FD apples, it also affected the structural integrity. In general terms, freeze-drying of apple caused structural modifications, such as cell wall collapse, texture breakage, membrane breakdown and more and larger intercellular spaces.[39] However, the structural modifications were mainly controlled by the ice crystal size, which is related to freezing rate. Thus, conventional freezing at -28 °C (Figure 2c and d) induced the slow formation of bigger crystals, destroying the native cell structure and giving rise to the most open structure. Thus, structure of FD28 samples (Figure 2c and d) was even more degraded than the one showed by HAD samples (Figure 2a and b). On the contrary, FD196 apples (Figure 2g and h) presented a better microstructure preservation, with less damage on cell walls and less cell collapse.

3.2. Effect of processing on enzymatic activity and antioxidant potential of dried apple

Aiming to characterize the dried material before carrying out the phenolics infusion, not only the microstructure of dried samples was analyzed but also other properties, such as the PPO and PO activities and the antioxidant potential (TPC and AC).

279 Blanching of fresh material affected the apple microstructure as
280 aforementioned and, at the same time, had a significant ($p<0.05$) influence on
281 the enzymatic activity. This pre-treatment completely inactivated the PO and
282 PPO, providing dried materials free of active enzymes. In general terms, non-
283 blanched apples showed higher PPO (Figure 3b) than PO (Figure 3a) activity,
284 except the NB-HAD samples where the drying temperature seemed to be more
285 effective in the denaturing of PPO enzymes. Drying temperature significantly
286 ($p<0.05$) affected the enzymatic content, as previously reported.[40] HAD at high
287 temperature would positively contribute to inactivate the enzymes.
288 Nevertheless, low temperatures applied during FD would preserve enzymes in
289 latent state, recovering their activities when they are placed in contact with
290 aqueous mediums. Thus, the NB-FD samples showed the highest PO (Figure
291 3a) and PPO (Figure 3b) activity. Even, the influence of freezing method was
292 also appreciated since the faster the ice crystal formation the higher the PO
293 (Figure 3a) and the lower the PPO activity (Figure 3b). However, it is important
294 to highlight that this effect was significant ($p<0.05$) only in the case of PPO.

295 The cell damage suffered during drying and freezing not only promoted
296 the further release of PO and PPO enzymes but also of other intracellular
297 compounds, such as apple polyphenols. Thereby, processing could manage the
298 extractability of polyphenols, making them more or less available for extraction
299 [41] and so, affecting the antioxidant potential of samples.[42] Previous studies
300 have reported that processing causes no change to antioxidant potential of fruit
301 and vegetables or enhances it due to the improvement of antioxidant properties
302 of naturally occurring compounds or formation of novel compounds.[43]

303 Nevertheless in this study, this fact was not observed and all processing
304 conditions reduced the TPC of fresh material (Figure 4a). The degradation of
305 the TPC was consistent with other works where the impact of apple drying on
306 the phenolic content was studied. [44, 45] Regarding the AC (Figure 4b), the effect
307 was different depending on the previous processing of apple. On the one hand,
308 its reduction was significant ($p<0.05$) for HAD samples regardless the pre-
309 treatment (B or NB), probably due to the high sensitivity of apple polyhenols to
310 high temperatures. On the other hand, the FD samples previously blanched
311 were the only ones able to keep the AC (Figure 4b) despite the TPC decrease.
312 This fact could be linked to the capacity of phenolics compounds to interact
313 among them to provide new polyphenols with higher AC than the initial ones, so
314 the decrease of TPC could result in the increase of the AC as consequence of
315 the new phenolics formed. In NB-FD samples, the reduction of both TPC and
316 AC should be consequence of the residual enzymatic activity (Figure 3a and b).
317 Regarding the freezing method, no influence was observed in the antioxidant
318 potential of FD samples.
319
320 *3.3. Phenolics infusion into dried apple and final stabilization by drying*
321 Dried apple cubes were vacuum impregnated with tea extract rich in
322 antioxidant compounds (Figure 1). Afterwards, in order to obtain stable
323 products, the drying of impregnated samples was performed and the TPC, AC
324 and microstructure were analyzed.
325 The microstructural analysis highlighted that, regardless apple pre-
326 treatments, the structure of the impregnated-dried samples was similar, this

being characterized by a total tissue collapse (Figure 5). This fact could be explained by the vacuum treatment during impregnation. Vacuum causes an expansion and a further release of the occluded internal gas.[46] Then, the recovery and holding of the atmospheric pressure during the impregnation pushes the solvent (tea) into the spaces initially occupied by the gas keeping the sample volume. However, when the water is removed by the final drying, samples lose their integrity since there is neither air nor liquid to keep the structure, resulting in compact fruit tissues. The undesirable structural changes as a consequence of the vacuum infusion have been also observed by other authors who attributed the structural changes suffered by apple cylinders to the vacuum application during the penetration of water into the samples.[47] Although it has also been reported that the structural collapse could be, in certain way, controlled by the vacuum level.[48]

The combination of drying-impregnation-drying provided stable products with much higher antioxidant potential (Figure 6) than those found in the dehydrated raw apple (Figure 4), which confirms the results obtained in previous works.[6] Blanching had a significant ($p<0.05$) effect in both TPC (Figure 6a) and AC (Figure 6b) when samples were dried by FD before the impregnation. FD samples improved the TPC and AC by prior blanching due to its influence on PPO and PO activity. This result highlighted the influence of the residual enzymatic activity on the antioxidant potential, a hypothesis already proposed.[6] In addition, in blanched samples, it was possible to study the influence of microstructure on the phenolics infusion. Thus, the structure, determined by the freezing and drying method, affected significantly ($p<0.05$)

TPC and AC when PPO and PO were denatured. The highest antioxidant potential (TPC of 16.7 ± 0.2 mg GAE/g d. m. and AC of 78.5 ± 0.9 mg Trolox/g d.m.) was found in samples with the most porous structure (Figure 2g), the FD196-I-HAD apples. This fact would confirm the hypothesis of a previous work [6] where it was suggested that polyphenols infused in an open structure are more exposed to dehydration conditions due to their weak interaction with the poorly consolidated solid matrix of FD samples previously frozen by a conventional method (-28 °C).

For NB samples, no clear influences were observed due to the dual effect of the enzymatic activity and structure in FD samples. It was previously postulated that HAD is better than FD to obtain final dried products with high TPC and AC (Figure 6) due to it involves a combined thermal/drying treatment.[6] The present study agreed with this result, although the differences between drying methods were similar probably due to the different operating conditions of FD and the sensitivity of phenolics compounds (olive leaves or tea extract) to the processing conditions.

Regarding the drying applied after the impregnation of apple pieces, it inactivated the PPO and PO of NB-FD samples (Figure 3). Thus, the enzymes were not found in any final dried product, providing materials completely stable.

4. Conclusions

Blanching, freezing and drying affected the microstructure, PPO and PO activity of dried apple, which are key factors to preserve the phenolics compounds infused into the solid matrix. Thus, latent oxidative enzymes in

freeze dried materials contributed to the degradation of impregnated polyphenols. Meanwhile, a more porous and well consolidated structure protected the infused compounds by reducing their exposition to drying conditions. The combination of blanching and freezing with liquid N_2 prior to the freeze drying provided impregnated apples with the highest antioxidant potential.

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559

Figure captions

Figure 1. Sequence of the different pre- and treatments undergone by apple samples.

Figure 2. Effects of blanching, freezing and drying on apple microstructure. B: Blanching, NB: Non-Blanching, HAD: hot air drying, FD28: freezing at -28 °C in a conventional freezer and then freeze drying, FD30: freezing at -30 °C in a blast freezer and then freeze drying, and FD196: freezing at -196 °C in liquid N_2 and then freeze drying.

Figure 3. Peroxidase (PO) and Polyphenol oxidase (PPO) activities of non-blanched (NB) dried apples. Means ± LSD intervals (95%) are plotted. HAD: hot air drying, FD28: freezing at -28 °C in a conventional freezer and then freeze drying, FD30: freezing at -30 °C in a blast freezer and then freeze drying, and FD196: freezing at -196 °C in liquid N_2 and then freeze drying.

Figure 4. Influence of processing on (a) the total phenolic content (TPC) and (b) antioxidant capacity (AC) of dried apple. Means ± LSD intervals (95%) are plotted. B: Blanching, NB: Non-Blanching, F: Fresh, HAD: hot air drying, FD28: freezing at -28 °C in a conventional freezer and then freeze drying, FD30: freezing at -30 °C in a blast freezer and then freeze drying, and FD196: freezing at -196 °C in liquid N_2 and then freeze drying.

Figure 5. Effects of processing on microstructure of dried apples previously pretreated and vacuum impregnated with tea extract. B: Blanching, NB: Non-Blanching, I: Impregnation, HAD: hot air drying, FD28: freezing at -28 °C in a conventional freezer and then freeze drying, FD30: freezing at -30 °C in a blast freezer and then freeze drying, and FD196: freezing at -196 °C in liquid N_2 and then freeze drying.

Figure 6. Antioxidant potential (TPC and AC) of vacuum impregnated dried apples with tea extract. Means ± LSD intervals (95%) are plotted. B: Blanching, NB: Non-Blanching, I: Impregnation, HAD: hot air drying, FD28: freezing at -28 °C in a conventional freezer and then freeze drying, FD30: freezing at -30 °C in a blast freezer and then freeze drying, and FD196: freezing at -196 °C in liquid N_2 and then freeze drying.

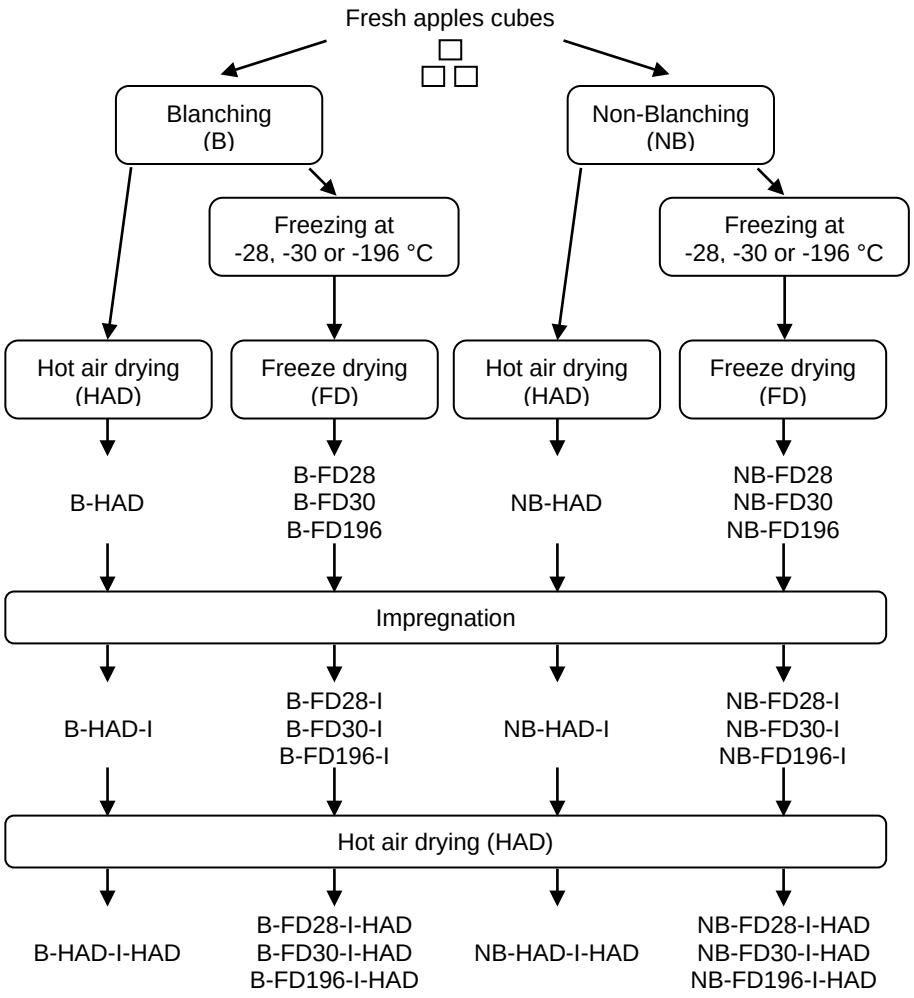
Fresh apples cubes
Blanching (B)
Non-Blanching (NB)
Freezing at -28, -30 or -196 °C
Freezing at -28, -30 or -196 °C
Hot air drying (HAD)
Freeze drying (FD)
Hot air drying (HAD)
Freeze drying (FD)
B-HAD
B-FD28
B-FD30
B-FD196
NB-HAD
NB-FD28
NB-FD30
NB-FD196
Impregnation
B-HAD-I
B-FD28-I
B-FD30-I
B-FD196-I
NB-HAD-I
NB-FD28-I
NB-FD30-I
NB-FD196-I
Hot air drying (HAD)
B-HAD-I-HAD
B-FD28-I-HAD
B-FD30-I-HAD
B-FD196-I-HAD
NB-HAD-I-HAD
NB-FD28-I-HAD
NB-FD30-I-HAD
NB-FD196-I-HAD

Figure 1

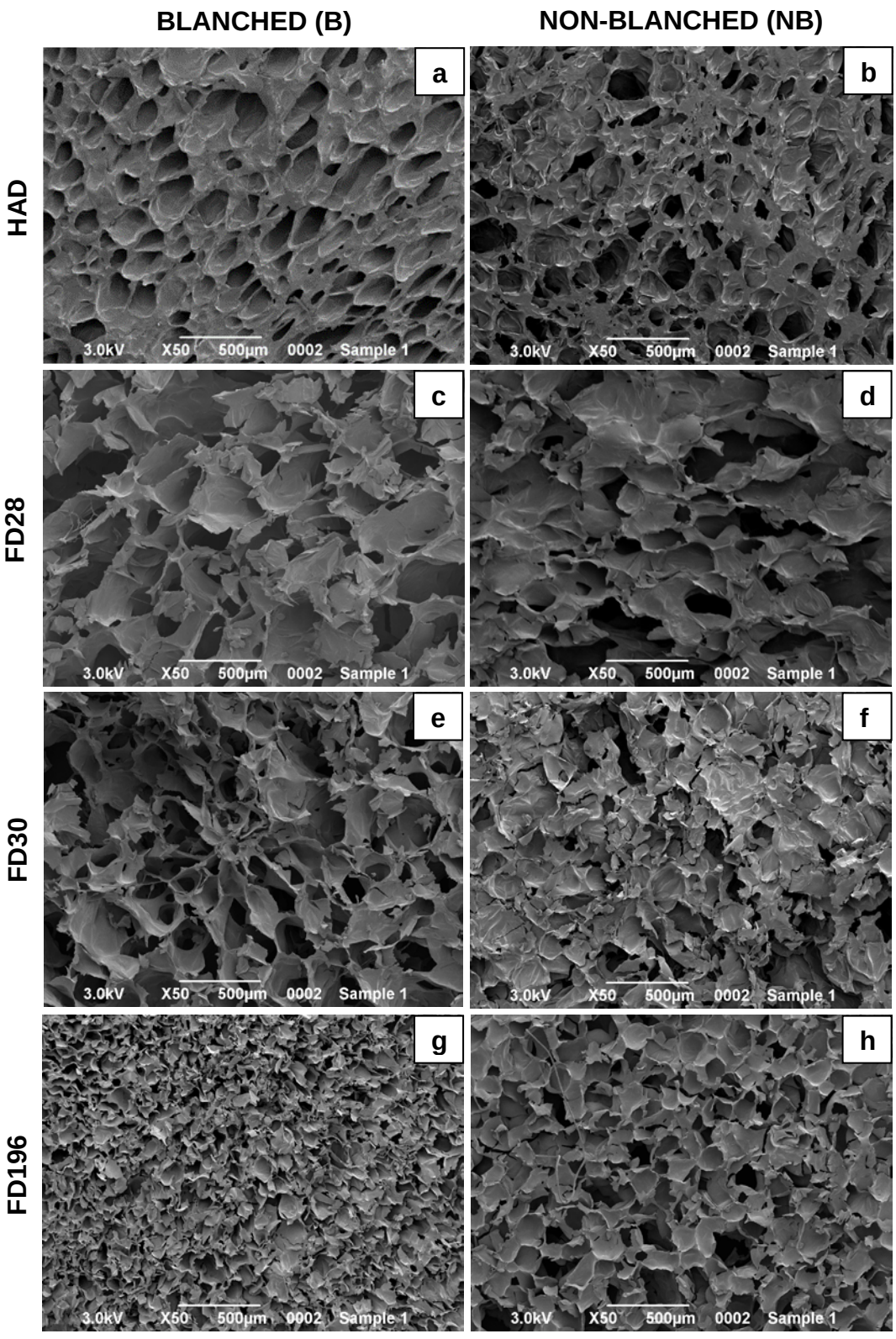
BLANCHED (B)
NON-BLANCHED (NB)
HAD
FD28
FD30
FD196
a
b
c
d
e
f
g
h
3.0kV X50 500µm 0002 Sample 1

Figure 2

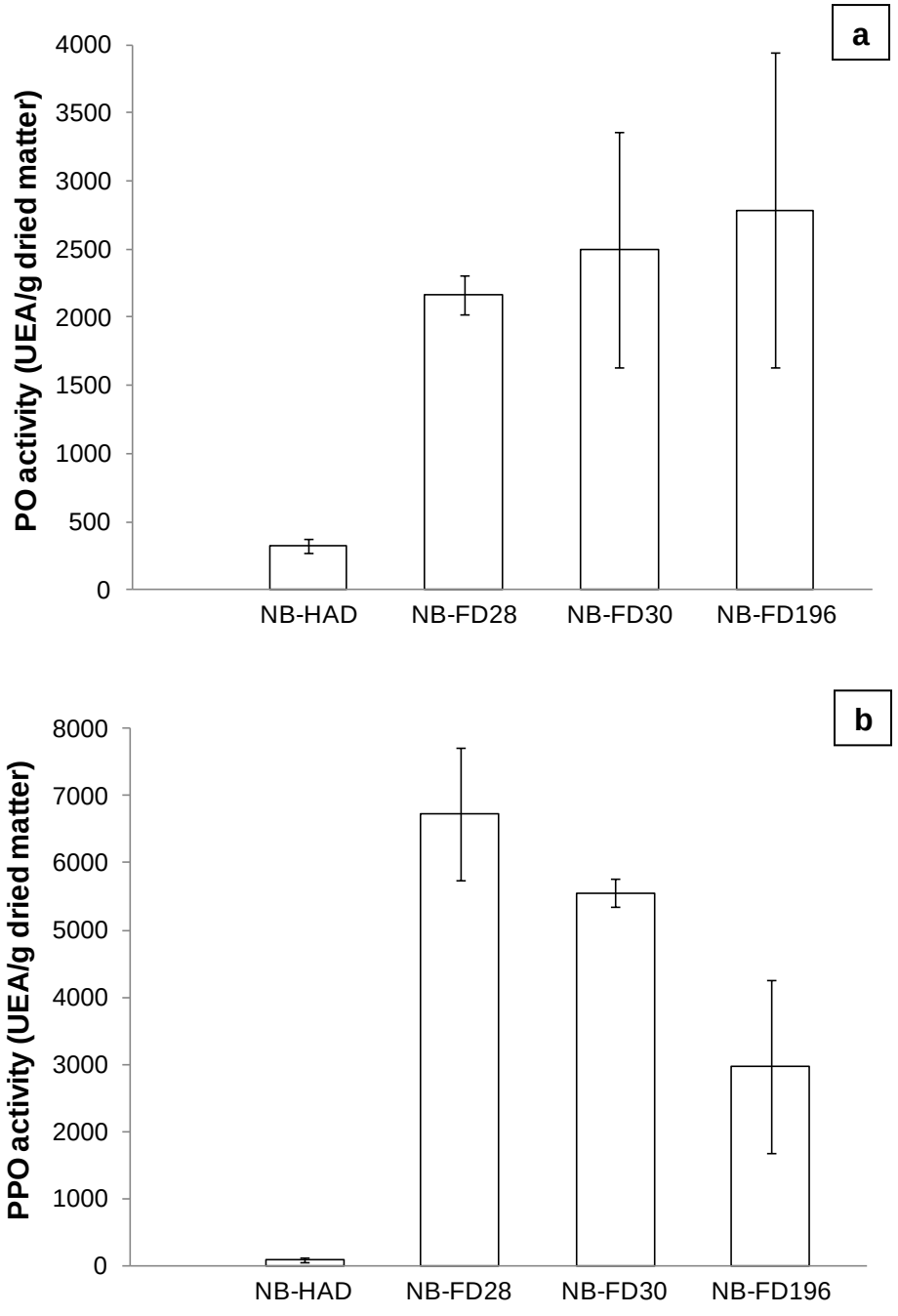
a
PO activity (UEA/g dried matter)
4000
3500
3000
2500
2000
1500
1000
500
0
NB-HAD
NB-FD28
NB-FD30
NB-FD196
b
PPO activity (UEA/g dried matter)
8000
7000
6000
5000
4000
3000
2000
1000
0
NB-HAD
NB-FD28
NB-FD30
NB-FD196

Figure 3

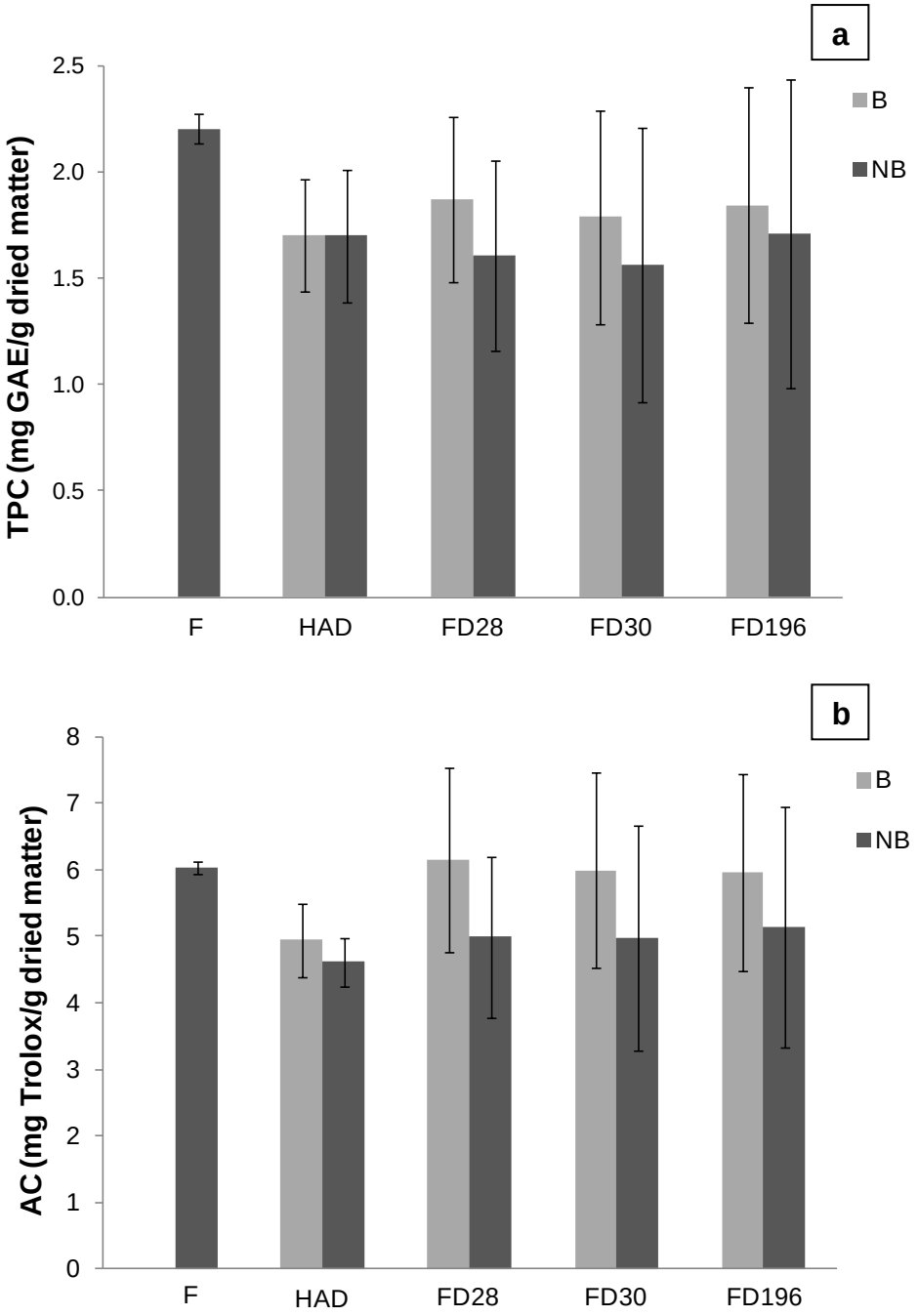
a
TPC (mg GAE/g dried matter)
2.5
2.0
1.5
1.0
0.5
0.0
B
NB
F
HAD
FD28
FD30
FD196
b
AC (mg Trolox/g dried matter)
8
7
6
5
4
3
2
1
0
B
NB
F
HAD
FD28
FD30
FD196

Figure 4

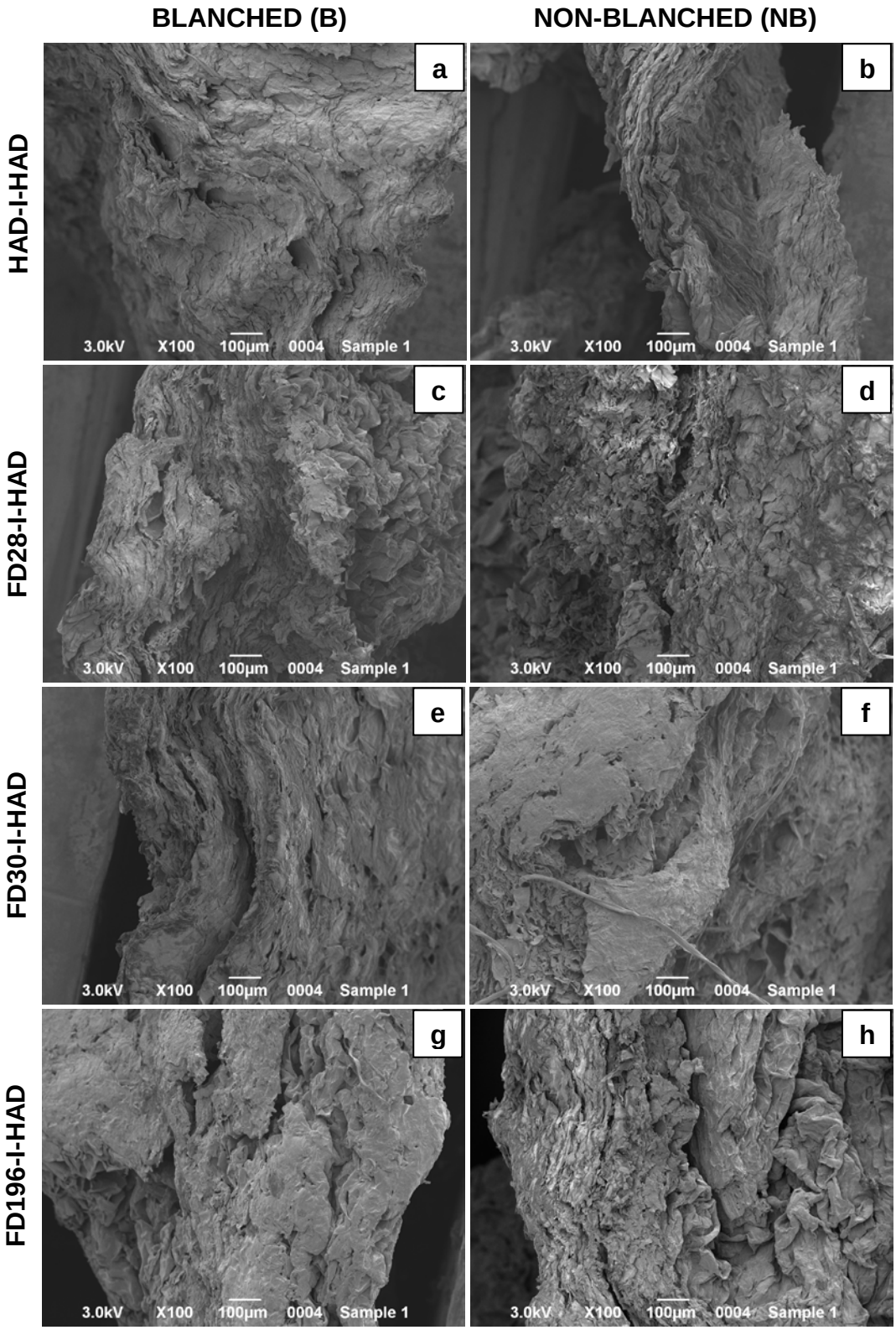
BLANCHED (B)
NON-BLANCHED (NB)
HAD-I-HAD
FD28-I-HAD
FD30-I-HAD
FD196-I-HAD
a
b
c
d
e
f
g
h
3.0kV X100 100µm 0004 Sample 1

Figure 5

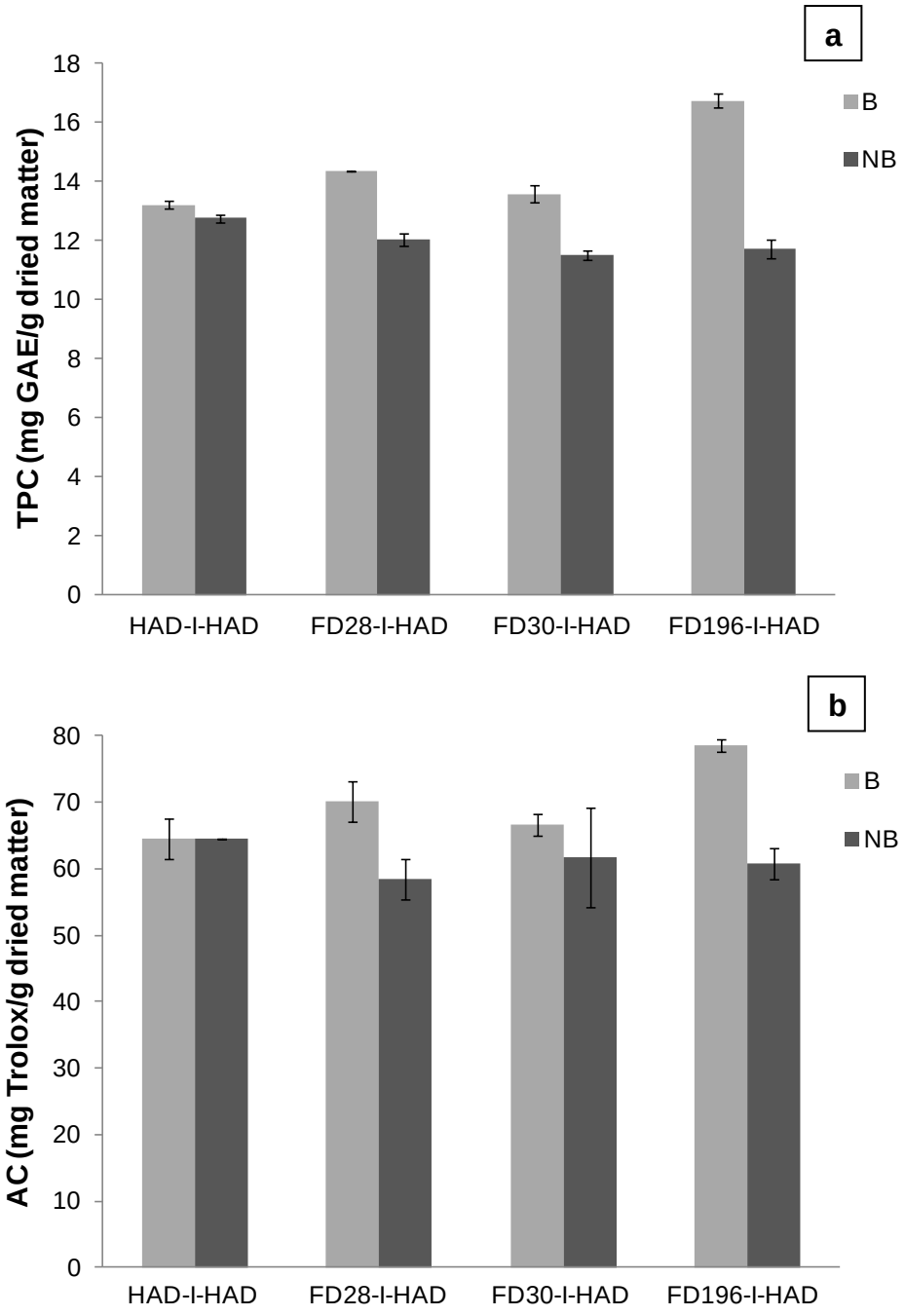
a
TPC (mg GAE/g dried matter)
18
16
14
12
10
8
6
4
2
0
B
NB
HAD-I-HAD
FD28-I-HAD
FD30-I-HAD
FD196-I-HAD
b
AC (mg Trolox/g dried matter)
80
70
60
50
40
30
20
10
0
B
NB
HAD-I-HAD
FD28-I-HAD
FD30-I-HAD
FD196-I-HAD

Figure 6