Effect of autoclaving cocoa nibs before roasting on the precursors of the Maillard reaction and pyrazines

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Summary Nibs of raw cocoa (N) were roasted (R) at 150 °C for 38 min. Some samples were autoclaved at 121 °C for 15 min (A) and roasted (AR) at 150 °C for 38 min. The samples were then analysed for moisture content, water activity (a_w), reducing sugar and amino group (α-N) levels and, absorption at 275 nm (A_{275}) for a trichloro acetic acid (TCA) extract and at 280 nm (A_{280}) for the distillate. Tri- and tetramethylpyrazines were quantified by solid phase micro extraction-gas chromatography (SPME-GC). There was a reduction in the levels of α-N, in the order N > A > R > AR. The same sequence was observed for reducing sugars. The tetramethylpyrazine had an $Area_{pyrazine}/Area_{internal\ standard}$ of N < R < A < AR and for trimethylpyrazine the order was N < A < R < AR. After autoclaving, the A_{tetra}/A_{tri} decreased from 31.30 (N) to 11.06 (A). With roasting, this ratio was 2.18 (R) and 2.58 (AR). Autoclaving nibs before roasting significantly influenced the levels of compounds which contribute to cocoa flavour formation and increased the concentration of tri- and tetramethylpyrazines in the headspace of cocoa samples. Autoclaving prior to roasting also affected the sensory properties of the samples.

Keywords Autoclaving, cocoa, flavour, processing, SPME, *Theobroma cacao*.

Introduction

Cocoa (*Theobroma cacao* L.) is used as raw material in many products and is highly appreciated for its flavour. Agronomic factors such as genotype, soil, climate and harvest procedures associated with processing conditions (fermentation, drying and roasting) are of great importance in determining the characteristics of cocoa, although the final users have little control over these factors. Cocoa from different origins and from different varieties have a distinct intrinsic composition, even after passing through the same processes. This distinctiveness contributes to the heterogeneity of the raw material. A better understanding of how processing influences the composition of cocoa and of how the constituents of cocoa are related to its sensory characteristics, is very important to the cocoa industry.

Flavour formation in cocoa is related, partly to thermal treatment (roasting) during which several modifications occur, including the Maillard reaction, which is responsible for the accentuated decrease in the concentration of reducing sugars and amino acids during roasting (Mermet *et al.*, 1992). The modifications associated with roasting are generally limited by factors inherent to the process itself.

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Cocoa samples from different origins show the same pyrazinic compounds but in different amounts. This may be related to the formation of flavour precursors during fermentation and to the type of roasting process and its intensity, as well as to agronomic factors. Major differences involve mainly the dimethyl, trimethyl and tetramethylpyrazines. The latter, together with 2,5-dimethylpirazine, have been suggested as indicators of roasting of cocoa beans (Muggler-Chavan & Reymond, 1967; van der Wal *et al*., 1971; Zamalloa *et al*., 1995).

Pyrazines are usually formed in proportion to the duration of treatment during the first 20–30 min of roasting at 150 °C, with each methylpyrazine having its own rate of development. After this the tetramethylpyrazine concentration begins to decrease and the increase of tri- is slow, indicating their involvement in other reactions or even the volatilization of pyrazines at a rate near or higher to their formation. Other methylpirazines such as the 2,3; 2,5 and 2,6-dimethyl show a constant increase until the beginning of over roasting (Chaveron *et al*., 1989). Pezoa García (1989) reported significant concentrations of methylpyrazines in the gases emitted during roasting.

Pyrazine formation is seen with roasting at 70 °C for 30 min (Reineccius *et al*., 1972). Dimethylpyrazines, especially 2,5-dimethylpyrazine, predominate in over-roasting. This increase, associated with the correlation between the degree of roasting and taste, made it possible to develop a roasting index based on the ratio between 2,5-dimethylpyrazine and the tri- and tetramethylpyrazines. The origin of the cocoa had little influence on this ratio (Ziegleder & Sandmeier, 1983; Chaveron *et al*., 1989; Pezoa García, 1989; Plumas *et al*., 1996; Jinap *et al*., 1998).

In this work, we studied the alterations in compounds that participate in the Maillard reaction as well as the formation of pyrazines during autoclaving of cocoa nibs, and the association of this process with roasting.

Materials and methods

Samples

Fermented cocoa beans of the Forastero variety collected from the Experimental Station of the IAC in Pariquera Açu, São Paulo, Brazil were used as the sample. The fermented material was broken into nibs (3–6 mm) prior to treatment. A raw sample was used as the control (N). A fraction of the material was roasted at 150 °C for 38 min (R). The rest of the material was autoclaved at 121 °C for 15 min (sample A), then roasted at 150 °C for 38 min (sample AR). A sample roaster (PRE 1Z, Probat-Werke, Emmerich-Rhein, Germany) with a high accuracy digital temperature controller was used for roasting the cocoa. This protocol was performed three times.

Analyses

After processing, the samples were ground in an analytical mill (IKA A10, Janke & Kunkel, Staufen, Germany) and analysed for moisture at 100 °C (AOAC, 1997). The reducing sugars were determined by the Dinitro Salicylic Acid (DNSA) method and the values expressed as glucose content (mg g^{-1}) (Chaplin, 1986). The amino groups (α-N) were extracted with a mixture of trichloroacetic acid/sodium acetate/acetic acid (0.11:0.22:0.33 M) (Murthy *et al*., 1997) and determined spectrophotometrically, after reacting with *o*-phthaldialdeyde (Church *et al*., 1985). The values were expressed as glycine content (mg g^{-1}). The absorbance of the resulting extract was also read at A_{275} to determine levels of amino acids and peptides with aromatic groups (Murthy *et al*., 1997). The volatile acid content was determined as described by Lopez (1983). The absorbance at 280 nm (A_{280}) was determined in distillates resulting from the samples. Distillation was also carried out as described by Lopez (1983). Water activity (a_w) was determined using a CX-2 (Decagon Devices, Washington, DC, USA).

All analyses were performed three times and the results were compared by ANOVA and the Tukey–HSD test was used to check for significant differences amongst samples ($P < 0.05$).

SPME-GC of the pyrazines

A study of several factors had been made to increase sensitivity of the SPME technique: these include extraction time and fibre coating, sample/headspace equilibration time, temperature effects and ionic strength.

Extraction time and fibre coating
One gram (1.0 ± 0.05) of sample was placed in a 15-mL V-vial flask with a magnetic stirrer and a silicone septum, and 10 mL of distilled water doped with 100 μg of standards (Aldrich, Milwawkee, WI, USA) tri- and tetramethylpyrazine. Some SPME fibres were exposed to the headspace equilibrated for 15 min for periods ranging from 5 to 60 min. The maximum efficiency was reached after 45 min of extraction using a Carbowax-PDMS (65 μm, Supelco, Belefonte, PA, USA) fibre.

Sample/headspace equilibration time
The effect of the sample/headspace equilibration time, in the range between 5 and 60 min, was evaluated as in *a* above but adopting 45 min as extraction time. This parameter was defined as 15 min for the further assays.

Temperature
Extractions with temperatures between 25 and 81 °C were carried out; an exponential increase in the extraction efficiency was observed in this range. Therefore, 60 °C was defined as the operational temperature for the final extraction procedure.

Ionic strength
The effect of ionic strength was evaluated replacing the 10 mL of distilled water (see *a*) by the same volume of aqueous NaCl solutions, with concentrations up to 353 g L^{-1} (corresponding to saturated solution). The amount of extracted pyrazines is linearly dependent on the NaCl concentration: an increase of c. 100% is observed when saturated NaCl is used instead of pure water.

The tri- and tetramethylpyrazine area was reported relative to the area of the internal standard (pyrazine, 250 μL of a solution with 0.72 mg mL^{-1}). All analysis were made in triplicate and standard deviation ranged between 0.6 and 10% (expected values for SPME works).

A gas chromatograph (AutoSystem XL, Perkin Elmer Co, Norwalk, CT, USA) was used for the chromatographic analysis. Desorption of the compounds present in the fibre occurred in the injector in the splitless mode, at 240 °C for 4 min. Helium (25 mL min^{-1}) was used as the carrier gas. The chromatographic column (PE-WAX (Perkin Elmer, Norwalk, USA), 30 m length, 0.53 mm internal diameter and 0.5 μm film thickness) was maintained at 45 °C for 10 min and then heated at a rate of 10 °C min^{-1} up to 210 °C and held at this temperature for 5 min. The detector temperature (flame ionization detector–FID) was 240 °C.

Sensory evaluation

The roasted nibs were milled in a cooled drum mill. The mass obtained was mixed with sugar and dairy cream (35% fat) in a proportion of 48.8:26.2:25 (w/w), respectively. The resulting product was cooled and cut into pieces (10 g weight) for sensory evaluation. Difference from the control test was assessed using sample R as the standard. Samples R and AR were presented to judges ($n = 30$) who were asked to indicate, on a 9-point scale (0 = no difference, up to 9 = extremely different), the extent to which the samples differed from the standard control. The results were compared by the *t* test (Meilgaard *et al.*, 1987).

Results and discussion

The temperature profile inside the roaster during the roasting process of samples N and A is shown in Fig. 1. The roasting behaviour of previously autoclaved samples and of standard control samples was similar.

After roasting the α-N value dropped in the order AR < R < A < N (Fig. 2). The same order was observed for reducing sugars (Fig. 2). There was a high correlation between the α-N value and the levels of reducing sugars from the control roasted and from the autoclaved roasted nibs ($R^2 = 0.991$ and 0.965, respectively), with a significant difference, $P < 0.05$, between them.

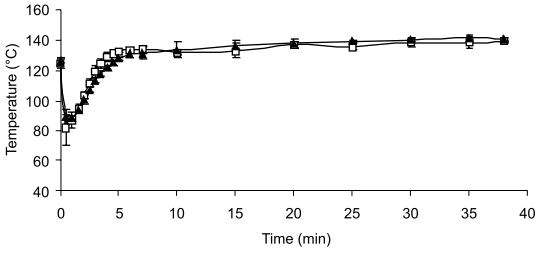

Figure 1 Temperature profile inside the roaster during the roasting of non-autoclaved cocoa (□) and autoclaved (▲) nibs (average ± s.d.).

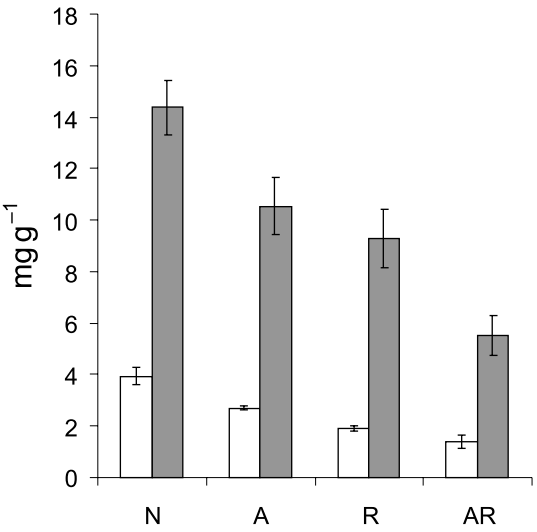

Figure 2 Reducing sugar (□) and amino group (■) concentrations expressed as glycine and glucose, respectively, for samples N, A, R and AR.

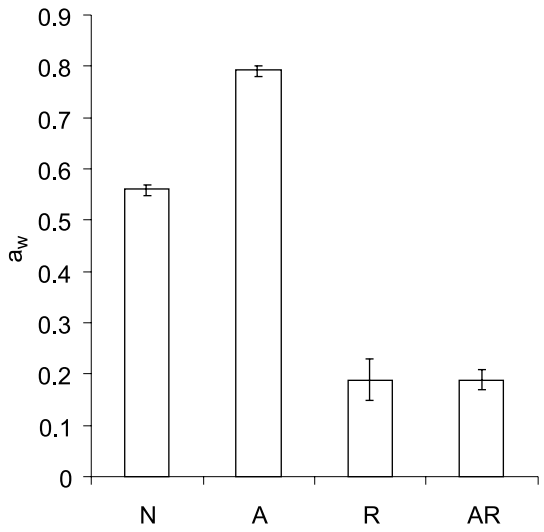

Figure 3 Water activity of samples N, A, R and AR.

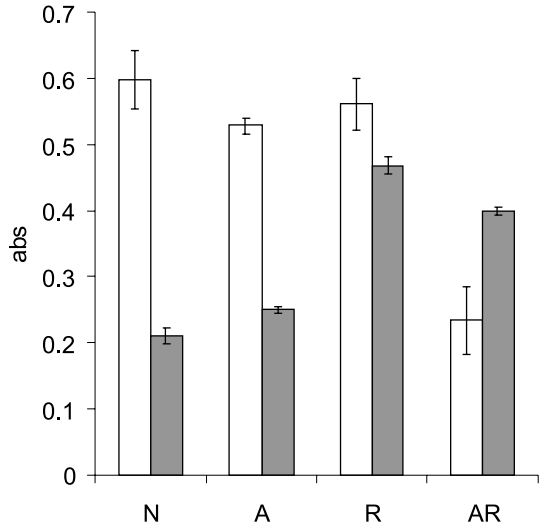

Figure 4 Absorption at 275 nm (□) for the TCA extract and at 280 nm (■) for the distillates of the samples N, A, R and AR.

The lower values for sugars and α-N in the group AR compared with the R group could indicate that autoclaving favours the Maillard reaction. In an experimental model system, with an increase in a_w the concentration of pyrrols increased, whereas the concentration of pyrazines, pyridines and furans decreased (Heinzler & Eichner, 1991). A process involving humidity changes and pre-thermal treatment prior to roasting resulted in cocoa with a better, more agreeable and longer lasting flavour than that of cocoa which was only roasted. This process would be indicated mainly for middle quality nibs (Oberparlaiter & Ziegleder, 1997). In the present work, the a_w content of the autoclaved sample was higher than that of the standard control sample (Fig. 3). This could explain the greater consumption of reducing sugars and α-N in the autoclaved sample.

The A_{275}, which indicates the presence of amino acids and/or peptides with aromatic groups, decreased significantly in AR compared with the rest of the samples (Fig. 4). As with the reduction in α-N, this decrease could be related to a higher a_w value of A compared with N, and this would favour the Maillard reaction.

Volatile acidity was reduced in the group AR (Fig. 5). This may be explained by a higher loss of water and volatile acids (vaporization) during roasting as the autoclaved samples had a higher moisture content (7.57 ± 0.17 g 100 g^{-1}) than raw nibs (4.44 ± 0.33 g 100 g^{-1}). Another possible explanation could be a higher permeability of nib tissues as a result of autoclaving. During roasting, there is a significant change in the acid content of cocoa butter, with unroasted cocoa butter containing a threefold higher concentration of volatile acids than that of roasted cocoa (Carlin *et al.*, 1986).

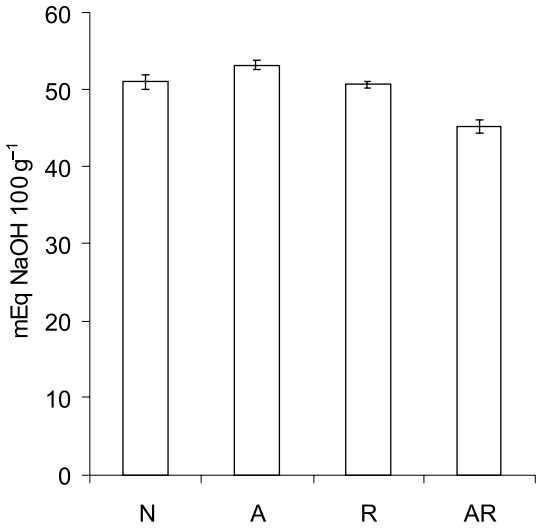

Figure 5 Volatile acidity in samples N, A, R and AR.

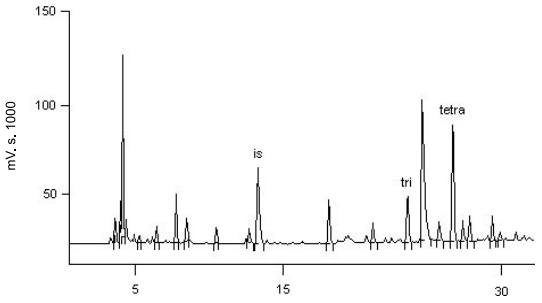

Figure 6 A typical SPME-GC chromatogram of a sample from the headspace of a roasted cocoa sample.

Figure 6 shows a SPME-GC chromatogram of the cocoa headspace. This procedure allows fast and easy evaluation of the compounds present in the headspace of the samples compared with the steam and/or solvent based extractions normally used.

Tetramethylpyrazine production in the treatments followed the order N < R < A < AR (Fig. 7), indicating that autoclaving produces tetramethylpyrazine in quantities similar to the direct roasting of nibs, which in turn showed a 9% increase relative to the raw material. In samples in which autoclaving preceded roasting, the tetramethylpyrazine content increased nearly 42%.

For trimethylpyrazine, the order of production was N < A < R < AR (Fig. 7). The values

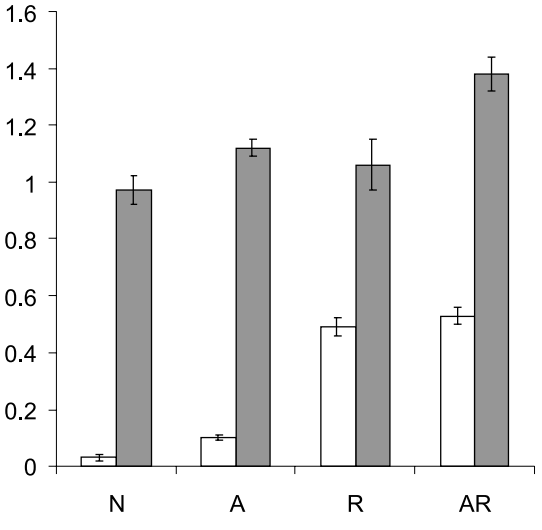

Figure 7 Ratio between the tri (□) and tetramethylpyrazine (■) area and the internal standard area for samples N, A, R and AR.

obtained represent an increase of about 1471% and of 1623% for R and AR, respectively. However, although sample AR showed values higher than R, the A_{tetra}/A_{tri} ratio did not differ significantly (2.60 and 2.16, respectively). A tetra/tri ratio equal or close to one indicates normal roasting, higher values indicate weak roasting and lower values reflect over-roasting (Hashim & Chaveron, 1994). So far, a ratio of one has only been achieved by methods using steam stripping. In the present work, tetra/tri ratio was obtained in the sample headspace.

Figure 4 shows the A_{280} values for the determination of volatile acidity. Pyrazines present in the distillate are the main compounds responsible for the UV activity (Ziegleder & Sandmeier, 1983). Figures 4 and 7 show a direct relationship between the A_{280} and pyrazine formation, especially trimethylpyrazine. This simple measurement could be useful for the inexpensive evaluation of changes as a result of thermal processing of cocoa.

The sensory test applied to products formulated with R and AR indicated that the samples differed significantly from one another ($F_{calculated} = 22.53$; $F_{tabulated(1,29)} = 4.20$, $P < 0.05$). This finding indicated that the extent of chemical changes as a result of autoclaving processing do influence cocoa flavour.

Conclusions

Autoclaving cocoa nibs prior to roasting increased the amount of compounds involved in flavour formation. The significant decrease in reducing sugars and α-N in autoclaved samples suggested that this treatment favoured the Maillard reaction. Cocoa nib autoclaving influenced the pyrazine concentration in the sample headspace both before and after roasting. Autoclaving the cocoa nibs before roasting increased the levels of tetramethylpyrazine, the principal pyrazine of cocoa, by 42.3% whereas in roasted cocoa the increase was 9.3%. For trimethylpyrazine these values were 1623% and 1471%, respectively. These results suggest that the use of SPME-GC to determine pyrazine levels in the headspace of cocoa by SPME-GC, could provide a suitable chemical test for evaluating the roasting process. Most chemical changes during autoclaving contribute to a product with a distinct flavour, as confirmed in a sensory evaluation.

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